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Research article

Analysis of needlestick and sharps injuries (NSSI) in a tertiary level hospital: A 6-year review study

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Abstract

This study aims to determine health care workers' (HCW) causes of injuries, and changes in these injuries according to years by analyzing notification forms made by HCWs in a tertiary hospital between 2018 and 2023 due to needlestick and sharps injuries (NSSI) and provide suggestions to managers for prevention of injuries. The study was a descriptive, single-center, retrospective study. It included data on the number of injuries reported by HCWs in a training and research hospital during 2018-2023. The year of injury to HCWs, the unit in which the injury was sustained and the type of injury instrument were evaluated. NSSI rate was determined using the "Healthcare Quality Standards Indicator Management Guide". Obtained data were analyzed and interpreted through tables created with SPSS 26.0 statistical software and Microsoft® Excel software. The study found that a total of 74, 105, 69, 55, 82 and 118 NSSIs were reported in 2018, 2019, 2020, 2021, 2022 and 2023, respectively. For 2018-2023, rates were 11.1%, 15.8%, 20.1%, 11.7% and 12.2%, respectively. Considering all years, the highest NSSI rate was found in 2020 (20.1%) by year. lowest NSSI rate was found in 2018 (11.1%). In 6 years between 2018 and 2023, the highest number of NSSI reports came from clinics/services. These units are followed by intensive care units, emergency departments, and operating rooms. It was observed that nurses/midwives (54) and doctors (25) were most likely to be exposed to NSSIs based on title. It was found that HCWs were exposed to the majority of needle-tipped NSSIs in all years. The results of the study indicate that most at-risk occupational groups are nurses among health workers. It is recommended to increase training activities, especially in high-risk groups, and to facilitate follow-up and reporting procedures after notification to prevent NSSI.

Keywords: *Health institutions; health workers; Needlestick and Sharps Injuries (NSSI); tertiary hospital*

1. Introduction

Needlestick and sharps injuries (NSSI) present a significant occupational hazard for healthcare workers (HCWs) due to the nature of their work (Mohamud et al., 2023; Li et al., 2024). These injuries have the potential to transmit over 20 different bloodborne pathogens, with the risk of contracting serious infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Although effective treatments exist, the risk of these infections can lead to psychological issues such as anxiety, depression, and post-traumatic stress disorder among HCWs, along with a decreased quality of life (Cooke and Stephens, 2017; Gauba, 2023). The estimated transmission rates of HIV, HCV, and HBV following a needlestick injury are 0.2%, 1.8%, and 30%, respectively, with only HBV being preventable through vaccination. Despite the implementation of preventative measures, including improved equipment design and enhanced training, sharps injuries continue to occur during all phases of handling sharps (Yunihastuti et al., 2020). The United States Occupational Health and Safety Administration (OSHA) states that 5.6 million HCWs are at risk of exposure to bloodborne pathogens through NSSIs (Alfulayw et al., 2021). Globally, approximately three million HCWs are exposed to blood-borne

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pathogens through needlestick injuries each year, resulting in approximately two million HBV infections, 0.9 million HCV infections, and 170.000 human immunodeficiency virus (HIV) cases, according to the World Health Organization (WHO) (WHO, 2024). Over 90% of these infections occur in developing countries. Despite the implementation of advanced prevention strategies in developed nations, such as real-time injury monitoring and standard operating protocols, NSSIs remain a global concern (Jahangiri et al., 2016).

HCWs experience a high incidence of NSIs and mucosal exposures, which are exacerbated by limited resources, inadequate infrastructure, insufficient personal protective equipment (PPE), and overwork in Türkiye. Studies in Turkish hospitals indicate that a significant proportion of HCWs have suffered NSSIs, despite high compliance with standard precautions (Duzgol et al., 2020; Kotanoglu, 2020; Korkmaz et al., 2022; Sari et al., 2023). These injuries are particularly prevalent among doctors and nurses, with surgical clinics and assistant doctors being notably affected. Further analysis in operating rooms and intensive care units highlights the necessity for the implementation of preventive measures, the maintenance of appropriate equipment, and the provision of comprehensive employee education with the aim of mitigating risks and ensuring safety (Thakur and Rao, 2024).

The risk of occupational blood-borne infections for HCWs in low- and middle-income countries remains high due to several factors. These include overcrowded hospitals, high patient-to-HCW ratios, limited awareness of the risks involved, inadequate PPE, a lack of sharps containers, insufficient understanding and use of post-exposure prophylaxis, low adherence to universal precautions, a high prevalence of bloodborne viral infections among patients, and low hepatitis B vaccination rates among HCWs (Dafaalla, 2016). It is imperative that continuous efforts be made to improve safety protocols, increase awareness, and enhance reporting mechanisms in order to protect HCWs from NSSIs globally (Kepenek and Sahin Eker, 2017). Furthermore, effective strategies for preventing NSSI rates in healthcare settings include implementing standardized training programs, ensuring adequate availability and proper utilization of personal protective equipment (PPE), establishing safety protocols, and promoting a culture of incident reporting and feedback mechanisms. The literature underscores the paramount importance of comprehensive safety measures for reducing injury rates, emphasizing risk factors such as inadequate training, fatigue, and insufficient personal protective equipment (PPE) (Abdalkareem Jasim et al., 2023; Abdo Almoliky et al, 2024). The prompt administration of post-exposure prophylaxis (PEP), the implementation of pathogen-specific protocols, and the undertaking of regular training initiatives are essential elements in preventing occupational exposure to bloodborne pathogens (Kutubudin et al., 2024; Manenzhe and Singh, 2024). The utilization of safety needles, such as fully passive safety needles, has demonstrated favorable outcomes in the prevention of NSSIs among healthcare professionals, exhibiting high success rates and positive feedback on safety features (Praise et al., 2023; Picakciefe et al., 2024). Quality improvement projects that concentrate on incident reporting, root cause analysis, and policy modifications have also been shown to be effective in reducing needle stick injuries among healthcare personnel (Kumah and Forkuo-Minka, 2023; Ebrahimi and Khosravi, 2024).

The Quality Standards in Health (QSH) initiative, spearheaded by the Ministry of Health, aims to enhance the quality of healthcare in Türkiye, standardize care and develop relevant policies. The Quality Standards in Health İndicator Management Guide has been established to ensure employee health and safety in accordance with the QSH, and NSSI situations in healthcare institutions are monitored under the title of "Reducing the risks of needle sticks and sharps injuries" (Ministry of Health, 2020). In accordance with the Quality Standards in Health (QSH), it is obligatory to monitor the incidence of sharps injuries rate (NSSIR) in healthcare institutions as an indicator, with the initiation of improvement activities when necessary. In this context, the determination of the frequency, type, and causes of NSSI in healthcare personnel will contribute to the reduction of the risk of exposure to these injuries (Ministry of Health, 2023). The objective of this study was to determine the causes of NSSI and the changes in the number of injuries over time, based on the notification forms completed by healthcare workers in a tertiary hospital between 2018 and 2023. The findings were used to provide valuable information and recommendations to managers on the prevention of these injuries.

2. Materials and methods

The descriptive, single-center, and retrospective study encompasses the data on NSSI reported by healthcare professionals in a training and research hospital over a six-year period between 1 January 2018 and 31 December 2023. The population of the study consisted of all notification forms pertaining to NSSI in the relevant period. İn the registration forms, healthcare workers were categorized according to their occupational groups, including physicians, nurses, midwives, health officers, cleaning personnel, interns and others. The year of injury, the unit in which the healthcare workers were injured and the type of injury instrument were evaluated.

In order to determine the NSSIR, the "Health Quality Standards Indicator Management Guide" was employed as a reference point, with the injury rate calculated in accordance with the following methodology: (Ministry of Health, 2023).

$$\textbf{NSSIR:} \frac{\textit{Number of NSSI Notification}}{\textit{Patient Load Coefficient}} \times 100$$

$$\textbf{Patient Load Coefficient:} \frac{\textit{In the relevant period total number of patient applications}}{\textit{In the relevant period active HCW number}}$$

The number of patient applications is the sum of the number of inpatients, emergency, and outpatient applications in the relevant period. Each application of patients with more than one application is included in the calculation.

The number of personnel actively working in the relevant period includes the personnel assigned through service procurement, interns, and intern students. Personnel working on administrative duty and personnel on temporary duty and unpaid leave are excluded.

In accordance with the ethical standards of the study, institutional permission was obtained from the Hospital Administration on 6 May 2024 (permission number 242972901). Additionally, ethical committee approval was obtained from the Antalya Training and Research Hospital

Clinical Research Ethics Committee on 9 May 2024 (decision number 128). Furthermore, the study was conducted in accordance with the Declaration of Helsinki and no falsification was made of the data. The data were obtained from the Quality and Statistics units of the relevant hospital and analyzed and interpreted through tables created with the SPSS 26.0 statistical program and Microsoft® Excel program.

3. Results

In this study, the NSSIR was determined according to the number of NSSIs reported between 2018 and 2023 in a tertiary hospital. Table 1 presents information on the number and rates of NSSIs according to years and months. According to the table, a total of 74 NSSIs were reported in 2018, resulting in an NSSIR of 11.1%. The lowest number of notifications was recorded in October (3), while the highest number was observed in December (12). The months with the highest NSSIR were December (19.5%), November (18.9%), and April (12.7%) in 2018; 105 NSSIs were reported, resulting in an NSSIR of 15.8% in 2019. The fewest notifications were recorded in April, July, and August, with five notifications each, while December had the highest count, with 17 notifications. The months with the highest NSSI rates were December (28.9%), February (25.6%), and November (23.8%) for 2019, There were 69 reported NSSIs, with an NSSIR of 20.1% in 2020; May had the fewest notifications, with only one, and August had the most, with 11 notifications. April (46.1%), August (42.6%), and October (24.3%) had the highest NSSI rates same year.

A total of 55 NSSIs were reported, resulting in an NSSIR of 11.7% in 2021. The months with the lowest number of notifications were September, October, and November, each with two notifications, while August recorded the highest, with seven notifications. The months with the highest NSSI rates in 2021 were January (25.4%), February (23.5%), and May (18.0%).

A total of 82 NSSIs were reported, yielding an NSSIR of 12.2% in 2022. The lowest notification counts were observed in January, February, and May, with four notifications each, while April recorded the highest, with 12 notifications. The months with the highest WACR were April (20.0%), September (15.0%), and October (14.4%).

A total of 118 NSSIs were reported, resulting in an LWRR of 18.2% in 2023. June had the fewest notifications, with two, and August had the highest, with 18 notifications. The months with the highest NSSIR in 2023 were August (35.3%), July (31.5%), and April (26.0%). Fig. 1 presents the monthly NSSIR figures for each year. A review of all years revealed that the highest NSSIR was observed in 2020 (20.1%), with the highest levels occurring in April-August. The lowest NSSIR was determined to be in 2018 (11.1%) and in the months of January to October.

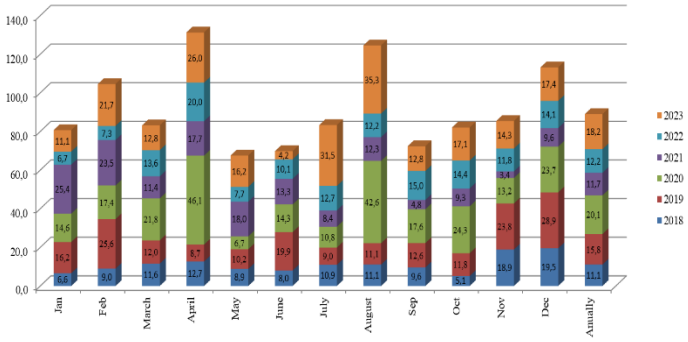
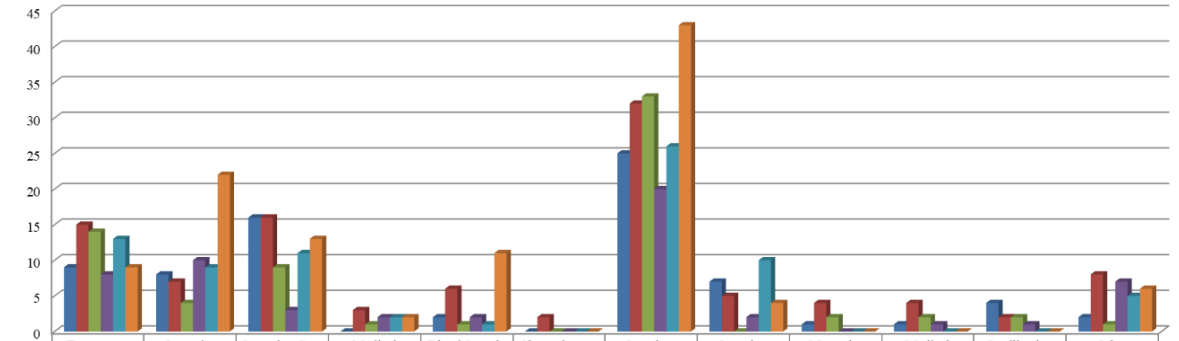


Fig. 1. NSSI rates.

Fig. 2 illustrates the distribution of NSSI notifications by units. Over the six-year period between 2018 and 2023, the highest number of NSSI notifications were made from inpatient units, which are clinics/services. These units are followed by intensive care units, emergency departments, and operating theatres. The number of notifications by units fluctuates according to the number of years, with the operating theatre ranking second in NSSI notifications in 2023.

Fig. 3 A-B illustrates the distributions based on title and the instrument causing injury by years. In 2018, a total of 73 HCWs reported that they were exposed to NWİ. The categories of nurse-midwife-health officer (34 people) and supporting staff (22 people) were the most prevalent in terms of exposure to NSSI injuries. In 2019, a total of 104 HCWs reported being exposed to NSSI. The data indicates that the greatest number of incidents involved nurse-midwife-health officers (41 people) and supporting staff (25 people). It was observed that the number of trainee injuries increased in this year compared to previous years. In 2020, a total of 69 healthcare workers reported being exposed to NSSI. The greatest number of HCWs exposed to NSSI were nurse-midwifes (27 people) and supporting staff (25 people). In 2021, a total of 55 HCWs reported being exposed to



	Emergency Department	Operating Rooms	Intensive Care Units	Medical Laboratories	Blood Sample	Chemotherapy Unit	Inpatient Clinics	Outpaient Clinics	Maternity Service	Medical Imaging Service	Sterilization Unit	Other
2018	9	8	16	0	2	0	25	7	1	1	4	2
2019	15	7	16	3	6	2	32	5	4	4	2	8
2020	14	4	9	1	1	0	33	0	2	2	2	1
2021	8	10	3	2	2	0	20	2	0	1	1	7
2022	13	9	11	2	1	0	26	10	0	0	0	5
2023	9	22	13	2	11	0	43	4	0	0	0	6

Fig. 2. NSSI distribution by units.

Table 1
NSSIR date by years.

Year	Data	Jan	Feb	March	April	May	June	July	August	Sep	Oct	Nov	Dec	Annually
2018	NSSI number	4	5	7	7	5	4	6	5	5	3	11	12	74
	Total Number of Patient Admissions to the Hospital	242.871	224.356	247.645	230.355	234.129	204.541	225.339	192.741	219.794	249.703	248.432	254.142	2.774.048
	Number of Active HCW	4019	4021	4097	4166	4148	4081	4106	4278	4227	4284	4265	4130	4152
	Patient Load Coefficient	60	56	60	55	56	50	55	45	52	58	58	62	668
	NSSIR	**6.6**	**9.0**	**11.6**	**12.7**	**8.9**	**8.0**	**10.9**	**11.1**	**9.6**	**5.1**	**18.9**	**19.5**	**11.1**
2019	NSSI number	10	14	7	5	6	9	5	5	7	7	13	17	105
	Total Number of Patient Admissions to the Hospital	263.904	236.691	252.874	248.227	254.172	199.920	247.176	200.652	243.299	258.035	241.950	259.839	2.906.739
	Number of Active HCW	4264	4333	4333	4335	4314	4415	4431	4435	4369	4339	4437	4423	4369
	Patient Load Coefficient	62	55	58	57	59	45	56	45	56	59	55	59	665
	NSSIR	**16.2**	**25.6**	**12.0**	**8.7**	**10.2**	**19.9**	**9.0**	**11.1**	**12.6**	**11.8**	**23.8**	**28.9**	**15.8**
2020	NSSI number	9	9	8	6	1	4	3	11	4	5	3	6	69
	Total Number of Patient Admissions to the Hospital	273.335	231.210	170.546	57.547	69.523	129.594	129.594	138.639	127.154	127.091	118.017	125.035	1.697.285
	Number of Active HCW	4448	4481	4640	4423	4639	4648	4658	5372	5580	6180	5193	4939	4933
	Patient Load Coefficient	61	52	37	13	15	28	28	26	23	21	23	25	344
	NSSIR	**14.6**	**17.4**	**21.8**	**46.1**	**6.7**	**14.3**	**10.8**	**42.6**	**17.6**	**24.3**	**13.2**	**23.7**	**20.1**
2021	NSSI number	**6**	6	4	6	5	5	3	7	2	4	2	5	55
	Total Number of Patient Admissions to the Hospital	105.535	114.583	157.960	152.403	124.560	169.313	161.089	257.315	189.967	195.955	282.784	239.671	2.151.135
	Number of Active HCW	4470	4488	4508	4508	4473	4491	4523	4520	4568	4532	4790	4600	4539
	Patient Load Coefficient	24	26	35	34	28	38	36	57	42	43	59	52	472
	NSSIR	**25.4**	**23.5**	**11.4**	**17.7**	**18.0**	**13.3**	**8.4**	**12.3**	**4.8**	**9.3**	**3.4**	**9.6**	**11.7**
2022	NSSI number	4	4	8	12	4	6	6	6	9	8	7	8	82
	Total Number of Patient Admissions to the Hospital	236.369	215.413	232.610	236.025	205.421	244.491	189.253	228.837	244.603	255.954	273.542	299.998	2.862.516
	Number of Active HCW	3988	3954	3944	3938	3940	4104	3998	4661	4072	4623	4623	5278	4260
	Patient Load Coefficient	59	54	59	60	52	60	47	49	60	55	59	57	672
	NSSIR	**6.7**	**7.3**	**13.6**	**20.0**	**7.7**	**10.1**	**12.7**	**12.2**	**15.0**	**14.4**	**11.8**	**14.1**	**12.2**
2023	NSSI number	6	11	8	13	9	2	16	18	7	10	8	10	118
	Total Number of Patient Admissions to the Hospital	293.043	248.584	306.301	245.561	282.288	237.335	262.463	263.655	277.416	295.136	283.403	292.685	3.287.870
	Number of Active HCW	5408	4898	4907	4904	5087	5027	5168	5165	5085	5047	5056	5080	5069
	Patient Load Coefficient	54	51	62	50	55	47	51	51	55	58	56	58	649
	NSSIR	**11.1**	**21.7**	**12.8**	**26.0**	**16.2**	**4.2**	**31.5**	**35.3**	**12.8**	**17.1**	**14.3**	**17.4**	**18.2**

NSSI. The majority of these were nurse-midwife-health officers (27 people) and supporting staff (16 people). In 2022, a total of 81 HCWs reported being exposed to NSSI. Of these, 38 were nurse-midwife-health officers and 20 were supporting staff. In 2023, a total of 113 HCWs reported NSSI exposure. The highest number of NSSI exposures by title was nurses-midwives-health

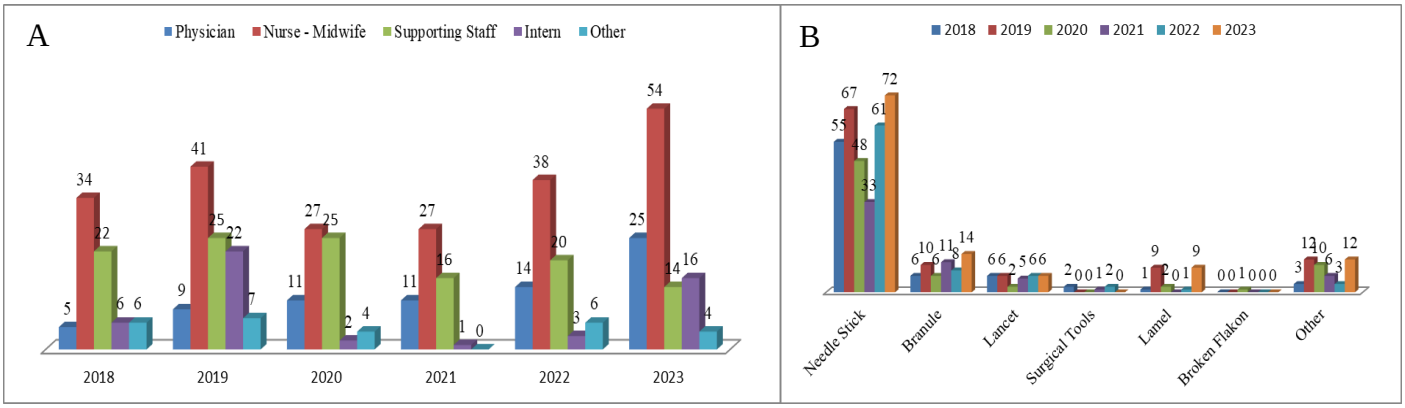


Fig. 3. (A-B) Classification of NSSI by title and tool causing injury.

officers (54 persons) and doctors (25 persons) (Fig. 3A). The instruments that were reported by HCWs and caused injury were: needle tip, branula, scalpel, surgical instrument, slide/lamel, and broken glass materials. It was determined that HCWs were mostly exposed to NSSI with a needle tip in all years. The years 2018 (67 notifications) and 2023 (72 notifications) were the years in which NSSI exposure due to needle tip was reported (Fig. 3B).

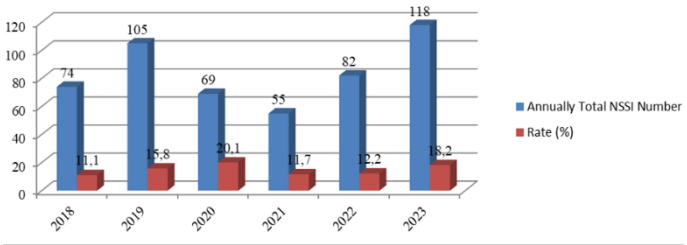


Fig. 4. The number and rates of NSSI by years.

Fig. 4 presents the number and rate of NSSIs by year. Upon analysis, it was observed that while the number of injuries in 2020 was 69 and the injury rate was 20.1%, the rate was determined to be 18.2% in 2023 despite 118 notifications. It can be concluded that the injury rate is directly proportional to the number of patients and active staff.

4. Discussion

The objective of this study was to analyze the notification forms completed by HCWs in a tertiary hospital between 2018 and 2023 due to NSSI. The aim was to determine the characteristics of the employees, the causes of the injuries, and the changes in the number of injuries over the years. Additionally, the study aimed to provide suggestions to the managers to prevent these injuries. The rationale for selecting a tertiary hospital as the subject of this study is that such institutions represent one of the highest levels of care, with a high patient density and a focus on complex and severe cases. This may result in a greater number and variety of medical procedures and interventional procedures, thereby increasing the risk of injuries. The assessment of such injuries will provide valuable information to policymakers and managers in terms of implementing precautions. Indeed, a study found that there were more notifications in a tertiary education and research hospital among the health facilities in Bolu province compared to other health facilities (Esen et al., 2024) which confirms this situation.

Similar studies conducted in healthcare institutions have demonstrated that occupational accidents involving NSSI occur with considerable frequency, particularly among nurses. These studies have revealed that these accidents are commonly precipitated by several factors, including a lack of attention, suboptimal working conditions, negligence, fatigue, and a tendency to work at a fast pace. The most common tools causing injuries in these accidents are needles, scalpels, medical devices and medical instruments. (Barnes et al., 2020; Reda et al., 2021; Abalkhail et al., 2022; Hosseinipalangi et al., 2022). HCWs, particularly support staff with less than five years' experience, are the most affected occupational group and are frequently exposed to injuries from improper handling and collection of medical waste and materials. İn addition, lack of knowledge and the normalization of accidents among HCWs contribute to both the occurrence and underreporting of these incidents. This situation highlights the need to develop prevention strategies and structured protocols following non-sharps safety incidents (NSSIs) to reduce such injuries. (Joukar et al., 2018; Papadopoli et al., 2019; Song et al., 2023).

The study found that the highest rate of NSSIR in 2020 was 20.1%, with the peak occurring between April and August. It is well known that the COVID-19 pandemic in 2020 put unprecedented pressure on healthcare systems worldwide. HCWs, especially those at the frontline, were required to work long hours. Due to the increased workload, stress, and difficulties associated with the use of personal protective equipment, workers may have been more susceptible to NSSI. Indeed, Esen et al. (2024) examined NSSI reports over a five-year period and found that a total of 614 incidents were reported during this period. The study identified 2022, the post-COVID-19 pandemic period, as the year with the highest reporting rate, with 28.99% (n:178) of incidents reported. İn the study by Karakoc et al. (2019), which examined NSSI reports from 2013 to 2016, the annual NSSI rates were found to be 4.3%, 9.6%, 4.4%, and 8.6%, respectively. The study, which spanned the six-year period from 2018 to 2023, revealed that inpatient units, such as clinics/wards, intensive care units, emergency departments, and operating rooms, were the units with the highest number of NSSI reports. In the study by Harman-Gunerken (2023) examining NSSI exposure, the highest number of injuries occurred in operating rooms (22.3%), emergency departments (18.8%), intensive care units (18.3%), and surgical wards (12.6%). Among the cases, 117 (62.5%) were due to needlestick injuries, and 45 (24%) were due to injuries from sharp instruments.

The study identified that the instruments causing injuries included needle tips, cannulas, scalpels, surgical instruments, slides/coverslips, and broken glass materials, with needle tips being the most common cause of NSSI across all years. The

years 2018 (67 reports) and 2023 (72 reports) had the highest number of NSSI incidents due to needle tips. The occupational groups most frequently exposed to injuries were nurses, midwives, health officers, and cleaning staff. In the study evaluating NSSI from 2013 to 2018, there were 393 reports, with the highest incidence among nurses (39.7%), followed by cleaning staff (36.3%), doctors (10.4%), other workers (7.4%), and laboratory technicians (5.9%) (Saadeh et al., 2020).

In a study spanning eight years, Ceylan and Celik (2022) observed NSSI exposure in a total of 132 individuals. Of these, nurses constituted the most frequently affected occupational group (69.7%). Injuries were more commonly observed in the emergency department (21.2%) and blood collection units (17.5%). The most frequent cause of injuries was needle stick exposure (76.5%). Kurttekin and Tacgin (2019) observed that NSSI was the most prevalent type of occupational accident among HCWs, with 80% of percutaneous injuries resulting from needlestick incidents.

Among the occupational groups experiencing exposure, nurses are the most frequently subjected to NSSIs. This is due to their close contact with patients during medical treatment and care, as well as their frequent use of syringes, cannulas, and ampoules. Consequently, nurses experience higher rates of NSSI compared to other healthcare workers. Furthermore, the global shortage of nurses, coupled with long and intensive working hours, contributes to the prevalence of NSSI (Ozturk Mentese and Karaca, 2021). Bozdemir and Bahar (2023) highlight that the incidence of injuries among nurses increases with longer shifts and advancing age.

Research indicates that 50% or more of NSSIs are underreported among HCWs. NSSIs not only carry the risk of transmitting infectious diseases but also impose economic burdens due to the testing and prophylaxis required after risky exposures. This can lead to anxiety and lack of motivation among HCWs, thereby negatively affecting their performance (Ozturk Mentese and Karaca, 2021). To minimize the often-irreparable injuries that can result from NSSIs, it is essential to provide periodic training to relevant healthcare personnel and to inform them about the procedures for reporting incidents.

Studies have shown that the number, rate, occupational groups, and instruments causing NSSIs vary across countries, regions, and the status of healthcare institutions (Martins et al., 2012; Cho et al., 2013; Bekele et al., 2015; Saadeh et al., 2020; Mohamud, 2023). In developed countries, the implementation of improved reporting systems and safety protocols has been associated with elevated NSSI reporting rates. Conversely, in developing countries, lower reporting rates may be observed due to a lack of awareness, insufficient training, inadequate safety equipment, and deficiencies in reporting systems (Jagger et al., 2008).

Regional disparities may emerge because of the configuration of healthcare systems, cultural influences, and the educational attainments of healthcare professionals. For example, the rates of non-suicidal self-injury (NSSI) in large hospitals situated in urban areas are likely to be higher than in other hospitals (Lee and Hassim, 2005). Furthermore, differences are observed among various occupational groups. It has been demonstrated that healthcare professionals, including nurses, doctors, surgeons, and laboratory technicians, exhibit varying degrees of risk for NSSI (Barnes et al., 2020; Cruz-Ausejo et al., 2022).

This study has certain limitations that need to be mentioned. The study was conducted at a single center, a tertiary research and training hospital with 1,270 beds. Consequently, the results are specific to this hospital and cannot be extrapolated to all hospitals. It is possible that the results of the study may not be generalizable to other healthcare institutions due to differences in the rates and causes of NSSI across different regions and geographical areas.

5. Conclusion

The study revealed significant variations in the rates of NSSI and the number of NSSIs reported from 2018 to 2023 at a tertiary hospital. The highest NSSIR was observed in 2020 (20.1%), coinciding with the COVID-19 pandemic, and the lowest in 2018 (11.1%). The occupational group most frequently affected was that of nurses, midwives, and health officers, due to their close patient contact and frequent use of needles. The most common instruments causing injuries were needle tips, followed by cannulas, scalpels, surgical instruments, slides, and broken glass. The study highlights the need for improved reporting and preventive measures to mitigate the risk of NSSIs among HCWs.

Preventive measures, such as occupational health and safety training on sharp object injuries, post-injury follow-up, root cause analyses, ensuring the appropriateness of personal protective equipment usage, prevention of injuries due to inadequate equipment usage, consideration of occupational conditions (stress, number of shifts, intra-team communication, working conditions, shift durations, etc.), and addressing material shortages (continuing the use of excessively filled sharp object containers) are crucial in mitigating such incidents. These measures are also pivotal in enhancing the quality of healthcare services. It is recommended that managers and units responsible for incident reporting, in particular, review safety policies and training among high-risk groups. Educational programs should be tailored to different occupational levels and learning paces, incorporating methods and techniques used in adult education such as animations and mobile applications. Regular repetition of such training programs and ensuring easy access for healthcare workers to participate are essential. It is recommended that policymakers, managers, and relevant stakeholders examine the issue in-depth and implement policies and measures accordingly. To ascertain whether the findings of this study reflect national trends, multicenter studies at the national level should be conducted.

Conflict of interest: The author declares that she has no conflict of interests.

Ethical Approval: The study was approved by the Ethical Committee of the Antalya Training and Research Hospital, (Approval number: 2024/128).

References

Abalkhail, A., Kabir, R., Elmosaad, Y. M., Alwashmi, A. S. S., Alhumaydhi, F. A., Alslamah, T., Almoammar, K. A., Alsalamah, Y. A., & Mahmud, I. (2022). Needle-Stick and sharp injuries among hospital healthcare workers in saudi arabia: a cross-sectional survey. *International Journal of Environmental Research and Public Health, 19*(10), 6342.

Abdalkareem Jasim, S., Thaeer Hammid, A., Turgunpulatovich Daminov, B., Kadhem Abid, M., Lateef Al-Awsi, G. R., Afra, A., ... & Mohammadi, M. J. (2023). Investigation ways of causes needle sticks injuries, risk factors affecting on health and ways to preventive. *Reviews on Environmental Health, 38*(4), 629-636.

Abdo Almoliky, M., Elzilal, H. A., Alzahrani, E., Abo-Dief, H. M., Saleh, K. A., Alkubati, S. A., ... & Sultan, M. A. (2024). Prevalence and associated factors of needle stick and sharp injuries among nurses: A cross-sectional study. *SAGE Open Medicine, 12*.

Alfulayw, K. H., Al-Otaibi, S. T., & Alqahtani, H. A. (2021). Factors associated with needlestick injuries among healthcare workers: implications for prevention. *BMC Health Services Research, 21*(1), 1074.

Barnes, L. A., Eng, A., Corbin, M., Denison, H. J., Mannetje, A., Haslett, S., McLean, D., Jackson, R., & Douwes, J. (2020). The prevalence of cardiovascular risk factors in different occupational groups in New Zealand. *Annals of Work Exposures and Health, 64*(6), 645-658.

Bekele, T., Gebremariam, A., Kaso, M., & Ahmed, K. (2015). Factors associated with occupational needle stick and sharps injuries among hospital healthcare workers in Bale Zone, *Southeast Ethiopia. PloS one, 10*(10), e0140382.

Bozdemir, M, & Bahar, Z. (2023). The effect of some features of nurses on sharp injuries. *Aydin Health Journal, 9*(2), 80-101.

Ceylan, M. R, Celik, M. (2022). Sharp and cutting tool injuries in healthcare workers: a second stage hospital experience. *Abant Medical Journal, 11*(1), 37-44.

Cho, E., Lee, H., Choi, M., Park, S. H., Yoo, I. Y., & Aiken, L. H. (2013). Factors associated with needlestick and sharp injuries among hospital nurses: a cross-sectional questionnaire survey. *International Journal of Nursing Studies, 50*(8), 1025-1032.

Cooke, C. E., & Stephens, J. M. (2017). Clinical, economic, and humanistic burden of needlestick injuries in healthcare workers. *Medical Devices: Evidence and Research, Volume 10*, 225-235.

Cruz-Ausejo, L., Vera-Ponce, V. J., Torres-Malca, J. R., & Roque-Quesada, J. C. (2022). Occupational accidents in healthcare workers: a bibliometric analysis in Scopus database 2010-2019. *Revista Brasileira de Medicina Do Trabalho, 20*(04), 642-649.

Dafaalla, M. D. (2016). Knowledge, attitude and practice towards needle stick injury among health care workers in a tertiary Sudanese hospital. *Texila International Journal of Clinical Research, 3*(1), 88-96.

Duzgol, M., Kara Aksay, A., Durgun, E., Yaman, Y., Demiray, N., Gulfidan, G., Ayhan, Y., Bayram, S. N., & Devrim, I. (2020). Risk groups for needlestick injury among healthcare workers in Children's Hospital: A cross-sectional study. *Journal of Pediatric Infection, 54*(4), 212-217.

Ebrahimi, H., & Khosravi, A. (2023). Needlestick injuries among nurses. *Journal of Research in Health Sciences, 7*(2), 56-62.

Esen, H. , İlce, A. ,Yigit, U. (2024). The effect of covid 19 pandemic on sharps injuries among health workers. *Health Care and Rehabilitation Journal, 3*(1), 1-11.

Gauba, A. (2023). Needle stick injuries in health workers. *International Journal of Advanced Research, 11*(03), 548-550.

Harman-Gunerken, R. (2023). Evaluation of sharps injuries of health professionals working in a tertiary care hospital during five years. *Klimik Journal/Klimik Dergisi, 36*(1), 27-31.

Hosseinipalangi, Z., Golmohammadi, Z., Ghashghaee, A., Ahmadi, N., Hosseinifard, H., Mejareh, Z. N., Dehnad, A., Aghalou, S., Jafarjalal, E., Aryankhesal, A., Rafiei, S., Khajehvand, A., Nasab, M. A., & Kan, F. P. (2022). Global, regional and national incidence and causes of needlestick injuries: a systematic review and meta-analysis. *Eastern Mediterranean Health Journal, 28*(3), 233-241.

Jagger, J., Perry, J., Gomaa, A., & Phillips, E. K. (2008). The impact of U.S. policies to protect healthcare workers from bloodborne pathogens: The critical role of safety-engineered devices. *Journal of Infection and Public Health, 1*(2), 62-71.

Jahangiri, M., Rostamabadi, A., Hoboubi, N., Tadayon, N., & Soleimani, A. (2016). Needle stick injuries and their related safety measures among nurses in a university hospital, Shiraz, Iran. *Safety and Health at Work, 7*(1), 72-77.

Joukar, F., Mansour-Ghanaei, F., Naghipour, M., & Asgharnezhad, M. (2018). Needlestick injuries among healthcare workers: Why they do not report their incidence? *Iranian Journal of Nursing and Midwifery Research, 23*(5), 382.

Karakoc, Z. C., Kocak, Y., & Simsek, B. (2019). Sharps injuries: A single-center experience. *Klimik Journal, 31*(3), 181-184.

Kepenek, E., & Sahin Eker, H. B. (2017). An assessment of sharp injuries to workers in a state hospital. *Klimik Journal, 30*(2), 78-82.

Korkmaz, N., Senturk, G. C., Tekin, A., Gurbuz, Y., Sevinc, G., Tutuncu, E. E., & Sencan, I. (2022). Rates of underreported needlestick and sharps injuries among healthcare workers in Turkey: in the light of Infection Control Committee data. *International Journal for Quality in Health Care, 34*(2).

Kotanoglu, M. S. (2020). Analysis of work related sharp object injuries affected by the health staff working in the operating room and intensive care units. *Ankara Training and Research Hospital Med. Journal, 53*(Additional Number 1), 27-32.

Kumah, A., Forkuo-Minka, A. O. (2023). Advancing staff safety: assessment of quality improvement interventions in reducing needlestick injuries among staff at Nyaho Medical Centre. *Global Journal on Quality and Safety in Healthcare, 6*(2), 55-61.

Kurttekin, A, Tacgin, E. (2019). An evaluation on occupational accidents in health services and cutter-drilling instrument injury investigations. *Performance and Quality in Health Journal, 17*(2), 135-182.

Kutubudin, A. F. M., Shafei, M. N., İbrahim, M. I., & Yaacob, N. M. (2024). Development and validation of the needlestick injury prevention (N-SİP) module. *Cureus, 16*(7), e64445.

Lee, L. K., & Hassim, I. N. (2005). Implication of the prevalence of needlestick injuries in a general hospital in Malaysia and its risk in clinical practice. *Environmental Health and Preventive Medicine, 10*(1), 33-41.

Li, X., He, Q., & Zhao, H. (2024). Situation and associated factors of needle stick and sharps injuries among health-care workers in a tertiary hospital: a cross-sectional survey. *BMC Health Services Research 24*(1), 1002.

Martins, A., Coelho, A. C., Vieira, M., Matos, M., & Pinto, M. L. (2012). Age and years in practice as factors associated with needlestick and sharps injuries among health care workers in a Portuguese hospital. *Accident Analysis & Prevention, 47*, 11-15.

Manenzhe, G. I., & Singh, T. (2024). Prevalence of needle stick and sharps injuries and associated factors among nursing students in Gauteng province, South Africa. *Occupational Health Southern Africa, 30*(2), 56-62.

Ministry of Health, (2020). Quality Standards in Health (QSH)-Hospital Version 6.

Ministry of Health. (2023). Health Quality Standards Indicator Management Guide. In General Directorate of Health Services Department of Quality, Accreditation and Employee Rights in Health.

Mohamud, R. Y. H., Mohamed, N. A., Doğan, A., Hilowle, F. M., Isse, S. A., Hassan, M. Y., & Hilowle, I. A. (2023). Needlestick and sharps injuries among healthcare workers at a tertiary care hospital: A retrospective single-center study. *Risk Management and Healthcare Policy*, 2281-2289.

Ozturk Mentese, H., & Karaca, A. (2021). Knowledge levels of nursing and midwifery students on needlestick and sharps injuries. *Klimik Journal, 34*(3), 156-163.

Papadopoli, R., Bianco, A., Pepe, D., Pileggi, C., & Pavia, M. (2019). Sharps and needle-stick injuries among medical residents and healthcare professional students: pattern and reporting in Italy—a cross-sectional analytical study. *Occupational and Environmental Medicine, 76*(10), 739-745.

Picakciefe, I. M., Kicali, R. U., Vatandas, M. O., & Ata, S. (2024). The effect of sociodemographic features, working conditions and excessive daytime sleepiness to needlestick and sharps injuries in health workers. *Nobel Medicus Journal, 20*(1).

Praisie, R., Anandadurai, D., Nelson, S. B., Venkateshvaran, S., & Thulasiram, M. (2023). Profile of splash, sharp and needle-stick injuries among healthcare workers in a Tertiary Care Hospital in Southern India. *Cureus, 15*(7), e42671.

Reda, S., Gebrehiwot, M., Lingerew, M., Keleb, A., Mekonnen, T. Chane, Wagaye, B., Atamo, A., Daba, C., Feleke, A., & Adane, M. (2021). Occupational blood exposure beyond needle stick injuries: hospital-based cross-sectional study among healthcare workers in governmental hospitals of Northern Ethiopia. *BMC Health Services Research, 21*(1), 1136.

Saadeh, R., Khairallah, K., Abozeid, H., Al Rashdan, L., Alfaqih, M., & Alkhatatbeh, O. (2020). needle stick and sharp injuries among

healthcare workers: a retrospective six-year study. *Sultan Qaboos University Medical Journal [SQUMJ], 20*(1), 54.

Sari, H., Dayan, S., Balkan, H., Cicek, Y., & Ozel, M. (2023). Assessment of sharps penetrating injury, mucosal exposure and compliance with standard precautions of health workers at a University Hospital in Turkey. *Saudi Medical Journal, 44*(6), 588-593.

Song, J., Jeon, Y. Bin, Jang, J. H., Cho, J. S., Choi, J. Y., & Choi, W. S. (2023). Severity of grinder injuries and related factors compared with other high-rotation cutting tool injuries: a multicenter retrospective study from 2011 to 2018. *Journal of Trauma and İnjury, 36*(1), 32-38.

Thakur, H., & Rao, R. (2024). Emphasis of infection prevention and control: a review. *J Popul Therap Clin Pharmacol, 31*, 2238-49.

WHO, (2024). World Health Report 2024: Reducing Risks, Promoting Healthy Life, https://eldis.org/, Last Accessed on September, 2024.

Yunihastuti, E., Ratih, D. M., Aisyah, M. R., Hidayah, A. J., Widhani, A., Sulaiman, A. S., Karjadi, T. H., & Soejono, C. H. (2020). Needlestick and sharps injuries in an Indonesian tertiary teaching hospital from 2014 to 2017: a cohort study. *BMJ Open*, *10*(12), e041494.

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Research article

Characterization of some local and commercial bread wheat (*Triticum aestivum* L.) genotypes with allele-specific DNA markers

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Abstract

Landraces play a significant role as genetic reservoirs in wheat breeding studies. Advances in functional marker technology have facilitated early and more precise selection processes. This study involved the characterization of a total of 96 bread wheat genotypes, comprising 76 landraces and 20 registered cultivars, utilizing allele-specific DNA markers targeting various genes including those for gluten strength, yellow rust resistance, stem rust resistance, dwarfness, rye translocation, hardiness. Molecular analysis revealed the presence of 148 alleles, with an average of 21.14 alleles per marker, and an average polymorphic information content (PIC) value of 0.5625. Specific genes such as the rye translocation gene were identified in genotypes 161 and 884, while the grain hardiness gene was found in genotypes 672, 3088, 3384, 3414, and 3541. The stem rust resistance gene was detected in the cultivar Adana-99, the yellow rust resistance gene in genotypes 1635 and 2115, and the grain hardiness gene in 31 genotypes including the cultivar Masaccio. Based on the dendrogram analysis, genotype 3652 exhibited around 93% genetic similarity with the cultivar Masaccio, while genotypes 2190, 2715, and 2897 showed similarity to genotype 2946. Genotypes 2959 and 2960 and genotypes 3334 and 3359 shared approximately 91% genetic similarity.

Keywords: *Allele specific marker; bread wheat; genetic diversity; landraces*

1. Introduction

Grains constitute one of the primary sources of protein and calories globally, serving as crucial food staples. Among grain products, wheat stands out as a fundamental food source for many countries worldwide due to its ease of cultivation, transportation, nutritional value, storage convenience, and broad adaptability (Rao and Poonia, 2023; Sertse et al., 2023).

Wheat is categorized into three groups based on chromosome numbers: diploid (2n=2x=14), tetraploid (2n=4x=28), and hexaploid (2n=6x=42). Diploid wheat contains only one A, B, or D genome. *Triticum durum* (durum wheat), classified as tetraploid, possesses both A and B genomes. *Triticum aestivum* (bread wheat), a hexaploid species, harbors the A, B, and D genomes together (Kaya, 2018; Afshari-Behbahanizadeh et al., 2024).

As time progresses, our country's wheat cultivation areas have expanded and reached their maximum capacity. With no further room for expansion, the focus has shifted towards enhancing yield per unit area. However, a mere yield increase is perceived as inadequate under current wheat consumption conditions. In addition to high productivity per unit area, there is a growing demand for crops demonstrating resilience against biotic and abiotic stress factors, along with superior quality characteristics. Given our country's diverse soil and climatic conditions, developing varieties capable of adapting to various climate conditions is crucial while maintaining high-quality standards. This is because wheat production is significantly influenced by factors such as variety, agricultural practices, and environmental conditions (Aktas et al., 2017; Gungor and

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Dumlupinar, 2019; Filip et al., 2023).

Breeding programs are labor-intensive, costly, and time-consuming studies. The fundamental principles of breeding emphasize the importance of clear objectives, meticulous observation, suitable locations, and appropriate parent selection to develop superior varieties. To succeed in breeding programs, the presence of potential sources of variation and their effective utilization are essential. Over the past quarter-century, the advent of DNA technologies and molecular markers in breeding programs has led to the rapid advancement of marker-assisted selection (MAS). Particularly in cereal crops, studies on quality, yield, and resistance to pests and diseases have increased. These efforts have yielded successful results. Marker-assisted selection (MAS) has enabled breeding programs to be completed with less labor and shorter timeframes. Additionally, MAS offers the advantage of requiring smaller population sizes than traditional breeding methods (Gupta and Varshney, 2000; Babu et al., 2004; Song et al., 2023; Habib et al., 2024).

With the adoption of molecular marker technologies, breeding durations have been reduced in recent years. One commonly used marker technology is Simple Sequence Repeats (SSR) (Dede, 2007; Turkoglu et al., 2023). Short Tandem Repeats (STRs) refer to the consecutive repeat sequences randomly occurring in the genetic material of a locus. Markers consisting of 1-6 base pair repeats are also known as SSRs or microsatellites (Ozsensoy and Kurar, 2012). Allele sizes in microsatellite markers can be determined through electrophoresis. Microsatellites are co-dominant, meaning they can distinguish heterozygous characters from homozygous characters. Consequently, SSR markers are easily standardized, exhibit high polymorphism rates and repeatability, and are co-dominant. Due to these superior characteristics, microsatellites are frequently preferred in characterization studies (Gebologlu and Furan, 2017; Merga and Getu, 2023; Undal and Ahir, 2023).

This study characterized 96 bread wheat genotypes (76 local genotypes and 20 standard varieties) using seven allele-specific DNA markers.

2. Materials and methods

The research used 76 landrace bread wheat genotypes from different provinces of Turkey and ten winter and ten summer registered bread wheat cultivars from the National Seed Gene Bank within the Plant Genetic Resources of the Aegean Agricultural Research Institute. Information about genotypes is presented in Ocaktan (2021).

2.1. Leaf sampling procedure

The wheat varieties planted in pots were sampled during the two-leaf stage, and leaf samples were collected. These samples were then placed into 2 ml Eppendorf tubes and stored at -80°C until DNA isolation. DNA isolation was carried out using the cetyl trimethyl ammonium bromide (CTAB) method described by Oliver et al. (2010).

2.2. DNA isolation

The leaf samples from bread wheat varieties were stored at -80°C and then crushed in 2 ml Eppendorf tubes using a sterile rod immersed in liquid nitrogen. Following this, 1 ml of isolation solution consisting of 1 M Tris-HCl (pH 8.0), 0.5 M EDTA (pH 8.0), 5 M NaCl, 2% w/v cetyltrimethylammonium bromide (CTAB), 2% Polyvinylpyrrolidone 40, and 5% sarcosine was added to the ground leaf samples in the Eppendorf tubes. The tubes were then incubated in a water bath at 65°C for one hour, and were homogenized by inversion for every 20 minutes. After incubation, a mixture of 1 ml chloroform and isoamyl alcohol (24:1) was added to the samples and mixed by inversion in the dark for 30 minutes. Subsequently, the samples were centrifuged at 10,000 rpm for 20 minutes. The clear liquid supernatant was carefully pipetted into empty sterile tubes, and then 1 ml of isopropanol, pre-chilled at -20°C, was slowly added and mixed gently by inversion. After centrifugation at 10,000 rpm for 30 minutes, the supernatant was removed without disturbing the pellet. The remaining pellets in the tubes were washed by adding 2 ml of 70% ethanol and centrifuged at 13,000 rpm for 2 minutes. The ethanol was then carefully removed without disturbing the pellet. The dried pellets were dissolved by adding ten mM Tris-HCl (pH 8.0) and RNAse to eliminate RNA. Finally, the quantity and quality of the extracted DNA were measured using a Nanodrop device.

2.3. DNA primers

The study utilized DNA markers, including Bx7OE (Gluten Strength gene), Sun104 (Yellow Rust Yr51 gene), Sun209 (Stem Rust Sr49 gene), DF-MR2 (Dwarfness Rht-D1 gene), RYE-NOR (Rye Translocations gene), Pina-D1 (Grain hardiness Pina gene), and Sun1 (Waxy Wx-A1 gene), to determine quality-related traits, certain diseases, and degrees of relatedness in 96 bread wheat varieties. The DNA markers used in the study are provided in Table 1 below.

Table 1
DNA primers used in molecular characterization.

No	Primer Name	Primer Sequence (5′-3′)	Gene Region	Reference	Expected Band Length (bp)	Marker Type
1	Bx7OE_F Bx7OE_R	CCTCAGCATGCAAACATGCAGC CTGAAACCTTTGGCCAGTCATGTC	Gluten Strength	Butow et al., 2003	563	Co-dominant
2	Sun104_F Sun104_R	TGCTATGTGCGTGATGATGA TTACATGCTCCAGCGACTTG	Stripe Rust *Yr51*	Randhawa et al., 2014	225	Dominant
3	Sun209_F Sun209_R	AG CTATGAGCTTCGCTATTG GTGATTGGTTCGGATTACTTA	Stem Rust *Sr49*	Bansal et al., 2015	148	Co-dominant
4	DF-MR2_F DF-MR2_R	CGCGCAATTATTGGCCAGAGATAG CCCCATGGCCATCTCGAGCTGCTA	Dwarfness *Rht-D1*	Ellis et al., 2002	254	Dominant
5	RYE-NOR_F RYE-NOR_R	GCATGTAGCGACTAACTCATC CCCAGTTTTCCATGTCGC	Rye Translocation	Koebner, 1995	400, 600, 700, 800	Dominant
6	Pina-D1_F Pina-D1_R	CCCTGTAGAGACAAAGCTAA TCACCAGTAATAGCCAATAGT	Grain Hardiness Pina	Gautier et al., 1994	330	Dominant
7	Sun1_F Sun1_R	CGCTCCCTGAAGAGAGAAAGAA ATAGGCACAACCCCTAAC	Waxy *Wx-A1*	Shariflou and Sharp, 1999	Xsun-7A, 219, 233, 260, 271, 275, 285 and 289	Co-dominant

2.4. Polymerase chain reactions (PCR) and fragment analysis

The polymerase chain reaction (PCR) procedure involved preparing a 20 µl solution with the following components added to 96-well PCR plates: 1 µl of dNTP mixture (2.5 mM mixture of A+T+G+C), 2 µl of 10x buffer, 0.1 µl of $MgCl_2$, DNA primer pair (5 µl each of forward and reverse primers), 5 µl of genomic DNA (60 ng), 1.8 µl of ddH_2O, and 0.1 µl of DNA polymerase (5U/µl, Fermentas). PCR reactions were performed using an ABI-brand PCR machine. The reactions commenced with an initial denaturation step at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 2 minutes. Finally, the reactions were completed with a final extension step at 72°C for 10 minutes. The finished PCR products were stored at -20°C until further use. Following the PCR process, the obtained products were utilized for fragment analysis. The analysis was conducted using the QIAxcel Advanced System, a fragment analysis device from Qiagen, to obtain DNA bands corresponding to the varieties.

2.5. Evaluation of data

According to the fragment analysis results, scoring precision was accepted as ± 4 base pairs, and alleles were evaluated accordingly. The similarities of these varieties were calculated using the Dice index (Dice, 1945) in the NTSYSpc 2.21q (Rohlf, 2005) program. DNA bands of the obtained varieties were coded as "0" or "1" and a binary data matrix was created. With the help of the created matrix, a dendrogram showing the similarities of the varieties was obtained using UPGMA (Unweighted Pair Group Method Arithmetic Average).

2.6. Calculation of polymorphism information contents

Polymorphism information contents for each DNA marker to be used in molecular analyses were calculated with the formula given below according to Weir (1996);

$$PIC=1-\sum Pi^2$$

Pi; is the frequency of the i^{th} allele in 96 bread wheat genotypes studied in the research.

3. Results and discussion

3.1. Polymorphism information content (PIC) and allele numbers of the markers used

This study used seven functional DNA markers to screen 76 landraces and 20 standard bread wheat cultivars for polymorphism. As a result of the screenings, a total of 148 alleles were detected, all of which exhibited polymorphic characteristics. The average number of alleles was determined to be 21.14. The primer "RYE-NOR" produced the highest number of alleles, generating 43 polymorphic alleles, while the primers DF-MR2 and SUN104 produced the lowest number of alleles, generating six polymorphic alleles each.

After the study, the average Polymorphic Information Content (PIC) value was determined to be 0.5625. The highest PIC value calculated was 0.9651, while the lowest PIC value was found to be 0.1172. Table 2 presents the calculation of PIC values for 96 bread wheat genotypes, the information content of DNA primers used in DNA screening, and the number of alleles.

Table 2
DNA primers, allele numbers and polymorphism information content (PIC) values used in screening genotypes.

No	Primer Name	Allele Numbers	Expected Band Length (bp)	PIC Value
1	$Bx7^{OE}$	35*	563	0.9651
2	Sun104	6	225	0.1172
3	Sun209	16	148	0.9206
4	DF-MR2	6*	254	0.1172
5	RYE-NOR	43	400, 600, 700, 800	0.4946
6	Pina-D1	31	330	0.4373
7	Sun1	11	Xsun-7A, 219, 233, 260, 271, 275, 285 and 289	0.8855
8	**Mean**	**21.14**		**0.5625**

***** PIC value has been calculated using non-specific alleles.

Bx7OE primer is a marker used to detect genes related to gluten strength. Butow et al. (2003) stated that the Bx7OE marker corresponds to a co-dominant marker for the encoded gene region of 750 bp, with alleles of 563 bp obtained for lines lacking the Glu-B1al (520 bp) gene, with a difference of 43 bases. In the study, scanning 96 bread wheat genotypes (76 local and 20 standard) using the Bx7OE primer did not yield the desired gene region. Previous studies by Kocyigit et al. (2021) and Uysal and Dumlupinar (2022) also failed to obtain the desired gene region using the Bx7OE primer. The Sun104 primer is a marker to detect genes resistant to yellow rust (Yr51) disease.

Randhawa et al. (2014) utilized the Sun104 primer for detecting yellow rust (Yr51) disease, obtaining an allele of 225 bp and determining that this band length was associated with the gene for resistance to yellow rust disease. Our study detected the yellow rust disease resistance gene in the varieties 1635 and 2115 (Fig.1; Table 3). In previous studies regarding the Sun104 primer, Kocyigit et al. (2021) reported obtaining the desired gene region in two hybrid combinations. Our study appears to be consistent with the findings of the previous research.

The Sun209 primer is a marker used to detect the black rust trait. Bansal et al. (2015) utilized the Sun209 marker to detect the Sr49 gene associated with black rust, determining the desired band length to be 148 bp. In our study, the presence of the gene for resistance to black rust disease was detected in the genotype Adana-99 (Table 3). In studies on the Sun209 primer, Uysal and Dumlupinar (2022) reported obtaining the desired gene region in 33 genotypes, while Kocyigit et al. (2021) reported success in two genotypes.

The DF-MR2 primer is a marker used to detect dwarfness (short) traits. Ellis et al. (2002) reported an allele of 254 bp length in the gene region encoded by the DF-MR2 marker and noted its association with dwarfness (Short). However, the desired gene region was not obtained in our study when scanning 96 wheat genotypes (76 local and 20 standard cultivars) using the DF-MR2 primer. In studies related to the DF-MR2 primer. Kocyigit et al. (2021) also reported not obtaining the desired gene region, indicating consistency with our findings. The RYE-NOR primer is a marker used to detect rye translocation traits. Koebner (1995) identified alleles of 400, 600, 700, and 800 bp length using the RYE-NOR marker. Our study detected the rye translocation gene in varieties 161 and 884 (Table 3). Kocyigit et al. (2021) reported obtaining the desired gene region in 3 hybrid combinations, consistent with our findings in studies

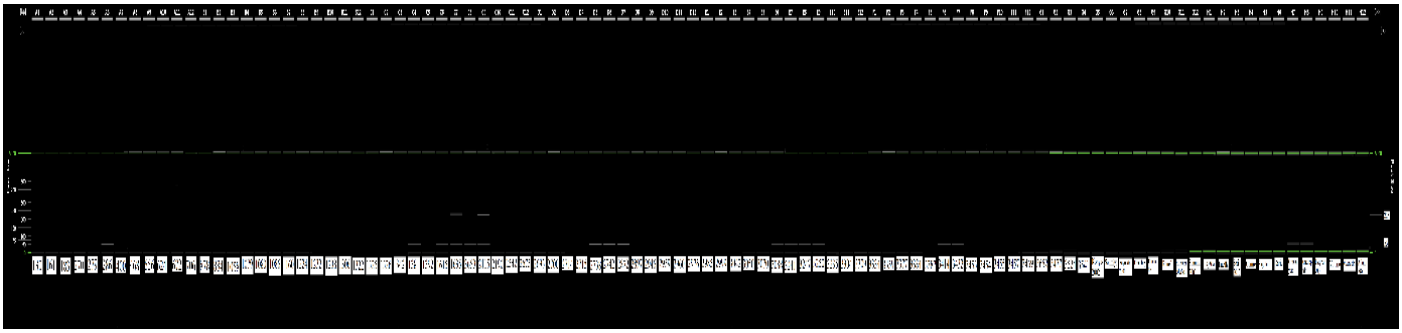

Fig. 1. Gel image of Sun 104 primer.

Table 3
Distribution of bands giving specific allele genes as a result of DNA marker screening of 96 bread wheat genotypes.

No	Genotypes	Sun1 219 bp Waxy Wx-A1	Sun104 Yr51, 225 bp Stripe Rust	Sun209 Sr49, 148 bp Stem Rust	Pina-D1 330 pb Grain Hardiness	Bx7OE 563 bp Gluten Strength	DF-MR2 254 bp Dwarfness (Short)	RYE-NOR 400, 600,700, 800 bp Rye Translocations
1	130							
2	161							+
3	183							
4	270							
5	279							
6	286							
7	400							
8	468							
9	556							
10	624							
11	672				+			
12	706							
13	878							
14	884							+
15	1058							
16	1079							
17	1080							
18	1086							
19	1160	+						
20	1224							
21	1232	+						
22	1238							
23	1306							
24	1322							
25	1325							
26	1326							
27	1342							
28	1381							
29	1392							
30	1615	+						
31	1635	+	+					
32	3659	+						
33	2115		+					
34	2190	+						
35	2542							
36	2675	+						
37	2693	+						
38	2700	+						
39	2714							
40	2715	+						
41	2738							
42	2742							
43	2872	+						
44	2897	+						
45	2946	+						
46	2959	+						
47	2960	+						
48	2976							

49	2985						
50	2999	+					
51	3042						
52	3050	+					
53	3070	+					
54	3088	+				+	
55	3241	+					
56	3247	+					
57	3250						
58	3295	+					
59	3304	+					
60	3309	+					
61	3334	+					
62	3359	+					
63	3377						
64	3384	+				+	
65	3387						
66	3414					+	
67	3432						
68	3453	+					
69	3454						
70	3455	+					
71	3457						
72	3469						
73	3652	+					
74	3477						
75	3524						
76	3541					+	
77	Konya-2002						
78	Selimiye						
79	Bayraktar - 2000						
80	Tosunbey						
81	Flamura-85						
82	Rumeli						
83	Krasunia odes'ka						
84	Sönmez- 2001						
85	Esperia						
86	Lucilla						
87	Seri-2013						
88	Cemre						
89	Sagittario						
90	Dariel						
91	Osmaniyem						
92	Karatopak						
93	Ceyhan-99						
94	Vittorio						
95	Masaccio	+					
96	Adana-99			+			

related to the RYE-NOR primer. The Pina-D1 primer is a marker used to detect grain hardiness traits. Gautier et al. (1994), Tranquilli et al. (1999), and Teniente Pérez et al. (2017) reported alleles of 330 bp, 331 bp, and 260 bp, respectively, associated with grain hardiness. Our study detected the grain hardiness gene in varieties 672, 3088, 3384, 3414, and 3541 (Table 3). However, Kocyigit et al. (2021) reported not obtaining the desired gene region in studies on the Pina-D1 primer, suggesting potential differences in genetic properties among the studied varieties. The Sun1 primer is a marker to detect the Waxy (Wx-A1) trait. Maryami et al. (2014) reported alleles of 230 bp and 265 bp, while Shariflou and Sharp (1999) reported alleles of various lengths (219 bp, 233 bp, 260 bp, 271 bp, 275 bp, 285 bp, and 289 bp) associated with the Waxy gene. Our study detected the Waxy gene in genotypes 1160, 1232, 1615, 1635, 3659, and others (Table 3). Like other markers, Kocyigit et al. (2021) reported not obtaining the desired gene region with the Sun1 primer, suggesting potential differences in genetic properties among the studied genotypes.

3.2. Dendrogram created based on obtained data

After screening 96 bread wheat genotypes using seven different DNA markers, the collected data were utilized to construct a phylogenetic tree employing the UPGMA (Unweighted Pair Group Method with Arithmetic Averages) method based on Weir's (1996) genetic similarity matrix (Fig.2). According to the resulting dendrogram, genotype 3652 shares approximately 93% genetic similarity with the genotype Masaccio. Genotypes 2190, 2715, and 2897 demonstrate about 91% similarity with genotype 2946. Similarly, genotypes 2959 and 2960, as well as genotypes 3334 and 3359, exhibit approxi-

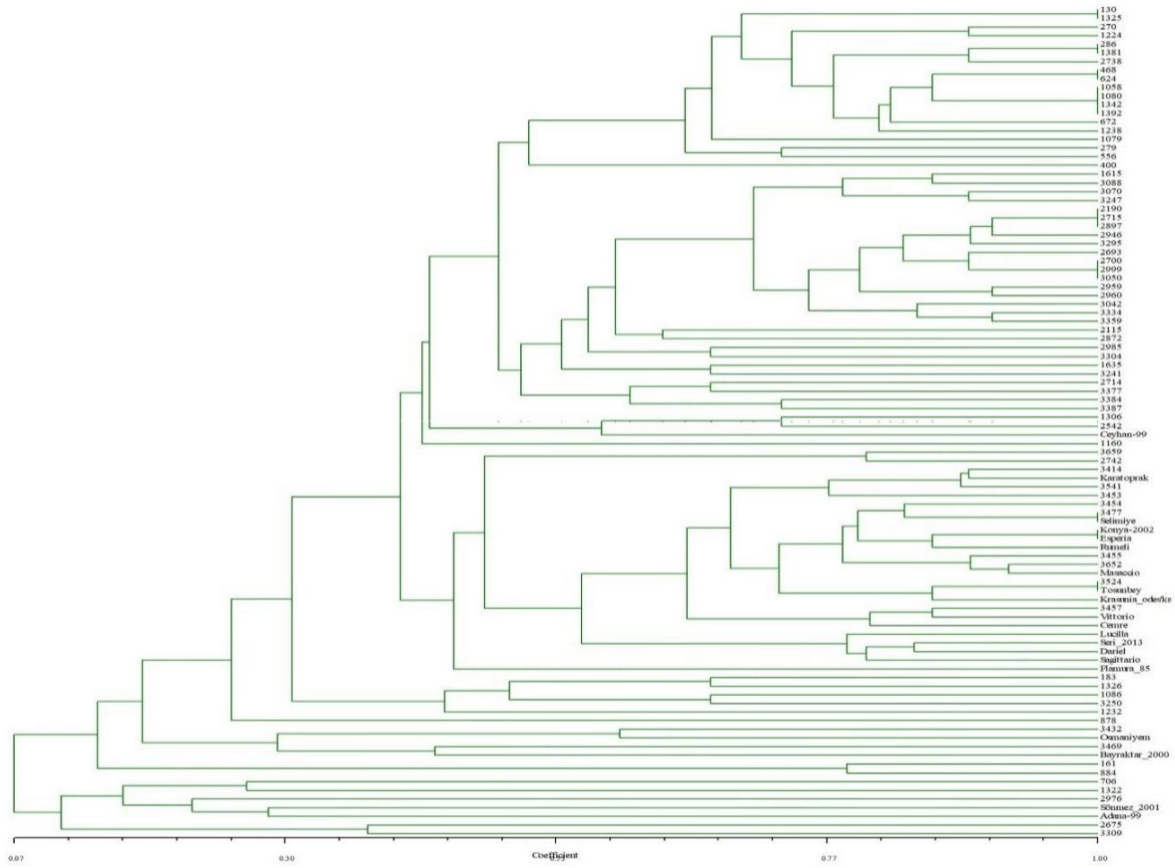

Fig. 2. Dendrogram constructed with 148 alleles obtained from 7 DNA markers of bread wheat genotypes.

mately 91% similarity. Moreover, genotypes 270 and 1224 and 286 and 1381 are similar to genotype 2738. Genotypes3070 and 3247, 2700, 2999, and 3050, as well as genotype 3414, also share approximately 89% similarity with genotype 3652 and the genotype Masaccio. Furthermore, genotypes Konya-2002 and Esperia are similar to the genotype Rumeli, while genotypes 3524 and Tosunbey are similar to the genotype Krasunia odes'ka. Similarly, genotypes 3457 and Vittorio, as well as genotypes 3042, 3334, and 3359, are similar, along with genotypes 1058, 1080, 1342, and 1392, to genotypes 468 and 624, and genotypes 1615 and 3088 share about 84% similarity. Besides, genotypes Seri-2013 and Dariel demonstrate approximately 83% similarity, while genotypes 1238 and 672, as well as genotypes 1058, 1080, 1342, and 1392, show about 80% similarity to genotypes 3659 and 2742, as well as genotype 3457 and Vittorio, and genotypes Seri-2013 and Dariel with Sagittario, respectively.

4. Conclusion and recommendations

In this study, local and commercial bread wheat genotypes were characterized using functional DNA markers, resulting in the identification of 148 alleles and the determination of polymorphism information content. The dendrogram created allowed the determination of the relatedness among genotypes and the identification of genotypes carrying genes for rye translocation, Pina-D1 grain hardiness, black rust Sr49, yellow rust Yr51, and Wx-A1 waxy traits. The results obtained in this study are likely effectively utilized in breeding programs.

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Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

References

Afshari-Behbahanizadeh, S., Puglisi, D., Esposito, S., & De Vita, P. (2024). Allelic variations in Vernalization (Vrn) Genes in *Triticum* spp. *Genes*, *15*(2), 251.

Aktas, H., Karaman, M., Oral, E., Kendal, E., & Tekdal, S. (2017). Evaluation of some bread wheat genotypes of yield and quality parameters under rainfall condition. *Journal of Field Crops Central Research Institute*, *26*(1), 86-95.

Babu, R., Sudha, K., Nair, B., Prasanna, M., & Gupta, S. (2004). Integrating marker assisted selection in crop breeding prospects and challenges. *Current Science*, *87*(5), 607-619.

Bansal, U.K., Muhammad, S., Forrest, K.L., Hayden, M.J., & Bariana, H.S. (2015). Mapping of a new stem rust resistance gene Sr49 in chromosome 5B of wheat. *Theoretical and Applied Genetics*, *128*, 2113-2119.

Butow, B. J., Ma, W., Gale, K. R., Cornish, G. B., Rampling, L., Larroqueand, O., & Bekes, F. (2003). Molecular discrimination of Bx7OE alleles demonstrates that a highly expressed high-molecular-weight glutenin allele has a major impact on wheat flour dough strength. *Theoretical and Applied Genetics*, *107*(8), 1524-1532.

Dede, B. (2007). Identification of bread wheat landraces using microsatellite DNA markers, Master Thesis, Gaziosmanpasa University, Tokat, Turkiye.

Dice, L. R. (1945). Measures of the amount of ecologic association between species. *Ecology*, *26*(3), 297-302.

Ellis, M., Spielmeyer, W., Gale, R. Rebetzke, G. J., & Richard, A. (2002). "Perfect" markers for the *Rht-B1b* and *Rht-D1b* dwarfing genes in wheat. *Theoretical and Applied Genetics*, 105, 1038-1042.

Filip, E., Woronko, K., Stępień, E., & Czarniecka, N. (2023). An overview of factors affecting the functional quality of common wheat (*Triticum aestivum* L.). *International Journal of Molecular Sciences*, *24*(8), 7524.

Gautier, M. F., Aleman, M. E., Guirao, A., Marion, D., Joudrier, P. (1994). *Triticum aestivum* puroindolines, two basic cystine-rich seed proteins: cDNA sequence analysis and developmental gene expression. *Plant Molecular Biolgy*, *25*(1), 43-57.

Gebologlu, M. D., & Furan M. A. (2017). Determination of genetic diversity among some Turkish spring bread wheat varieties using SSR markers. *Yuzuncu Yil University Journal of Agricultural Sciences*, *27*(1), 132-138.

Gungor, H., & Dumlupinar, Z. (2019). Evaluation of some bread wheat (*Triticum aestivum* L.) cultivars for yield, yield components and quality traits in Bolu conditions. *Turkish Journal of Agricultural and Natural Sciences*, *6*(1), 44-51.

Gupta, P. K., & Varshney, R. K. (2000). The development and use of microsatellite markers for genetic analysis and plant breeding with emphasis on bread wheat. *Euphytica*, *113*, 163-185.

Habib, M., Hina, A., Sajjad, M., Ahmad, M. Q., Azhar, M. T., & Qayyum, A. (2024). Marker-assisted selection in wheat. In *Advances in Wheat Breeding: Towards Climate Resilience and Nutrient Security* (pp. 597-621). Singapore: Springer Nature Singapore.

Kaya, Y. (2018). Culturing wheat. Siirt University, Faculty of Agriculture Department of Field Crops, Information Note 1. URL: https://www.siirt.edu.tr/dosya/personel/bugdayin-kulture-alinmasi-siirt-201865164933406.pdf, Last accessed on July, 2024.

Kocyigit, B. K., Yuce, I., Baskonus, T., Dokuyucu, T, Akkaya, A., & Dumlupinar, Z. (2021). Evaluation of F4 individuals belong to Seri 82 × B35 Bread Wheat (*Triticum aestivum* L.) cross population using functional DNA markers. *KSU Journal of Agriculture and Nature*, *24*(3), 586-593.

Koebner, R. M. D. (1995). Generation of PCR-based markers for the detection of rye chromatin in a wheat background. *Theoretical and Applied Genetics*, 90(5), 740-745.

Maryami, Z., Fazeli, A., & Mehrabi, A. A. (2014). Investigation of diversity of waxy-A1 gene using amplification in different spices in a genome wheats. *Advances in Environmental Biology*, *8*(7), 2004-2007.

Merga, W., & Getu, A. (2023). The application of genetic marker for diversity assessment and conserving of plant genetic resource. *Journal of Experimental and Molecular Biology*, *24*(3), 195-206.

Ocaktan, H. (2021). Diallele analysis of Bread Wheat (*Triticum aestivum* L.) genotypes determined by allele specific DNA markers and agronomical traits, Ph.D. Thesis. Kahramanmaras Sutcu Imam University, Kahramanmaras. Turkiye.

Oliver, R. E., Obert, D. E., Hu, G., Bonman, J. M., O'Leary-Jepsen E, Jackson E. W. (2010). Development of oat-based markers from barley and wheat microsatellites. *Genome*, *53*(6), 458-471.

Ozsensoy, Y., & Kurar, E. (2012). Marker systems and applications in genetic characterization studies. *Journal of Cell and Molecular Biology*, 10(2), 11-19.

Randhawa, M., Bansal, U., Valárik, M., Klocová B., Doležel J., & Bariana H. (2014). Molecular mapping of stripe rust resistance gene Yr51 in chromosome 4AL of wheat. *Theoretical and Applied Genetics*, *127*, 317-324.

Rao, V., & Poonia, A. (2023). Protein characteristics, amino acid profile, health benefits and methods of extraction and isolation of proteins from some pseudocereals-a review. *Food Production, Processing and Nutrition*, *5*(1), 37.

Rohlf, F. J. (2005). NTSYSpc (Numerical Taxonomy & Multivariate Analysis System). Version 2.2, Exeter Software, Applied Biostatistics Inc., New York.

Sertse, D., You, F. M., Klymiuk, V., Haile, J. K., N'Diaye, A., Pozniak, C. J., Cloutier, S., & Kagale, S. (2023). Historical selection, adaptation signatures, and ambiguity of introgressions in wheat. *International Journal of Molecular Sciences*, *24*(9), 8390.

Shariflou, M. R., & Sharp, P. J. (1999). A polymorphic microsatellite in the 3'end of 'Waxy' genes of wheat, *Triticum aestivum*. *Plant Breeding*, *118*, 275-277.

Song, L., Wang, R., Yang, X., Zhang, A., & Liu, D. (2023). Molecular markers and their applications in marker-assisted selection (MAS) in bread wheat (*Triticum aestivum* L.). *Agriculture*, *13*(3), 642.

Teniente-Pérez, D. K., Hernández, J. L., Chavira, M. M., & Melesio, R. (2017). Study of the diversity and genetic footprint of 17 wheat varieties using molecular markers for industrial quality. *Seguridad Alimentaria: Aportaciones Científicas y Agrotecnológicas*, 267.

Tranquilli, G., Lijavetzky, D., Muzzi, G., & Dubcovsky, J. (1999). Genetic and physical characterization of grain texture-related loci in diploid wheat. *Molecular and General Genetics*, 262, 846-850.

Turkoglu, A., Haliloglu, K., Mohammadi, S. A., Ozturk, A., Bolouri, P., Ozkan, G., Bocianowski, J., Pour-Aboughadareh, A., & Jamshidi, B. (2023). Genetic diversity and population structure in Türkiye bread wheat genotypes revealed by simple sequence repeats (SSR) markers. *Genes*, *14*(6), 1182.

Undal, V. S., & Ahir, M. R. (2023). The methods of molecular markers in genome analysis: a brief biotechnological approach. *Weser Books*, 149.

Uysal, A., & Dumlupınar, Z. (2022). Characterization of foreign and turkish originated bread and durum wheat landraces by disease resistance and quality using functional markers. *KSU Journal of Agriculture and Nature*, *25*(4), 766-777.

Weir, B. S. (1996). Genetic data analysis II: methods for discrete population genetic data. Sinauer Associates, Inc., Sunderland.

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Research article

Determination of heavy metals and trace element contents in *Veronica grisebachii* S. M. WALTERS

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Abstract

This study aimed to determine the concentrations of Al (aluminum), Ba (barium), Co (cobalt), Cr (chromium), Cu (copper), Fe (iron), Mn (manganese), Ni (nickel), Pb (lead), S (sulphur), and Zn (zinc) in soil and *Veronica grisebachii* S. M. WALTERS samples. The research focused on the heavy metal and essential nutrition element contents of these plant species. Plant samples were collected from southeastern Aksaray province, Türkiye, at the geographical coordinates 38°13'54.5"N 34°08'28.8" E and an elevation of 1276 m above sea level. Standard methods were used to determine the plant (root, stem, and leaf parts) and soil elements. The numerical values of essential elements and heavy metals in the species were quantified using ICP-MS. XRF device was also used to determine the elements in the soil. The results showed that the amounts of Cr, Cu, Fe Pb, S, and Zn in the soil were within the optimum range, while the concentrations of Al, Co, Mn, and Ni were above the optimum values. The levels of Al, Co, Mn, and Ni in the soil of the plant's natural habitat were above the reference values. This species has a high capacity to absorb and accumulate heavy metals such as Al, Co, Mn, and Ni from the soil.

Keywords: *Heavy metals; ICP-MS; trace elements; Türkiye; Veronica*

1. Introduction

Veronica is a large genus belonging to the Plantaginaceae family, with approximately 500 species, generally distributed in Southern and Central Europe as well as Türkiye (Ozyigit et al., 2013; Salehi et al., 2019; Hai et al., 2024). Plantaginaceae includes 94 genera and 1900 species in the world (Yaylaci et al., 2018). It consists herbs, subshrubs or rarely small trees, with exstipulate, opposite, alternate or whorled leaves. The flowers are hermaphrodite, and are typically borne solitary in the leaf axils. Additionally, they can be found in racemose, spicate, or paniculate cymes (Oztunca, 2009).

Veronica grisebachii, called Kesan blue in Türkiye, is an annual plant. This plant (Fig. 1) has an upright stem and can grow from 4 cm to 20 cm (Ulukus and Tugay, 2008). Generally, its branches are abundant and densely pubescent. Leaves with 3-7 mm petiole, lower leaves ovate to elliptic, shallowly lobed, or entire. Its upper part is palmate or pinnatifid, with 3-5 lobes. The dense flowers are glandular and pubescent. Corolla bright to deep blue and 8-12 mm diam. Seeds are almost 4-angled, thick, rugulose, and yellowish (Davis et al., 1978).

V. grisebachii S. M. WALTERS is a species that can grow at altitudes of 700-1600 meters above sea level. Its distribution areas cover habitats specific to the Eastern Mediterranean phytogeographic region, including sparse maquis, *Pinus* forests, steppes, maquis, and meadows. Aksaray, Antalya, Ankara, Burdur, Bursa, Eskisehir, Edirne, Izmir, Karaman, Nevsehir, Sinop, Tekirdag and Usak are among the important distribution areas of this species in Türkiye (Turkish Plants Data Service, 2023).

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Fig. 1. A- General view of *Veronica grisebachii*, B- Close up view of the flower, C-Leaves of plant.

An examination of the existing research on *V. grisebachii* reveals a significant focus on floristic studies on the species. However, the research concerning its uptake of heavy metals and utilization of trace elements is notably limited. Moreover, the species' response to the pollution of heavy metals and its potential for phytoremediation is not yet fully understood. For this reason, this study's purpose is to research the heavy metal retention capacity and trace element utilization potential of *V. grisebachii*. In particular, metals such as Al, Ba, Co, Cr, Cu, Fe, Mn, Ni, Pb, S, and Zn were evaluated based on the interaction between soil and plants. These elements play a vital role both in determining the nutritional value of the soil and in the growth and development of the plant (Agac et al., 2024). During the uptake of these trace elements (Ba, Mn, and S) by plants, some heavy metals (Zn, Pb, Ni, Fe, Cu, Co, Cr, and Al) are also taken up and included in the food chain (Pugazhendhi et al., 2024). However, the excessive amounts of these heavy metals in the soil can cause environmental pollution and lead to toxic effects for both plants and other organisms that consume these plants (Ozyigit, 2021; Osma et al., 2023). In addition, heavy metals can disrupt the normal structure and function of cellular components, interfering with various metabolic and developmental processes. (Angon et al., 2024; Yazicioglu et al., 2024). Therefore, it is thought that determining the phytoremediation potential of this species will make a significant contribution to the literature both concerning combating heavy metal pollution and better understanding the ecological characteristics of the plant.

2. Materials and methods

2.1. Study area

Aksaray province (Fig. 2) is situated in the middle section of the Central Anatolia Region. The coordinates of Aksaray province are 8-39 N and 33-35 E. It borders Nevsehir, Nigde, Konya, Ankara, and Kirsehir. The province is also distinguished by the presence of Tuz Lake, Türkiye's second-largest lake, in its northwestern region (Doganay and Eskin, 2018; Kalkan and Terzi, 2023). According to the Population-Based Registration System Database, Aksaray has 433.055 population in a land area of 7.997 km^2 (Aksaray Governorship, 2024; Turkish Statistical Institute, 2024).

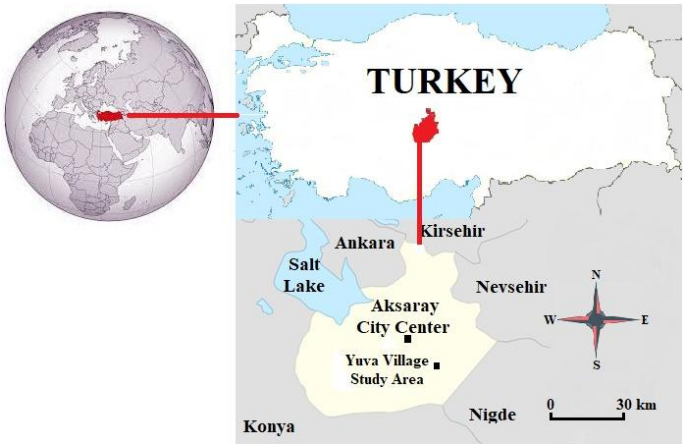

Fig. 2. Map of the research area (Location of Yuva Village in Aksaray, Türkiye).

2.2. Topography, soil and geology

The study site is located in a tectonic basin surrounded by mountains and a saline lake. The area exhibits a significant elevation difference, ranging from a low of 705 m to a high of 3,275 m. In addition, most of the Aksaray center and districts are covered with flat lands (Akyazi, 2019). Major soil groups of the research area include non-calcareous brown soils, brown soils, and colluvial soils (Uygur, 2014). Aksaray province is covered with lands formed by calcareous volcano tuffs formed in the tertiary. Stratigraphically, the metamorphics of the Kırşehir massif called the Kaman Group, are located at the bottom of the land. The metamorphites are overlain by gabbro and gabbronorite masses called Karakaya ultramaphite. Eocene and Oligocene aged cover units lie unconformably on these units (Kiraz, 2013).

2.3. Climate

Türkiye is climatically divided into regions with a Mediterranean climate and regions with a non-Mediterranean climate (Akman, 2011). The bioclimatic layer of Aksaray is the Mediterranean climate with semi-arid summers and very cold winters (Baser, 2014). In the study area, there is generally high temperature and very little precipitation in the summer months. The annual mean temperature was measured as 12.1°C (Table 1)

Table 1
Climate data for Aksaray (Between 1929-2022).

Month	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Average highest Temperature (°C)	5.5	7.5	12.5	18.1	23.1	27.1	30.7	30.7	26.7	21	13.8	7.7	18.7
Average temperature (°C)	0.5	2.1	6.3	11.5	16.2	20.2	23.5	23.3	18.8	13.3	7.2	2.6	12.1
Average low (°C)	-3.6	-2.2	1.2	5.6	9.7	13.1	16.2	15.9	11.4	6.9	2	-1.4	6.2
Precipitation (mm)	40.7	35.1	40.9	45.2	43.6	29.3	7.1	5.4	12.2	23.4	31.5	46.2	361
Average precipitation days	9.97	9.97	10.6	10.5	10.4	6.22	1.54	1.47	2.79	5.5	7.09	10.5	86.5

in about the last hundred years. Summer weather is warm, with high temperatures in July and August averaging 30.7°C. (MGM, 2023). According to these calculated climate analyses, Aksaray province is among the driest areas in Türkiye (Donmez and Yildiz, 2023).

2.4. Collection, preparation, and analysis of samples

Samples of *V. grisebachii* were collected from Yuva Village Aksaray, Türkiye in May during the period of flower. One location in the same neighborhood was determined for heavy metal status and mineral nutrients. 10 plants were taken from this location because the plant was too small. Soil samples taken from under each plant were mixed and turned into a single sample. Samples were taken in the area where the plant is densely populated. In addition, approximately 500 grams of soil samples were taken from a depth of 0-15 cm with a shovel. The plant samples were dried in the oven at 80°C for 48 hours. Then, dried samples were ground with a micro hammer and passed through a 1.5 mm mesh sieve. In the next stage, 0.2 grams of samples were placed in Teflon containers, and 4 ml of 65% HNO_3, was added. These samples were mineralized using a microwave oven (CEM MARS 5) following at the three-step temperature level (145°C for 5 minutes, followed by 165°C for 5 minutes, and finally 175°C for 20 minutes). After mineralization and cooling, the samples were filtered using Whatman filters and then were diluted to a final volume of 50 ml using ultrapure water and transferred to Falcon tubes. By preparing a multi-element standard solution at -1000 ppm (Merck) from the stock solution, the quantitative values of mineral elements and heavy metals were determined via Inductively Coupled Plasma Mass Spectrometry (ICP-MS; Thermo, Xseries 2 Serial number: SN02132C).

An ICP Multi-Element Standard solutions were created by diluting a 10 mg kg^{-1} stock solution to obtain final concentrations of 1, 5, 6, 10, 30, 50, 100, 150, and 200 µg kg^{-1}. Then, a calibration curve was created using equal concentrations of trace elements (Ahamad et al., 2017; Ozbay et al., 2023). In addition, numerical results for the same minerals in the soil were obtained by the XRF device (Pan Analytical, Axios Max Serial number: DY5970).

Numerical results for the same minerals in the soil were obtained by the XRF device (Pan Analytical, Axios Max Serial number: DY5970). In preparation stage, soil samples were ground to 20 µm particle size using a ball bearing tungsten carbide cap and a 5 g aliquot was homogenized with 1 g of Micropulver Wachs C. The mixture of wax and sample was pressed into pellets using a die attacher at 13 kg N^{-1}. The pressed pellet was analyzed with XRF to identify the heavy metals (Demir et al., 2021).

The $CaCO_3$ ratio in the soil was detected using a Scheibler calcimeter (Caglar, 1949). The soil pH was measured with a pH meter. Organic matters were measured according to Walkley-Black (Black, 1965). Additionally, the total phosphorus of the samples was determined with Olsen and Sommers methods (Olsen and Sommers, 1982). The photometric method was used to determine the amount of available potassium in the soil.

2.5. Statistical analyses

Pearson correlation coefficients were calculated for average and standard deviation values using SPSS (version 25). The significance level of the data was found to be statistically significant at **P<0.01 and *P<0.05 levels (2-tailed).

2.6. Bioconcentration factor

The Bioconcentration Factor (BCF) is a quantitative measure of the extent to which a plant species can take metals from the soil. It is determined by dividing the metal concentration in plant tissue by the corresponding concentration in the soil, based on the analysis of elements. Plants exhibiting a BCF value exceeding one (>1) are promising for extracting contaminants from soil through phytoremediation (Ghosh and Singh, 2005; Demir et al, 2021).

BCF = metal concentration in the plant tissue/metal concentration in the soil

3. Results and discussions

The mean numerical values of heavy metal and trace element distributions in soil and plant samples are shown in Table 2. The average values (mg kg^{-1}) for Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S, and Zn in the soil samples were 66900, 485.6, 100, 27, 25, 33400, 700, 51.5, 22.7, 24.8 and 60.3 respectively. The optimum reference values (mg kg^{-1}) in soil are as follows: Al: 10,000-40,000, Cr: 5-120, Co: 1-10, Cu: 5-30, Fe: 5,000-50,000, Mn: 270-525, Ni: 10-50, Pb: 10-30, S: 10-157, and Zn: 10-300 (Kabata-Pendias and Pendias, 2001; Barker and Pilbeam, 2007; Kabata-Pendias and Mukherjee, 2007; Kacar and Katkat, 2010; Blum et al., 2014). The results of the analysis showed that the concentrations of Cr, Cu, Fe, Pb, S, and Zn in the soil were within the optimum reference values. However, the values of Al, Co, Mn, and Ni were found to be above the optimum ranges. On the other hand, the element concentrations (mg kg^{-1}) in plant parts, without considering standard errors, were determined as follows: Al: 327.53-932.84, Ba: 18.54-84.01, Cr: 11.18-66, Co: 0.23-0.63, Cu: 3.71-5.15, Fe: 1009.2, Mn: 16.69-36.10, Ni: 0.74-3.80, Pb: 0.60-0.72, S: 0.55-1, and Zn: 19.21-20.72. Optimal reference values for mineral nutrient and heavy metal elements in plants (mg kg^{-1}) are as follows: Al: 7-3470, Cr: 0.1-0.5, Co: 0.02-0.5, Cu: 5-30, Fe: 5-250, Mn: 30-300, Ni: 0.1-5, Pb: 0.05-3, and Zn: 20-150 (Kabata-Pendias and Pendias, 2001; Jones and Jacobsen, 2005; Kabata-Pendias and Mukherjee, 2007; Blum et al., 2014).

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When the element concentrations in the plant parts were compared with the optimum values: It was observed that the concentrations of Al, Cu, Mn, Ni, Pb, and Zn complied with the reference values. This shows that these elements are at appropriate levels for plant growth and development. It was seen that the concentrations of Cr, Co, and Fe elements were above

Table 2
Mineral element and heavy metal concentration values in soil and plant samples.

Veronica grisebachii	Root	Stem and Leaf	Soil	Optimum Value in Soil	Optimum Value in Plant	Bioconcentration Factor (BCF)
Al (mg kg^{-1})	932.84	327.53	66900	10000-40000	7-3400	0.0139
Ba (mg kg^{-1})	18.54	84.01	485.6	-	-	0.0381
Cr (mg kg^{-1})	11.18	66	100	5-120	0.1-0.5	0.111
Co (mg kg^{-1})	0.63	0.23	27	1-10	0.02-0.5	0.0233
Cu (mg kg^{-1})	5.15	3.71	25	5-30	5-30	0.206
Fe (mg kg^{-1})	1009.2	247.28	33400	5000-50000	5-250	0.0302
Mn (mg kg^{-1})	36.10	16.69	700	270-525	30-300	0.0515
Ni (mg kg^{-1})	3.80	0.74	51.5	10-50	0.1-5	0.0737
Pb (mg kg^{-1})	0.72	0.60	22.7	10-30	0.05-3	3.1718
S (mg kg^{-1})	1	0.55	24.8	10-157	-	0.0403
Zn (mg kg^{-1})	19.21	20.72	60.3	10-300	20-150	0.3185

Refrences for bioconcentration factor (BCF) and optimum value in soil and plant (Kabata-Pendias and Pendias, 2001; Ghosh and Singh, 2005; Jones and Jacobsen, 2005; Barker and Pilbeam 2007; Kabata-Pendias and Mukherjee, 2007; Kacar and Katkat, 2010; Blum, Horak and Mentler, 2014)

the optimum values. This situation indicates that these minerals are taken up from the soil and stored by the plant. The fact that certain minerals are taken up from the soil and stored by the plant indicates that the plant can utilize these minerals for growth, development, and metabolic processes. The ion form of these minerals, taken up from the soil through *V. grisebachii* roots, can be stored in different tissues of the plant. This storage allows the plant to reserve the minerals it needs for growth periods or stress conditions. In addition, in *V. grisebachii*, values for Ba, Cr, and Zn are arranged as root < stem-leaf, while values for Al, Co, Cu, Fe, Mn, Ni, Pb, and S are arranged as root > stem-leaf. The concentrations of Ba, Cr, and Zn are the highest in the roots. The concentrations of Ba, Cr, and Zn are found at the highest levels in the roots, while the concentrations of Al, Co, Cu, Fe, Mn, Ni, Pb, and S are at the highest levels in the leaves of species. This situation shows that different elements are transported and stored differently within *V. grisebachii*. indicating that Ba, Cr, and Zn are needed more in the regions close to the roots, while Al, Co, Cu, Fe, Mn, Ni, Pb, and S are transported to the leaves for processes such as photosynthesis or other metabolic activities.

Also, Bioconcentration Factor (BCF) of the studied heavy metals varied 0,0139 for Al, 0,0381 for Ba, 0,111 for Cr, 0.0233 for Co, 0,206 for Cu, 0,0302 for Fe, 0,0515 for Mn, 0,0737 for Ni, 3,1718 for Pb, 0,0403 For S, 0,3185 for Zn (Table 2). The Pb ratio (3,1718) in this species is greater than 1 (>1). This shows that this plant may be a good hyperaccumulator of Pb.

Table 3 shows the physical analysis results of soil samples taken from the distribution area of *V. grisebachii*. When these results are evaluated, it can be observed that the natural habitat of *V. grisebachii* consists of clay-loamy and slightly alkaline soils with a pH of 7.89. The level of CaCO3 is low (1.25%), and the Saturation value is 0,028% (non-saline). In addition, the organic matter concentration of the soil was found to be of low (1.37 %) value. These findings indicate that *V. grisebachii* has a certain tolerance to soil type and chemical properties. Also, the result of correlation analysis between root and stem/leaves is a high level of positive correlation (>1.00, >0.82) between the root and shoot/leaf for the nutrient elements Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S, and Zn (Table 4). These nutrient elements indicate that they are transported and stored in a coordinated manner among different organs of the plant. In addition, the correlation between Al, Ba, Cr, Co, Cu, Pb in the root and Al, Ba, Cr, Co, Cu, Fe Mn Ni, and Zn in the stem/leaf (>0.58, >0.33) is low. This means that these elements are transported and stored differently between roots and stems/leaves.

Table 3
Physical analysis results of the soil samples of *Veronica grisebachii* habitats.

	Soil	
Analysis Type	**Numerical value**	**Status**
Texture (%)	52.58	Clayey-loamy
$CaCO_3$ (%)	1.25	Low Chalky
pH	7.89	Slightly Alkaline
Saturation (%)	0,028	Without salt
Potassium (K_2O) kg/da	177.55	Adequate
Phosphorus (P_2O5) kg/da	1.63	Very Low
Organic Matter (%)	1.37	Low

According to the result of the correlation between soil and plant parts is a high level of positive correlation between soil and plant parts for the nutrients Al, Ba, Cr, Co, Cu, Fe, Mn, Ni, Pb, S, and Zn (Table 5). While it is understood that the correlation is low (>0,001, >0.59) in some soils and stems/leaves, it is high (>1, >0.61) in others. This indicates that the element concentrations in the soil directly affect the element concentrations stored in different organs of the plant.

It is possible for the elements found in high concentrations in the soil to be more absorbed and stored by the roots, stems, and leaves of the plant (Phuong et al., 2023). This shows that the element concentrations in the soil can directly affect plant nutrient uptake and growth. The plant-soil interaction is a critical ecological factor. As a plant grows, the amounts of different elements in it change, which affects its whole life cycle.

In conclusion, the close relationship between Veronica and its habitat shows that this plant has the potential to be used in phytoremediation studies.

4. Conclusion

The levels of Ni, Mn, Co, and Al in the soil of the plant's natural habitat are above the reference values. This shows that the soil is exposed to heavy metal pollution. In addition, *V. grisebachii* accumulates Cr, Co and Fe nutritional elements. According to BFC factor the Pb ratio in this species is greater than 1. This result shows that the plant can be a good Pb hyperaccumulator. This research results indicate that there is a strong relationship between nutrients in *V. grisebachii* and soil.

Table 4
Correlation relationship between mineral nutrients in root and stem/leaf.

	Correlation Matrix (R)										
Pearson Correlation	**Al Root**	**Ba Root**	**Cr Root**	**Co Root**	**Cu Root**	**Fe Root**	**Mn Root**	**Ni Root**	**Pb Root**	**S Root**	**Zn Root**
Al Stem/Leaf	0.012**	1.00**	0.051	0.866	0.008	0.5	0.668	0.688	0.027	0.561	0.507
Ba Stem/Leaf	0.967	0.996	0.030*	0.822	0.817	0.427	0.551	0.692	0.054*	0.596	0.453
Cr Stem/Leaf	0.027*	0.026*	0.233	0.566	0.686	0.455	0.996	0.985	0.998	0.955	0.829
Co Stem/Leaf	0.001	0.009**	0.511	0.5	0.406	0.211	0.219	0.98	0.999	0.758	0.844
Cu Stem/Leaf	0.008**	0.005**	0.998	0.492	0.994	0.908	0.861	0.968	0.994	0.995	0.336
Fe Stem/Leaf	0.064	0.063	0.993	0.443	0.24	0.973	0.24	0.241	0.242	0.16	0.638
Mn Stem/Leaf	0.996	0.996	0.028*	0.823	0.079	0.429	0.148	0.464	0.052*	0.445	0.498
Ni Stem/Leaf	0.052	0.051*	0.994	0.453	0.644	0.637	0.619	0.146	0.996	0.997	0.329
Pb Stem/Leaf	0.672	0.671	0.705	0.211	0.793	0.705	0.784	0.858	0.721	0.977	0.265
S Stem/Leaf	0.189	0.181	0.971	0.327	0.314	0.191	0.314	0.313	0.976	0.561	0.727
Zn Stem/Leaf	0.063	0.065	0.999	0.554	0.676	0.599	0.572	0.665	0.547	0.559	0.661

Table 5
Correlation relationship between mineral nutrients in soil and plant parts.

	Correlation Matrix (R)										
	Al Soil	**Ba Soil**	**Cr Soil**	**Co Soil**	**Cu Soil**	**Fe Soil**	**Mn Soil**	**Ni Soil**	**Pb Soil**	**S Soil**	**Zn Soil**
Al Root	0.012 **	1.000**	0.082	0.866	0.114	0.24	0.199	0.167	0.363	0.09	0.904
Al Stem/Leaf	0.012**	1.000**	0.082	0.866	0.114	0.24	0.199	0.167	0.363	0.996	0.904
Ba Root	0.013**	0.001**	0.081	0.866	0.113	0.241	0.202	0.168	0.363	0.091	0.905
Ba Stem/Leaf	0.069	0.996*	0.163	0.822	0.195	0.16	0.118	0.086	0.285	0.009**	0.866
Cr Root	0.999	0.054**	0.991	0.427	0.952	0.981	0.996	0.993	0.949	0.999	0.472
Cr Stem/Leaf	0.118	0.024**	0.998**	0.476	0.996	0.963	0.974	0.98	0.921	0.993	0.402
Co Root	0.51	0.8674	0.427	0.05*	0.397	0.693	0.662	0.637	0.78	0.576	0.996
Co Stem/Leaf	0.999	0.002**	0.405	0.337*	0.258	0.97	0.979	0.985	0.931	0.995	0.427
Cu Root	0.999	0.002**	0.996	0.468	0.993	0.651	0.979	0.461	0.931	0.995	0.427
Cu Stem/Leaf	0.999	0.005	0.322	0.427	0.994	0.968	0.984	0.984	0.928	0.995	0.419
Fe Root	0.872	0.502	0.822	0.866	0.803	0.996**	0.948	0.937	0.988	0.907	0.821
Fe Stem/Leaf	0.997	0.061	0.999	0.443	0.998	0.953	0.964	0.937	0.531	0.822	0.367
Mn Root	0.996	0.064	0.999	0.449	0.998	0.766	0.964	0.391	0.905	0.987	0.365
Mn Stem/Leaf	0.067	0.996	0.929	0.996	0.938	0.337	0.121	0.233	0.966	0.011**	0.867
Ni Root	0.977	0.194	0.993	0.319	0.996	0.985	0.921	0.933	0.841	0.958	0.24
Ni Stem/Leaf	0.333	0.049	0.052	0.997	0.052	0.051	0.05	0.978	0.911	0.989	0.379
Pb Root	0.360	0.028	0.994	0.523	0.989	0.977	0.985	0.990	**0.941**	0.998*	0.451
Pb Stem/Leaf	0.672	0.671	0.793	0.211	0.812	0.557	0.591	0.617	0.721	0.676	0.291
S Root	0.5	0.497	0.904	0.576	0.993	0.72	0.748	0.77	0.96	0.817	0.082
S Stem/Leaf	0.996	0.067	0.994	0.327	0.997	0.907	0.924	0.99	0.846	0.96	0.248
Zn Root	0.85	0.538	0.526	0.886	0.948	0.911	0.934	0.922	0.981	0.889	0.845
Zn Stem/Leaf	0.998	0.536	0.989	0.554	0.984	0.899	0.994	0.99	0.953	0.999	0.484

This research is the first study on the phytoremediation possibilities of *V. grisebachii* species. Therefore, it is thought that the results of this research will make a significant contribution to the literature.

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References

Agac, H., Erdogan, G., & Yalcin, F. S. (2024). Effect of thermal power plant fly ash at different application levels on plant growth parameters of wheat. *Osmaniye Korkut Ata University Journal of The Institute of Science and Technology*, *7*(1), 276-293.

Ahamad, S.R., Raish, M., Ahmad A., & Shakeel, F. (2017). Potential health benefits and metabolomics of camel milk by GC-MS and ICP-MS. *Biological Trace Element Research*, 175, 322–330.

Akman Y. (2011). *Climate and bioclimate*. (pp. 1-352). Palme Publications.

Aksaray Governorship, (2024). Geographical Structure, http:// www.aksaray.gov.tr/, Last accessed on January 20, 2024.

Akyazi, N. (2019). Linking urban open and green spaces with ecological zones: Aksaray sample, Master's Dissertation, (pp. 1-101). Aksaray University.

Angon, P. B., Islam, M. S., Kc, S., Das, A., Anjum, N., Poudel, A., & Suchi, S. A. (2024). Sources, effects and present perspectives of heavy metals contamination: Soil, plants and human food chain. *Heliyon*. *10*(7), 1-15.

Barker A. V., & Pilbeam, D. J. (2007). *Handbook of plant nutrition*. (pp. 1-613). Boca Raton, USA: CRC Press.

Baser, M. (2014). Flora of Elmacık, Koçpınar and İncesu villages and envi-

ronment (Aksaray), Master's Dissertation, (pp. 1-105). Aksaray University.

Black, C. A. (1965). *Methods of Soil Analysis: Part I, Physical and Mineralogical Properties*. (pp. 1-1572). Madison, Wisconsin, American Society of Agronomy.

Blum, W. E. H., Horak, O., & Mentler, A. (2014). *Trace Elements*. Oxford, UK.: Environmental and ecological chemistry, Sabljic a. (Éditeur). Encyclopedia of life support systems (EOLSS), developed under the auspices of the UNESCO.

Caglar, K. O. (1949). *Soil Science*. Ankara University, Ziraat Publications.

Davis, P. H., Edmondson J. R., Mill, R. R., & Parris, B. S. (1978). *Flora of Turkey and the East Aegean Islands*. Volume 6, (pp. 1-825). Edinburgh University Press.

Demir, A., Baysal Furtana, G., Tekşen, M., & Tıpırdamaz, R. (2021). Determination of the heavy metal contents and the benefit/cost analysis of *Hypericum salsugineum* in the vicinity of Salt Lake. *Sains Malaysiana*, *50*(12), 3493-3503.

Doganay, S., & Eskin B. (2018). Urban transformation applications in Aksaray province. *Aksaray University Journal of the Faculty of Economics and Administrative Sciences*, *10*(4), 45-54.

Donmez, A. H., & Yildiz, O. (2023). Survival and growth success of Russian olive (*Elaeagnus angustifolia* L.), salt cedar (*Tamarix smyrnensis* Bunge) and white poplar (*Populus alba* L.) in gypsum and sulfur applied salt-sodic sites in Central Anatolia. *Anatolian Journal of Forest Research*, *9*(2), 184-194.

Ghosh, M., & Singh, S. P. (2005). Comparative uptake and phytoextraction study of soil induced chromium by accumulator and high biomass weed species. *Applied Ecology and Environmental Research*, *3*(2), 67-79.

Hai, Y., Qian, Y., Yang, M., Zhang, Y., Xu, H., Yang, Y., & Xia, C. (2024). The chloroplast genomes of two medicinal species (*Veronica anagallis-aquatica* L. and *Veronica undulata* Wall.) and its comparative analysis with related *Veronica* species. *Scientific Reports*, *14*(1), 13945.

Jones, C., & Jacobsen, J. (2005). *Plant Nutrition and Soil Fertility.* Nutrient management module 2. Montana State University Extension Service. Publication 4449-2.

Kabata-Pendias, A., & Pendias, H. (2001). *Trace elements in soils and plants*. (pp. 1-432). Boca-Raton, USA: CRC Press.

Kabata-Pendias, A., & Mukherjee, A. B. (2007). *Trace Elements from Soil to Human*. (pp. 1-576). New York, USA: Springer Science and Business Media.

Kacar, B., & Katkat, V. (2010). *Plant nutrition*. (pp. 1-659) Kızılay-Ankara, Türkiye: 5th Edition, Nobel Publication.

Kalkan, M., & Terzi, M.H. (2023). Assessment of heavy metal pollution of the soils in the southwest of Aksaray City. *Recep Tayyip Erdogan University Journal of Science and Engineering*, *4*(2), 219-231. https://doi.org/10.53501/rteufemud.1360204.

Kiraz, S.E. (2013). Flora of Mamasın Dam Surrounding (Aksaray), Master's Dissertation, (pp. 1-105). Aksaray University.

MGM, (2023). The Official Website of Turkish State Meteorological Service, https://www.mgm.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?k=A&m=AKSARAY, Last accessed on November 30, 2023.

Phuong, H. T., Ba, V. N., Thien, B. N., & Hong, L. T. T. (2023). Accumulation and distribution of nutrients, radionuclides and metals by roots, stems and leaves of plants. *Nuclear Engineering and Technology*, *55*(7), 2650-2655.

Pugazhendhi, A., Govindasamy, C., & Sharma, A. (2024). Heavy metal accumulation in root and shoot tapioca plant biomass grown in agriculture land situated around the magnesite mine tailings. *Environmental Research*, *257*, 1-9.

Olsen, S.R., & Sommers, L. E. (1982). *Phosphorus*, In: Page, A. L., et al., (eds). *Methods of Soil Analysis (Part 2). Chemical and Microbiological Properties*. (pp. 1-320). American Society of Agronomy.

Osma, E., Varol, T., & Elveren, M. (2023). Determination of concentration of some heavy metals (Ba, Cd, Co, Cr, Pb) in plants growing from different habitats (Gypsum, Limestone, Serpentine). *Osmaniye Korkut Ata University Journal of The Institute of Science and Technology*, *6*(2), 1562-1578.

Ozbay, S., Dikici, E., & Soylukan, C. (2023). Evaluation of biological (feed, water), seasonal, and geological factors affecting the heavy metal content of raw milk. *Journal of Food Composition and Analysis*, *121*, 105401.

Oztunca, H. (2009). Pharmacognostical investigations on *Veronica cuneifolia* D. Don subsp. *cuneifolia* and *Veronica cymbalaria* Bodard, Master's Dissertation, (pp. 1-188). Hacettepe University.

Ozyigit, I. I., Dogan, I., Demir, G., Eskin, B., Keskin, M., & Yalçın, I. E. (2013). Distribution of some elements in *Veronica scutellata* L. from Bolu, Turkey: soil-plant interactions. *Sains Malaysiana*, *42*(10), 1403-1407.

Ozyigit, I. I. (2021). Heavy metals in agricultural soils; origins, distribution and effects. *Necmettin Erbakan University Eregli Journal of Agricultural Science*, *1*(1), 46-72.

Salehi, B., Shivaprasad Shetty, M., V. Anil Kumar, N., Živković, J., Calina, D., Oana Docea, A., ... & Sharifi-Rad, J. (2019). *Veronica* plants—Drifting from farm to traditional healing, food application, and phytopharmacology. *Molecules*, *24*(13), 2454, 1-35.

Turkish Plants Data Service, (2023). *Veronica grisebachii S. M. WALTERS*. http://194.27.225.161/yasin/tubives/index.php?sayfa=1&tax_id=7214, Last accessed on August 23, 2023.

Turkish Statistical Institute, (2024). Population of province/district centers and towns/villages and annual population growth rate by province, 2021, 2022, https://data.tuik.gov.tr/Bulten/Index?p=The-Results-of-Address-Based-Population-Registration-System-2022-49685&dil=2, Last accessed on January 20, 2024.

Ulukus, D., & Tugay, O. (2008). The flora of region among Urgup, Derinkuyu and Hodul Mountain (Nevsehir/Turkey). *Biological Diversity and Conservation*, *7*(3), 42-57.

Uygur, A. (2014). The flora of area between the towns of Karaoren-Yuva-Karkın (Aksaray), Master's Dissertation, (pp. 1-109). Aksaray University.

Yaylaci, O. K., Sezer, O., Ozgisi, K., Ozturk, D., Erkara, I. P., Koyuncu, O., & Ocak, A. (2018). A new *Veronica* (Plantaginaceae) species from central Anatolia, Turkey. *Phytotaxa*, *362*(1), 55-67.

Yazicioglu, H., Hocaoglu-Ozyigit, A., Ucar, B., Yolcu, S., Yalcin, I. E., Suner, S., & Ozyigit, I. I. (2024). Physiological alterations and genotoxic damage under combined aluminum and cadmium treatments in *Bryophyllum daigremontianum* clones. *Molecular biology reports*, *51*(1), 1-21.

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Research article

Determination of biomarker candidates with proteomics approach in small cell lung cancer: NCI-H209 cell line

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Abstract

Proteins, the primary building blocks of the cell membrane, play crucial roles in communication between cells as well as interactions with the extracellular matrix. They make for an excellent resource for disease identification due to their potential as biomarkers. In order to perform the study, HEL-299 (CCL-137™) and NCI-H209 lung cells were incubated at 37°C in a chamber that contained 5% CO_2. Trypsinization was used to transfer the cells into Eppendorf tubes. Proteomics analyses were carried out using LC-QTOF equipment, and the corresponding procedures of denaturation, alkynylation, trypsinization, and purification were carried out by adding the required chemicals. The Searchquie and PeptideShacker software interfaces were used to assess the analysis findings. Proteins that differ across groups are displayed by classifying them based on their roles as cellular components, molecular activities, and biological processes. Proteomics data showed that the lung cancer cell line NCI-H209 lacked 14 proteins that were present in the healthy lung cell HEL-299. These are the proteins ANK3, PIK3R2, INPP5F, HSF1, VIM, NFAM1, SHROOM3, ETV4, RNF31, LMNA, BRD8, PRTN3, TERT, SMAD9. There were discovered to be 5 distinct proteins in the lung cancer group compared to healthy lung HEL-299 cells. These proteins are AHSG, NCOA6, VCP, DNAJC19, NCL. Given the heterogeneity of lung cancer, a thorough and in-depth investigation of lung cancer proteome profiling is necessary for effective target treatment. The examination of proteins as prospective lung cancer biomarker candidates shows that it will make up a viable source for clinical investigations. These proteins differ in the direction in this study. Potential clinical applications of the biomarkers identified in this study, such as early diagnosis, monitoring treatment response, and determining disease prognosis, may contribute to the development of personalized medicine approaches.

Keywords: *Biomarker; cancer; proteomics; small cell; lung cancer*

1. Introduction

Lung cancer was a relatively uncommon disease in the 1800s, but it is now one of the most common cancers, with an estimated 2.21 million new cases and 1.80 million deaths per year (Nadaf et al., 2022). Lung cancer accounts for more deaths in recent years than breast, colon, and prostate cancer combined. In India, the mortality rate from lung cancer has increased in women in the last ten years, in contrast to men, whose rate has started to decline more than 20 years ago (Ghosh et al., 2023).

The lack of early diagnostic techniques, along with smoking, environmental pollution, and genetic predisposition, is a major factor contributing to the rise in lung cancer incidence. Large populations are particularly challenging to screen for diseases in because of the general lack of knowledge on the benefits of routine blood work and physical examinations. The development of methods such as low dose computed tomography (LDCT), liquid biopsies, and advanced bronchoscopy techniques has become increasingly important in the biological early diagnosis of lung cancer (Rusch et al., 2020; Wang et al., 2021). The fact that lung cancer's overall five-year survival rate is just 14% suggests that the disease's individual

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death rates have not yet dropped considerably. As a result, it's critical to establish procedures for tracking the disease's progression and early diagnosis. Validated biomarkers are seen to be the most significant of these approaches, so finding novel, targeted biomarkers for lung cancer is necessary.

Proteomic studies have been extensively used in the investigation of many diseases, particularly in cancer research, and are now acknowledged as a potent tool for large-scale assessment of protein expression (Matsuda et al., 2020; Zhang et al., 2021; Chen et al., 2022; Lee et al., 2023).

A key component of contemporary proteomic research is the identification and confirmation of biomarkers, which enable early cancer diagnosis, treatment, monitoring, and prognosis. Since mRNA levels do not always fully reflect the number of molecules or proteins involved in biological processes, the use of protein-based biomarkers in cancer diagnosis and treatment has become increasingly important (Meyer et al., 2020; Zhang et al., 2022; Atasever, 2024).

Post-translational modifications (such as phosphorylation, glycosylation, and methylation) are more valuable and instructive than transcriptomics or genomics, as well as revealing the functions of tumors and their interactions with the tumor microenvironment. Proteomic analyses have been used to study malignancy in many types of cancer, including gastric, lung, colorectal, breast, prostate, and pancreatic cancer. There are still a lot of pre-, analytical, and post-analytical issues with MS techniques, despite their many benefits and insights into cancer biology (Liu et al., 2022; Zhao et al., 2023; Wang et al., 2023; Gao et al., 2024).

When applied to fresh tissue or blood samples, certain mass spectrometry techniques like MALDI/MS do not always allow for the direct identification of proteins. Bioinformatics biostatistical artifacts are the primary cause of post-analysis constraints. Because blood serum is easily accessible from patients through routine blood chemistry, it is most frequently employed in biomarker investigations. Many circulating protein fragments originating from the tumor microenvironment and biomarkers detected in biopsied cancer tissue are examples of potential biomarkers that are predominantly found in blood (Schumacher et al., 2021; Kalluri and LeBleu, 2022; Duffy et al., 2023).

The effects of meclofenamic acid on the KHAK cell line DMS114 were examined in the study carried out by Yanar et al. (2023). The findings of the study demonstrated that the administration of meclofenamic acid altered cellular energy metabolism. Glycolysis was inhibited, although oxidative phosphorylation and mitochondrial activity rose. Thus, the proteomics results changed. Proteomic analysis of lavage fluids from SCLC patients revealed 460 BALF proteins. In addition, CNDP2 and RNPEP were identified as potential subtype markers for ASCL1 and NEUROD (Vu et al., 2023). A further study carried out in 2023 to enhance the therapy of lung cancer revealed 73 microRNAs (miRNAs). Ten miRNAs were shown to be tumor suppressors in lung cancer and four miRNAs to be oncogenic based on patient survival data and tumors compared to normal lung tissues. DGKE and WDR47 were found to be significant in lung cancer among these microRNAs (Ye et al., 2023). 33 distinct proteins were found in the study of Gasparri et al. (2023) on the profiling of serum proteomics of early-stage lung cancer. ACTR3B, CD59, PRKCA, and ARSA are among these proteins. PRKCA and ARSA were highlighted as being crucial. The thorough proteogenomic characterization of small cell lung cancer (SCLC) has been established by Liu et al. (2024) study. This work has demonstrated the cancer biology resulting from genetic aberrations using integrated multi-omics analysis, emphasizing the carcinogenic functions of RB1 deletion, FAT1 mutation, and chromosomal 5q loss. HMGB3 and CASP10 are two prognostic biomarkers that have been found.

Moreover, it has been documented that HMGB3 overexpression stimulates SCLC cell migration by transcriptionally regulating genes linked to cell attachment. Four distinct subtypes have been identified using multi-omics clustering, each with unique treatment limitations. 67 of the 1162 circulating proteins that were examined in the 2023 study were linked to an increased risk of lung cancer. Reproducible correlations between lung cancer diagnosis risk and 36 proteins were found (Ahamed et al., 2024).

The 36 proteins were examined for their known mechanistic roles, and it was discovered that they performed a variety of molecular fuertnctions. These include chemokines, various growth factors, tumor necrosis factor receptors, and cytokines. The only protein that has been found to have a negative correlation with lung cancer is SCF. SCF may control cell survival, proliferation, and hematopoiesis, according to certain theories. In 2023, the proteomic profiles of plasma exosomes from patients diagnosed with metastatic lung cancer were examined using liquid chromatography-tandem mass spectrometry (LC-MS/MS). The samples comprised five healthy controls (HC), twenty-six solitary brain metastases (BM), sixteen solitary liver metastases (LM), and twenty-five non-metastatic locally advanced (LA) lung cancer cases. Lung cancer LM and BM showed deregulation of 143 and 120 exosome-based proteins, respectively. Additionally, it was found that SELL and MUC5B could be used as diagnostic markers of BM, while APOH, CD81, and CCT5 could be used to diagnose LM in LC patients (Li et al., 2023). In a study aiming to find invasive biomarkers as plasma proteins in the early diagnosis of lung cancer, 2941 proteins were analyzed in 131 cases, 237 controls, and numerous plasma samples taken one to 10 years anterior to diagnosis. Samples obtained 1-3 years before the diagnosis revealed that 240 proteins had been significantly changed in cases; samples taken 1-5 years before the diagnosis revealed 150 more proteins, 117 of which were significantly altered (Davies et al., 2023).

Currently, while there are no widely approved serum biomarkers for the early detection of lung cancer, ongoing research is focused on enhancing early and targeted identification as well as disease surveillance. Changes in protein glycosylation are ubiquitous indicators of malignant transformation and tumor advancement in human cancer, manifesting in every cell. Target protein research is therefore crucial to the treatment of cancer and the advancement of the disease. Characterization and biological function of proteins depend heavily on extensive and methodical protein analysis (Koch et al., 2020). Recent research has demonstrated that protein alterations in cancerous cells are significant during different phases of the tumor's development (Pérez-Rodríguez et al., 2021). Proteomic data sets are necessary to comprehend the biological significance of the distinct protein networks of healthy and malignant cells, to clarify their molecular interactions, to determine the functional architectures of proteins, and to determine how these relationships relate to diseases. To ascertain the function of tumor-associated chemicals as putative biomarkers in the NCI-H209 small cell lung cancer cell line, a proteomics investigation was carried out using the LC-QTOF/MS instrument (Chen et al., 2024).

2. Materials and methods

2.1. Cell proliferation

The study used the NCI-H209 cell line and HEL-299 (CCL-137TM) cell line. The Safe Fast Elite (EN 12469 2000) flow chamber was used to cultivate the cell line. Bovine serum-containing media (MULTICELL (FBS-HI-IIA)) was used for cell passage and culture, together with medium, 1% L-glutamine (MULTICELL (609-065-E2), and 1% penicillin-streptomycin [MULTICELL (450-201-Z2)]. The medium was kept at 37 °C and 5% CO2 in sterile incubators (PANASONIC). Up until the fifth passage, cultured cell lines were repeated; at that point, stocks were prepared for utilization. DMSO (MERCK 67-68-15) was placed in medium-containing cryotubes and kept at a temperature of -150°C (PANASONIC). All experiments began at the fifth passage of the cell lines and ended at the fifteenth passage. In this study, cells were standardized for proteomic analysis with three independent replicates on samples obtained from cell lines and grown under the same conditions. This process was designed to increase the reliability of the analyses and aimed to achieve statistical significance of the obtained data.

2.2. Determination of glycoproteins by proteomics study (LC-QTOF/MS)

First, urea solution (i.e., DTT) was added to the proliferating cells, and incubated at room temperature for a few hours to denature them. To prevent the formation of disulfide bonds, 1.5 µL of 200 mM iodoacetamide (IAA) was added, and the mixture was left to sit at room temperature in the dark for an hour. To reduce the urea content, 20 µL of 50 mM ABC/2 mM $CaCl_2$ was added to 100 µL of the reaction mixture. To separate the glycans, 2 µL of the PNGaseF enzyme was added, and the samples were incubated for 3-4 hours at 37ºC. The proteins were broken down into small pieces by trypsinization, which was followed by an overnight (16-18 hours) incubation at 37ºC for digestion. (An example would be to add 12.5 µL of 0.2 µg/µL trypsin solution to 100 mg of total protein to create a 1:40 ratio. After incubation, 10% TFA was added to stop the reaction. Purification was carried out in the SEP-PAK C18 solid-phase extraction system. Proteomics analysis was performed with three independent replicates from each cell line. The cartridge column was conditioned with methanol and 20 mM ammonium acetate solution. The elution was carried out with 20%-40%-75% acetonitrile: water (v:v), fractions were collected and evaporated under nitrogen and solubilized with 1% TFA. When the obtained data were evaluated on a repeat and fraction basis, it was aimed to obtain statistically significant results. Proteomics analysis was performed with Liquid Chromatography-Time-of-Flight Mass Spectrometry (AB-Sciex 4600 QTOF). The parameters of the method are presented in Table 1. The samples were injected into the LC-QTOF/MS system and the fragmentation ions were characterized by analyzing with high efficiency. There are many different interfaces available for proteomics analysis. However, in this study, SearchGUI (Compomics, 2024) was chosen as a search interface because it integrates various search tools (Fan, et al., 2024) into a single platform, rather than functioning as a database. By setting the search parameters, data was made available for search using various definition software techniques for the examination and interpretation of the collected data. SearchGui is an interface that allows the comparison of results using multiple search engines simultaneously and provides more reliable results. It offers advanced features for the integration and analysis of the obtained data. Precursor and product mass tolerance is applied at 10 ppm and carbamidomethylation of C (Cystine) is a fixed modification and oxidation of M (methionine) is a variable modification. In this study, a 1% False Discovery Rate (FDR) was applied to the SearchGui interface to increase the reliability of the proteins obtained by controlling the rate of false positive results, reduce the number of false positives, reveal proteins with real biological meaning more clearly and increase the statistical power of the analysis. For the processing and interpretation of search results; the PeptideShaker interface was used which contains post-translational modification (PTM) and peptide sequence matching (PSM) in the verification process. In addition, the PeptideShaker applies a comprehensive validation process to increase the reliability of the peptide and protein results obtained, reduces the false positive rate, helps to reveal results with real biological meaning, and allows researchers to easily visualize and understand the results thanks to its user-friendly interface (Compomics, 2024). In addition, the obtained analysis results allowed the comparison of the expression levels of certain proteins and contributed to the emergence of biologically significant findings.

Table1
Proteomics method and LC-QTOF parameters.

High-performance liquid chromatography parameters for proteomics method	
System	Agilent 1260 (Autosampler, Binary pump, Column Oven, Solvent Cabin)
Column	300SB-C18 column (Agilent Technologies) with 50 mm×1.0 mm, 3.5 µm particle size
Mobile phase (A)	Formic acid in water (0.1 %)
Mobile phase (B)	Formic acid in acetonitrile (0.1%)
Autosampler temperature	4°C
Flow rate Gradient:0 min. %5 B, 8.0 min. %5 B, 40 min. %40 B, 41 min. %80 B, 55 min %80 B, 56 min. %5 B, 75 min. %5 B	0.12 mL·min^{-1}
Column temperature	35°C
Injection volume	5 µl
Total run time	75 min
High resolution mass spectrometry parameters for proteomics method	
System	AB-Sciex 4600 QTOF
Ionization mode	Positive electrospray ionization
Ion source gas (1)	25 l\min
Ion source gas (2)	40 l\min
Curtain gas	30 l\min
Temperature	350°C
Ion spray voltage	5500 Volt
Declustering potential	120 Volt
Collision energy	35
Ms Method	300-2000 mass range
MsMs	IDA experiment, 50-1750 mass range

3. Results and discussion

The discovery of various tumor markers and other proteins associated with cancer through genomic and clinical studies has made the development of appropriate proteomic analyses of interest. It is of great importance to evaluate proteomic approaches for early diagnosis and treatment of cancer cells.

Table 2
Small cell lung cancer primary cell culture proteome: cancer-related proteins.

Group	Description	Chromosome	Gene Name	MW [kDa]	Molecular Function
Control	Ankyrin-3	10	ANK3	480,113	structural component of the cytoskeleton, cadherin binding, cytoskeletal protein binding
Control	Phosphatidylinositol 3-kinase regulatory subunit beta	19	PIK3R2	81,495	phosphotyrosine residue binding, protein heterodimerization, phosphatidylinositol 3-kinase regulatory activity, 1-phosphatidylinositol-3-kinase activity, 1-phosphatidylinositol-3-kinase regulatory activity, and protein binding—both protein phosphatase and protein binding are examples of these activities.
Control	Phosphatidylinositide phosphatase SAC2 (Fragment)	10	INPP5F	20,622	The enzymes that catalyze the breakdown of inositol monophosphate include phosphatase, phosphatidylinositol phosphate 4-phosphatase, phosphatidylinositol phosphate 5-phosphatase, protein binding, phosphatitol monophosphate 3-phosphatase, and phosphatidylinositol-4-phosphate phosphatase.
Control	Heat shock factor protein 1	8	HSF1	53,560	chromatin binding, sequence-specific single-stranded DNA binding, transcription repressor activity, protein binding, and STAT family protein binding.
Control	Vimentin	10	VIM	49,623	Double-stranded RNA binding, scaffold protein binding, keratin filament binding, protein domain-specific binding, structural molecule activity, and protein C-terminal binding are examples of structural cytoskeleton components.
Control	NFAT activation molecule 1	22	NFAM1	17,849	transmembrane signaling receptor activity
Control	Protein Shroom3	4	SHROOM3	207,922	actin filament binding
Control	ETS translocation variant 4	17	ETV4	48,120	Sequence-specific DNA binding, DNA-binding transcription activator activity and protein binding are all associated with the cis-regulatory region of RNA polymerase II.
Control	RBR-type E3 ubiquitin transferase (Fragment)	14	RNF31	102,332	transferase activity, protein binding, ubiquitin-protein transferase activity, and ubiquitin-protein ligase binding.
Control	Prelamin-A/C	1	LMNA	14,212	Similar protein binding and structural molecule action.
Control	Bromodomain-containing protein 8 (Fragment)	5	BRD8	31,591	transcription factor activity that binds DNA, nuclear receptor activity.
Control	Myeloblastin	19	PRTN3	27,789	protein binding, signal receptor binding, serine-type endopeptidase activity, and peptidase activity.
Control	Telomerase reverse transcriptase	5	TERT	126,916	protein nucleotidyltransferase activity, telomeric DNA binding, and protein C- and N-terminal binding.
Control	Mothers against decapentaplegic homolog 9	13	SMAD9	16,519	transcription factor activity specific to DNA binding, metal ion binding, protein binding, and RNA polymerase II specific.
Cancer	Alpha-2-HS-glycoprotein	3	AHSG	39,387	kinase inhibitor activity and cysteine type endopeptidase inhibitor activity.
Cancer	Nuclear receptor coactivator 6	20	NCOA6	219,008	nuclear receptor coactivator activity, transcription coactivator activity, retinoid X receptor binding, enzyme binding, chromatin binding, thyroid hormone receptor binding, protein binding.
Cancer	Vesicle-fusing ATPase	9	VCP	50,962	Protein-containing complex binding, ubiquitin-specific protease binding, hydrolase activity, RNA binding, and ubiquitin-like protein ligase binding are some of the binding mechanisms associated with the BAT3 complex.
Cancer	Mitochondrial import inner membrane translocase subunit TIM14	3	DNAJC19	5,333	protein binding
Cancer	Nucleolin	2	NCL	68,552	mRNA 5'-UTR binding, protein C-terminal binding, nucleic acid binding, telomeric DNA binding, DNA topoisomerase binding, protein, RNA, and identical protein binding.

Therefore, it was decided to examine protein differences by propagating HEL-299 (CCL-137™) and NCI-H209 lung cells under appropriate conditions and performing a proteomic analysis with the LC-QTOF/MS approach. After exporting the reports to Excel, an in-built tool was used to extract the percentage of each GO term in the Genome Ontology (GO) dataset for the cellular component of each protein. Unclassified proteins were validated by comparing with entries in Swiss-Prot, Human Protein Reference Database, and Bioinformatics Harvester to search for annotations for biological components.

The overlap between the proteins found in each cell line and between each cell line's three replicates was assessed using a method that was developed internally. Additionally, the Plasma Proteome Database was searched for all extracellular and membrane-bound proteins. According to the proteomics results, it was determined that 14 proteins found in the healthy lung cell HEL-299 were not found in the lung cancer cell line NCI-H209. These are ANK3, PIK3R2, INPP5F, HSF1, VIM, NFAM1, SHROOM3, ETV4, RNF31, LMNA, BRD8, PRTN3, TERT, SMAD9 proteins. In the lung cancer group, a total of 5 proteins were found to differ from those in healthy HEL-299 lung cells. These proteins are AHSG, NCOA6, VCP, DNAJC19, NCL. The results obtained are presented in Table 2.

Complex protein mixtures have traditionally been separated and compared using the proteomics approach as the main method. Nevertheless, there are significant variances in this method due to sample processing, protein loading, and gel staining (Kulasingam and Diamandis, 2007). Another disadvantage of 2DE in terms of proteomics is the inability to adequately recover proteins from the gel for MS. For this reason, techniques like multidimensional LC have been looked for as ways to enhance or replace 2DE (Sardana et al., 2008). High throughput protein identification is achieved using multidimensional LC-MS/MS analysis, in contrast to the relatively slow and laborious 2DE-MS/MS techniques. Through the analysis of complex protein combinations seen in biological fluids, tissues, or cell cultures, this technique has been utilized to find cancer biomarkers (Cho et al., 2007; Shaw et al., 2007; Li et al., 2023). The LC-QTOF/MS method was employed in this work to detect protein variations in two lung cell lines. Because lung cancer is a diverse disease, the secretomes of cell lines from various backgrounds were examined to better characterize the proteome of the disease and increase the possibility of finding biomarkers for its pathology (Gonzalez et al., 2021; Kawasaki et al., 2024).

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The AHSG protein exhibits distinct expression patterns in lung, colorectal, and breast cancers. Five potential biomarkers found in serum samples from lung cancer patients were analyzed using boxplots, and the median levels of the control group were compared to those in the small cell, adenocarcinoma, and squamous cell carcinoma. The AHSG levels of 55 lung cancer patients were found to be higher after the serum samples were analyzed (Petrik et al., 2008; Qiu et al., 2008).

It has been demonstrated that the 59 kDa glycoprotein AHSG, primarily produced in the liver, mediates growth signals in breast carcinoma cells (Ren et al., 2020). In contrast to the control group, the differential expression of AHSG was shown to be significant only in breast cancer in this study., and its abundance levels were also reduced. Petrik et al. (2008) used SELDI-TOF to find that in glioblastoma patients, AHSG is a peak that becomes less noticeable as tumor grade rises. Thus, in a separate cohort of 72 glioblastoma patients, AHSG was later verified as a survival marker in glioblastoma using ELISA.

It was found that across all cancer types examined, C3, which has a high AUC value, was elevated. Increased levels of this APP have been linked to cancer in the past, and C3 is a crucial part of the complement system in this study (Fang et al., 2020; Xing et al., 2023). Using gel electrophoresis, it was previously discovered that the serum of patients with pancreatic adenocarcinoma had higher levels of C3 than the sera of healthy individuals (Costantini et al., 2021).

The TCGA-LUAD database shows that AHSG expression is substantially greater in cancer tissues than in normal tissues. Additionally, a pan-cancer analysis identified aberrant AHSG expression in many tumor types. The expression of AHSG may be a separate predictor of overall survival in lung adenocarcinoma, according to a later study. According to survival analysis, patients in the low-expression group in the TCGA-LUAD database fared better overall than those in the high-expression group. The AHSG gene is involved in many physiological and pathological pathways in lung cancer cell lines. Xiong et al. discovered in their 2023 study that patients with lung cancer may use AHSG as a predictive indicator for OS. They also underlined the possibility that it is a therapeutic target and could sustain the biological behavior of lung adenocarcinoma (Zhou et al., 2021).

NCOA6 is mainly found in the colon of human fetal trophoblasts and also in endometrial transplants. Following NCOA6 knockdown, HTR-8/SVneo cell invasion and migration were markedly diminished, and transcriptional suppression led to a decrease in MMP9 secretion. Additionally, it was discovered that NCOA6 co-activates NF-κB-mediated MMP9 transcription. Furthermore, it was discovered that patients with early-onset sPE have altered NCOA6 expression in their placentas. The study's findings indicate that NCOA6 plays a crucial role in cytotrophoblast invasion and migration, possibly through its activation of NF-κB-mediated MMP9 transcription. Additionally, it has been discovered that NCOA6 downregulation may have a role in the pathophysiology of early-onset sPE (Wu et al., 2020).

Valosin-containing protein (VCP)/p97, a molecular chaperone of AAA ATPase, regulates critical cellular functions and protein processing. Furthermore, a recent study showed a relationship between the prognosis and course of non-small cell lung cancer (NSCLC) and the expression levels of VCP. Additionally, the precise function of VCP in the development and course of NSCLC was identified in this study. According to the study, VCP has also been shown to directly influence the levels of the proteins NFκB and p53, which may be a way to control the proliferation and advancement of tumor cells. The investigation further assessed the therapeutic potential of VCP inhibition and found that EerI therapy greatly inhibited the growth of NSCLC tumors in vitro and in xenograft mouse (athymic-naked) models. Thus, focusing on VCP in NSCLC might offer a fresh approach to raising p53 and NFκB levels, enhancing tumor development, and enhancing tumorigenesis—all of which could result in better clinical results (Costantini et al., 2021). Multiple driver oncogenes exist for NSCLC. Disease-associated protein DNAJC19 is a crucial part of the mitochondrial membrane translocation mechanism. The function of DNAJC19 in NSCLC cell proliferation and metastasis is described here. Immunohistochemistry (IHC) was used to investigate DNAJC19 expression in clinical samples of non-small cell lung cancer in this study. NCI-H1299 or A549 lung cancer cells were subjected to overexpression or knockdown experiments using lentiviral vectors. After their functionalities were evaluated, mouse xenograft and metastatic tumor models were created using DNAJC19-knockdown A549

cells. RNA-seq data, western blotting, PCR and IHC-based evaluations were performed. As a result, the study determined that DNAJC19 expression was higher in tumors than in non-cancerous tissues. A survival analysis revealed a correlation between lower DNAJC19 levels and higher progression-free survival. DNAJC19 was significantly decreased in terms of proliferation, survival, migration, and invasion by shRNA-mediated knockdown. Additionally, RNA-seq research revealed that the PI3K/AKT signaling pathway was involved in molecular events when shDNAJC19 was treated in A549 cells. By controlling PI3K/AKT signaling, DNAJC19 was discovered to significantly enhance NSCLC cell proliferation and lung metastasis, offering a potential therapeutic target for the treatment of NSCLC patients (Zhou et al., 2021).

Proteomics studies performed with LC-QTOF/MS analysis have some limitations. First of all, in proteomics analysis with LC-QTOF/MS, there may be difficulties in detecting low-abundance proteins, which may cause important biological information to be missed (Bache et al., 2018; Kim et al., 2020). In particular, proteins with post-translational modifications (e.g., phosphorylated and glycosylated proteins) may not be adequately represented in these analyses (Guan et al., 2019). In addition, chemicals used during proteomics analysis may have an effect on the integrity of the samples; the potential of some reactors or chemicals to disrupt protein structure may threaten the accuracy of the analysis results (Shin et al., 2021). Therefore, it is important to consider these limitations in future studies and discuss the potential effects in detail.

Based on the findings, this study fills a significant gap in the current literature and provides an innovative approach to proteomic profiling of small cell lung cancer. The applied methodology provides new insights into cancer biology by analyzing proteins that differ between both HEL-299 and NCI-H209 cell lines in detail. Furthermore, easy-to-use software interfaces and comprehensive analysis processes allow researchers to reliably evaluate the results. This innovative methodology provides a solid foundation for the discovery of potential biomarkers for future clinical studies and contributes to the development of personalized medicine approaches.

4. Conclusion

Although this study demonstrates that there is now a great deal more understanding of the molecular causes of cancer, there are still gaps in the knowledge of the disease's mechanisms and the creation of practical early detection and treatment plans. The most important element influencing a patient's ability to get successful therapy is an understanding of the pathophysiology of the illness in its later stages. Currently, it is standard procedure to use molecular techniques to diagnose biological samples based on many markers. Changes in these markers' concentrations can be detected to facilitate early diagnosis, prognosis prediction, monitoring of therapy response, and disease screening of high-risk people. According to the findings in this study, tiny cells may be biomarkers for lung cancer that can be detected early on. With the support of further studies, the use of target protein markers in the clinic can be made widespread.

Ethical approval: The development, acquisition, authentication, cryopreservation, and transfer of cell lines between laboratories were followed according to the guidelines published in the British Journal of Cancer, 2014.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

References

Ahamed, M. T., Forshed, J., Levitsky, A., Lehtiö, J., Bajalan, A., Pernemalm, M., ... & Andersson, B. (2024). Multiplex plasma protein assays as a diagnostic tool for lung cancer. *Cancer Science*, *115*(10), 3439-3454.

Atasever, S. (2024). Identification of potential *hub* genes as biomarkers for breast, ovarian, and endometrial cancers. *Front Life Sci RT*, 5(1), 7482.

Bache, N., Møller, H. D., Bjerregaard, M. R., & Müller, S. (2018). The impact of protein abundance on the detection of proteins in mass spectrometry. *Journal of Proteomics*, *177*, 1-9.

Chen, L., He, H., Wang, T., Wu, L., Xu, D., & Zhang, H. (2022). Mass spectrometry-based proteomics in cancer research: Challenges and opportunities. *Molecular Cancer*, *21*(1), 37.

Chen, X. P., Lu, Y. H., Xu, B., Wei, Y. X., Cui, X. L., Zhang, W. W., ... & Feng, C. G. (2024). Retention time-independent strategy for screening pesticide residues in herbs based on a fingerprint database and all ion fragmentation acquisition with LC-QTOF MS. *Analytical Methods*, *16*(45), 7831-7841.

Cho, C. K., Shan, S. J., Winsor, E. J., & Diamandis, E. P. (2007). Proteomics analysis of human amniotic fluid. *Molecular & Cellular Proteomics*, *6*(8), 1406-1415.

Compomics. (2024). PeptideShaker, https://compomics.github.io/projects/peptide-shaker Last accessed on December 25, 2024.

Costantini, S., Capone, F., Polo, A., Bagnara, P., & Budillon, A. (2021). Valosin-containing protein (VCP)/p97: A prognostic biomarker and therapeutic target in cancer. *International Journal of Molecular Sciences*, *22*(18), 10177.

Davies, M. P., Sato, T., Ashoor, H., Hou, L., Liloglou, T., Yang, R., & Field, J. K. (2023). Plasma protein biomarkers for early prediction of lung cancer. *EBioMedicine*, *93*.

Duffy, M. J., McDermott, E., & Clynes, M. (2023). Biomarkers for detection and treatment of cancer: A comprehensive review. *Clinical Cancer Research*, *29*(4), 655-663.

Fan, M., Liu, Q., Ma, X., Jiang, Y., Wang, Y., Jia, S., ... & Zhang, X. (2024). ZNF131-BACH1 transcriptionally accelerates RAD51-dependent homologous recombination repair and therapy-resistance of non-small-lung cancer cells by preventing their degradation from CUL3. *Theranostics*, *14*(18), 7241.

Fang, S., Wang, H., Lu, L., Jia, Y., & Xia, Z. (2020). Decreased complement C3 levels are associated with poor prognosis in patients with COVID-19: A retrospective cohort study. *International immunopharmacology*, *89*, 107070.

Gao, S., Xu, B., Sun, J., & Zhang, Z. (2024). Nanotechnological advances in cancer: Therapy a comprehensive review of carbon nanotube applications. *Frontiers in Bioengineering and Biotechnology*, *12*, 1351787.

Gasparri, R., Sabalic, A., & Spaggiari, L. (2023). The Early Diagnosis of Lung Cancer: Critical Gaps in the Discovery of Biomarkers. *Journal of Clinical Medicine*, *12*(23), 7244.

Ghosh, S., Bhan, M. K., & Ghosh, S. (2023). Trends in lung cancer incidence and mortality in India: A comprehensive review. *Asian Pacific Journal of Cancer Prevention*, *24*(2), 453-460.

Gonzalez, M., Guarino, V., & Friedrich, C. (2021). Proteomic analysis of lung cancer cell lines reveals unique secretome profiles. *Journal of Proteomics*, *240*, 104178.

Guan, P. P., Yu, X., Zou, Y. H., & Wang, P. (2019). Cyclooxygenase-2 is critical for the propagation of β-amyloid protein and reducing the glycosylation of tau in Alzheimer's disease. *Cellular & molecular*

immunology, 16(11), 892-894.
Kawasaki, T., Takeda, Y., & Kumanogoh, A. (2024). Proteomics of blood extracellular vesicles in inflammatory respiratory diseases for biomarker discovery and new insights into pathophysiology. *Inflammation and Regeneration, 44*(1), 38.
Kim, H., Lee, C., & Park, J. (2020). Challenges in proteomic analysis: The importance of sample preparation. *Proteomics, 20*(10), 1900076.
Kalluri, R., & LeBleu, V. S. (2022). The biology of the extracellular matrix: Role in cancer and therapy. *Nature Reviews Cancer, 22*(12), 753-767.
Kulasingam, V., & Diamandis, E. P. (2007). Proteomics analysis of conditioned media from three breast cancer cell lines: a mine for biomarkers and therapeutic targets. *Molecular & cellular proteomics, 6*(11), 1997-2011.
Lee, J., Kim, H., & Hwang, S. (2023). Emerging trends in cancer proteomics: Applications and innovations. *Journal of Proteomics, 250*, 104304.
Li, S., Qu, Y., Liu, L., Zhang, X., He, Y., Wang, C., Guo, Y., Yuan, L., Ma, Z., Bai, H., & Wang, J. (2023). Comparative proteomic profiling of plasma exosomes in lung cancer cases of liver and brain metastasis. *Cell & Bioscience, 13*(1), 180. https://doi.org/10.1186/s13578-023-01112-5.
Liu, Y., Zhang, Y., & Wang, H. (2022). Proteomics in cancer research: Opportunities and challenges. *Nature Reviews Cancer, 22*(6), 325-339.
Liu, Q., Zhang, J., Guo, C., Wang, M., Wang, C., Yan, Y., & Zhang, P. (2024). Proteogenomic characterization of small cell lung cancer identifies biological insights and subtype-specific therapeutic strategies. *Cell, 187*(1), 184-203.
Lung Cancer Cohort Consortium (LC3) (2023). The blood proteome of imminent lung cancer diagnosis. *Nature Communications, 14*(1), 3042. https://doi.org/10.1038/s41467-023-37979-8.
Matsuda, K., Ueha, S., & Mizuguchi, H. (2020). Recent advances in proteomic technologies for cancer research. *Cancer Science, 111*(6), 1950-1962.
Meyer, C., Baessler, A., & Schmid, J. (2020). Protein-based biomarkers in cancer: Recent advances and clinical applications. *Clinical Cancer Research, 26*(24), 6151-6160.
Nadaf, S. J., Killedar, S. G., Kumbar, V. M., Bhagwat, D. A., & Gurav, S. S. (2022). Pazopanib-laden lipid based nanovesicular delivery with augmented oral bioavailability and therapeutic efficacy against non-small cell lung cancer. *International Journal of Pharmaceutics, 628*, 122287.
Petrik, V., Saadoun, S., Loosemore, A., Hobbs, J., Opstad, K. S., Sheldon, J., & Papadopoulos, M. C. (2008). Serum α2-HS glycoprotein predicts survival in patients with glioblastoma. *Clinical Chemistry, 54*(4), 713-722.
Pérez-Rodríguez, M. L., González, R. A., & Martínez, A. (2021). The role of protein alterations in cancer progression: Insights from tumor development phases. *Nature Reviews Cancer, 21*(9), 617-630.
Ren, Q., Zhu, P., Zhang, H., Ye, T., Liu, D., Gong, Z., & Xia, X. (2020). Identification and validation of stromal-tumor microenvironment-based subtypes tightly associated with PD-1/PD-L1 immunotherapy and outcomes in patients with gastric cancer. *Cancer cell internat., 20*, 1-13.
Qiu, Y., Patwa, T. H., Xu, L., Shedden, K., Misek, D. E., Tuck, M., & Lubman, D. M. (2008). Plasma glycoprotein profiling for colorectal cancer biomarker identification by lectin glycoarray and lectin blot. *Journal of Proteome Research, 7*(4), 1693-1703.
Rusch, V., Asamura, H., & Wang, H. (2020). The role of low-dose computed tomography in lung cancer screening: A review. *Journal of Thoracic Oncology, 15*(2), 198-205.
Sardana, G., Jung, K., Stephan, C., & Diamandis, E. P. (2008). Proteomic analysis of conditioned media from the PC3, LNCaP, and 22Rv1 prostate cancer cell lines: Discovery and validation of candidate prostate cancer biomarkers. *Journal of Proteome Research, 7*(8), 3329-3338.
Schumacher, T. N., Klebanoff, C. A., & Gajewski, T. F. (2021). Cancer immunoediting and the tumor microenvironment: An overview. *Nature Reviews Immunology, 21*(10), 569-581.
Shaw, J. L., Smith, C. R., & Diamandis, E. P. (2007). Proteomic analysis of human cervico-vaginal fluid. *Journal of Proteome Research, 6*(7), 2859-2865. https://doi.org/10.1021/pr0701658.
Shin, T. H., Nithiyanandam, S., Lee, D. Y., Kwon, D. H., Hwang, J. S., Kim, S. G., ... & Lee, G. (2021). Analysis of nanotoxicity with integrated omics and mechanobiology. *Nanomaterials, 11*(9), 2385.
Vu, H. M., Mohammad, H. B., Nguyen, T. N., Lee, J. H., Do, Y., Sung, J. Y., & Kim, M. S. (2023). Quantitative proteomic analysis of bronchoalveolar lavage fluids from patients with small cell lung cancers. *PROTEOMICS-Clinical Applications, 17*(5), 2300011.
Wang, H., Zeng, Y., & Liu, C. (2021). Advancements in liquid biopsy for lung cancer: Opportunities and challenges. *Cancer Letters, 508*, 45-53.
Wang, L., Yang, Y., & Zhou, Y. (2023). Current trends in cancer proteomics: Implications for early diagnosis and therapy. *Frontiers in Oncology, 13*, 814253.
Wu, L., Zhao, K. Q., Wang, W., Cui, L. N., Hu, L. L., Jiang, X. X., ... & Sun, Y. P. (2020). Nuclear receptor coactivator 6 promotes HTR-8/SVneo cell invasion and migration by activating NF-κB-mediated MMP9 transcription. *Cell Proliferation, 53*(9), e12876.
Xing, Y., Zhang, D., Fang, L., Wang, J., Liu, C., Wu, D., ... & Min, W. (2023). Complement in Human Brain Health: Potential of Dietary Food in Relation to Neurodegenerative Diseases. *Foods, 12*(19), 3580.
Xiong, J., Chen, M., & Wang, Q. (2023). Advances in understanding the tumor microenvironment: Implications for lung cancer therapy. *Nature Reviews Clinical Oncology, 20*(1), 15-32.
Yanar, S., Kasap, M., Kanli, A., Akpinar, G., & Sarihan, M. (2023). Proteomics analysis of meclofenamic acid-treated small cell lung carcinoma cells revealed changes in cellular energy metabolism for cancer cell survival. *Journal of Biochemical and Molecular Toxicology, 37*(4), e23289.
Ye, Q., Raese, R., Luo, D., Cao, S., Wan, Y. W., Qian, Y., & Guo, N. L. (2023). Microrna, mrna, and proteomics biomarkers and therapeutic targets for improving lung cancer treatment outcomes. *Cancers, 15*(8), 2294.
Zhang, Y., Li, S., & Chen, L. (2021). Integration of proteomics and genomics in cancer research: A new perspective. *Nature Reviews Cancer, 21*(5), 307-322.
Zhang, Y., Zhang, X., & Zhao, J. (2022). Emerging protein biomarkers for cancer diagnosis and prognosis. *Nature Reviews Clinical Oncology, 19*(3), 166-182.
Zhao, J., Li, H., & Wang, C. (2023). Application of proteomic technologies in cancer diagnosis and therapy. *Journal of Proteomics, 266*, 104560.
Zhou, J., Peng, Y., Gao, Y. C., Chen, T. Y., Li, P. C., Xu, K., Liu, T., & Ren, T. (2021). Targeting DNAJC19 overcomes tumor growth and lung metastasis in NSCLC by regulating PI3K/AKT signaling. *Cancer Cell International, 21*(1), 338.

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Research article

Occupational health and safety in a hazardous waste disposal facility using Industry 4.0 technologies: A Fine Kinney risk analysis

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Abstract

Considering the environmental and human health impacts of rapidly growing industries and the uncontrolled increase in the amount of waste they generate; hazardous waste disposal facilities play a critical role in protecting public health. Hazardous waste disposal facilities include various multidisciplinary systems such as landfills, biogas plants, laboratories, medical waste landfills, wastewater plants, and incineration plants. This study aims to examine each facility in a sample hazardous waste disposal facility in terms of occupational health and safety to identify risks, and to offer recommendations for solutions. The sample facility was visited, and each section was examined separately, and preliminary information was obtained using a checklist. In light of the preliminary information obtained, risk analysis was performed and the results were presented with recommendations. Fine Kinney Risk Assessment method was preferred because it is more comprehensive than other risk analysis methods based on probability and severity and can adapt to multiple disciplines. In addition, it is stated that automated processes within the scope of Industry 4.0 can have a positive impact on the prevention of occupational accidents by leading to a reduction in human work and thus a reduction in worker-waste contact. As a result of the risk assessment, 68 risks were identified in the facilities visited, most of which were substantial.

Keywords: *Fine Kinney; industrial revolutions; hazardous waste; occupational health and safety*

1. Introduction

The increase in hazardous waste, which has become a major problem all over the world, poses a threat to environmental health if it is disposed of uncontrolled, as well as a threat to public health and therefore worker health. It is accepted that the importance of the concept of hazardous waste management can be understood by looking at the laws and regulations that have come into force in recent years, especially in developed countries. The participation of the European Union (EU) member countries in the Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal, which regulates the increase and disposal of hazardous wastes, which is considered a significant problem globally, has revealed that this issue is an important problem worldwide. Türkiye has internationally emphasized that it attaches importance to this issue by participating in this agreement in 1994 (Taser and Erdogan, 2010; Akkoyunlu et al., 2017).

In this period of accelerating industrial and technological progress, research into the management and disposal of hazardous waste is becoming increasingly important. The uncontrolled growth of fast-growing industries and the wastes

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generated by these industries pose serious risks to the environment and human health. At this point, the study and improvement of hazardous waste disposal facilities from an occupational health and safety point of view is of great importance (Gokcan et al., 2022; Harikaran et al., 2023). The process carried out by each facility presents different risks due to the nature of the work. For this reason, the study discusses in detail the operation and characteristics of each part of the facility, makes a risk assessment, and proposes solutions.

1.1. Concept of Industry 4.0

Industry 4.0 is considered to be the final point of industrial revolutions from the past to the present. The Fourth Industrial Revolution is one of the defining industrial and technological dynamics of today's working world. The First Industrial Revolution paved the way for mechanization and the widespread use of steam-powered machines, transforming an agrarian society into an industrial one. Then came the Second Industrial Revolution, when production processes became more automated, and productivity increased with innovations such as electricity and assembly lines. The Third Industrial Revolution led to a radical transformation of the business world with the spread of computers and digital technologies. Today, Industry 4.0 has led to significant changes in production processes with the combination of technologies such as digitalization, artificial intelligence, the Internet of Things, and automation (Celik and Can, 2019). This new industrial era aims to increase efficiency by making production processes more flexible and intelligent (Harmanci, 2024). Hazardous waste management facilities are an important example of the technologies used in Industry 4.0. While these facilities use technologies such as automation, artificial intelligence, big data analytics, and internet-connected devices to make production processes more intelligent, flexible, and efficient, they also create new types of risks in the area of occupational health and safety. These technologies, which form the basis of Industry 4.0, also have an impact on the risks and hazards faced by workers in hazardous waste management facilities. With the increase in human-robot interaction, the working conditions and safety requirements for workers are also changing. In this context, the impact of Industry 4.0 on occupational health and safety management in hazardous waste facilities should be closely examined. Traditional occupational safety methods and standards should be kept up to date to adapt to the new safety requirements brought about by Industry 4.0 (Caner, 2021; Oluk et al., 2023).

1.2. The concept of occupational health and safety

The needs of human beings have changed since the first day they appeared on Earth. The need for security is frequently encountered as one of the most basic needs. According to the Theory of Needs established by the American psychologist Maslow in 1943, human needs are defined as physiological needs, security needs, the need for belonging and love, the need for respectability, and the need for self-realization (Coban and Ozdemir, 2020). Given these needs in today's working world, it is considered the duty of occupational health and safety professionals to ensure that people who have to work and produce to sustain their lives are able to exist in the working environment in the best possible way, both physically and psychologically (Baskan Takaoglu et al., 2018; Oluk et al., 2022). According to the Occupational Safety and Health Law No. 6331, published in the Official Gazette in the year 2012, an occupational accident is defined as "an event that occurs in the workplace or as a result of the performance of work that causes death or mental or physical disability." Occupational health and safety is defined as the science of eliminating the health hazards and risks to which workers are or will be exposed during the performance of work because of the environmental conditions in the workplace, and reducing those hazards that cannot be completely eliminated because of the performance of work (Adsoy, 2020).

1.3. Concept of waste

The concept of waste was first legally defined in Environmental Law No. 2872, published in 1983, as "any substance that is generated, discarded or left in the environment as a result of any activity" in Türkiye. Furthermore, according to the "Waste Management Regulation" published in the Official Gazette in 2015, waste is defined as "any substance or material that is discarded or left in the environment by the producer or the natural or legal person who is in actual possession of it, or which is required to be discarded" (Aylanc, 2022).

1.4. Classification of waste

Waste is classified in the literature according to various criteria. These criteria include factors such as consumption habits, production processes, technical properties, chemical composition, physical properties, sources, origin, degree of hazard, and potential hazards (Tenikler, 2007). Waste interacts with the environment in which they are left, and as a result of this interaction, they can have positive or negative effects. It is possible to analyze waste in two groups as hazardous and non-hazardous according to their effects (Ercan, 2016).

Hazardous waste is defined as waste that has adverse effects on the environment and human health. These wastes present different hazards depending on their biological, chemical, and physical properties. For example, substances such as acids, lead, mercury, and arsenic compounds are included in this group. Hazardous wastes are substances that can be dissolved in water or transported in gaseous form, can be absorbed in the short term through respiration, digestion, or the skin, and can cause acute toxicity or chronic toxicity over time. These substances have carcinogenic or teratogenic effects and may pose a threat to the environment by inhibiting biological activities. Due to the negative effects of both human and environmental health effects, proper management of hazardous waste is important for the protection of the environment and human health (Durczak et al., 2024; Sikder et al., 2024).

Non-hazardous wastes are organic and inorganic wastes that are not considered harmful or hazardous wastes. Municipal solid wastes (MSW), food waste, cardboard, paper, ash, glass, plastic, and construction waste are included in this group. According to another definition, non-hazardous waste is waste that is not considered legally hazardous and can be recovered, stored, disposed of, or incinerated by separation within the scope of services provided by municipalities (Tenikler, 2007).

1.5. Waste management

In the Waste Management Regulation 2015, published in the Official Gazette, waste management is defined as "prevention of waste generation, reduction at source, re-use,

separation according to its characteristics and type, accumulation, collection, temporary storage, transport, interim storage, recycling, recovery including energy recovery, disposal, monitoring, control, and post-disposal control activities." Waste management is a process that includes the collection, transport, recovery, disposal, and treatment of waste, as well as post-disposal site controls (Ercan, 2016).

The fundamental objective of the waste management hierarchy (Fig. 1) is to recycle and recover energy from waste materials in an optimal manner, while concurrently minimizing any potential risks to human health and the natural environment (EPA, 2020; Sahin et al., 2021).

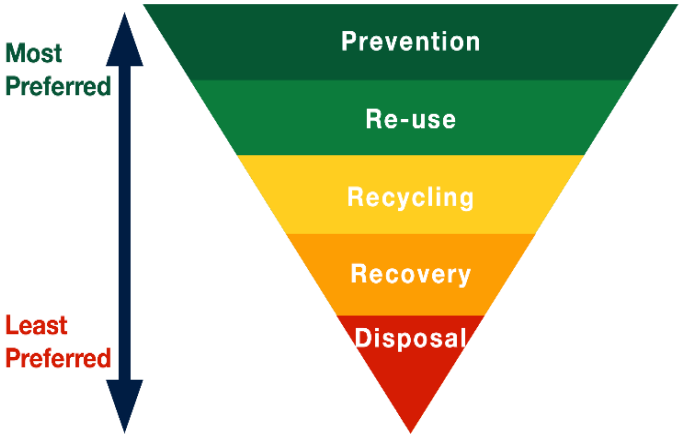

Fig. 1. Hazardous waste management hierarchy.

2. Materials and methods

The location of different hazardous waste disposal facilities in Türkiye, waste disposal and storage capacities, the number of waste disposal methods used in the facility, the operations carried out in other departments within the facility, the use of Industry 4.0 systems, and the size of the waste disposal capacity were taken as criteria for the selection of the facility to carry out the field study. Contact was established with the facility found suitable for the study. Prior to the risk assessment, the field study team obtained preliminary information by completing a checklist form. Subsequently, an inspection was conducted of the preceding accident reports of the enterprise, the occupational health and safety training documents of the employees, additional occupational health and safety forms, the control and maintenance documentation of the machinery and equipment utilized within the facility, the material safety data sheets and the preceding risk assessment reports.

The Fine Kinney method was employed as the risk assessment methodology (Kokangul et al., 2017). A risk assessment was conducted for each department based on the operations performed within the facility. The primary objective of conducting a risk assessment for each department individually is to ascertain the comparative risk and risk level between the departments and to mathematically quantify the extent to which the risks inherent to the nature of the operations performed affect the risk levels. A total of ten areas were examined in eight main sections and two sections within eight main sections at the facility in question. These areas included the site general area, the hazardous waste incineration plant and bunker area, the biogas plant, the landfill areas, the interim storage areas, the laboratory, the landfill leachate treatment plant, the medical waste plant, and the sterilization area.

The Fine Kinney risk analysis method is a qualitative risk assessment tool that enables the evaluation of past risk assessment data for enterprises and the current status of facilities following the implementation of corrective and preventive actions. The Fine Kinney method enables the attainment of more reliable and realistic results than those obtained through the use of other commonly employed matrix risk analysis methods. This is achieved by incorporating the probability and severity of damage, in addition to the frequency of exposure to hazardous areas or situations, into the risk score calculation (Oluk et al., 2023).

According to the Fine-Kinney Method, the risk level increases depending on three factors:

(P) Probability: The probability that the damaging event will occur.
(F) Frequency: The frequency of exposure to the harmful event.
(S) Severity: Possible consequences of the event.

For risk calculations, numerical values were assigned to these factors and the risk score was formulated by multiplying these values (Mogan and Gungor, 2023).

Risk=Probability x Severity x Frequency (Birgoren, 2017).

In accordance with the findings of the risk assessment and

Table 1
Probability, frequency, and severity grading according to the Fine Kinney method (Aker, 2020).

Probability Value	*Possibility (Probability of damage occurring)*	*Frequency Value*	*Frequency (Frequency of exposure to hazard over time)*	*Violence Value*	*Violence (Estimated harm to humans and/or the environment)*
10	Expected / Certain	**10**	Almost constantly, several times an hour	**100**	Multiple fatal accidents, environmental disaster
6	High, quite possible	**6**	Frequently, once or several times a day	**40**	Fatal accident, serious environmental damage
3	Possible	**3**	Occasionally, once or several times a week	**15**	Permanent damage, injury, job loss, environmental impairment
1	Low, but it is possible	**2**	Not often, once or a few times a month	**7**	Significant damage/injury, need for external first aid
0.5	Unexpected, but it is possible	**1**	Infrequently, a few times a year	**3**	Minor damage/injury, need for internal first aid
0.2	Unexpected	**0.5**	Frequently, a few times a year	**1**	Cheap bypass, no environmental damage

the established hierarchy of priorities, corrective measures are identified and implemented with respect to the identified hazards.

Table 2
Evaluation of the risk score according to the Fine Kinney method and the time allowed for correction (termination) (Aker,2020).

Risk Value	Risk Assessment Result	Time Allowed for Correction
400<R	Intolerable Risk The situation is reported to the employer as soon as possible so that he can take precautions.	In less than 1 month
200<R<400	Substantial Risk It should be improved in the medium term.	Within 1-3 months
70<R<200	Significant Risk It should be improved in the long term.	Within 6 months
20<R<70	Possible Risk It should be administered under supervision.	In 1 year
R<20	Insignificant Risk Prevention is not a priority.	Control

4. Results

Distribution percentages of the facility according to risk levels are given (Fig. 2). Of the risks identified;

1% at negligible (insignificant) risk level and with the explanation of "precaution is not prioritized",

18% with a possible (low) risk rating and the statement "should be implemented under supervision",

40% with the statement "should be improved in the long term (within 6 months)" in the degree of significant (medium) risk,

41% with the definition of "should be improved in the medium term (within 1-3 months)" in the substantial (high) risk rating and no extreme (intolerable) risk has been identified.

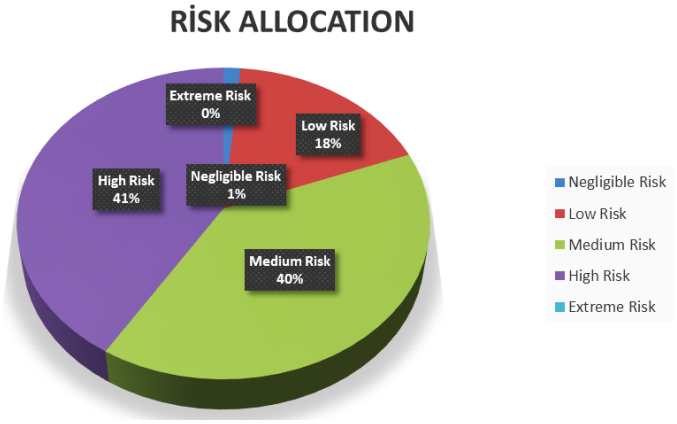

Fig. 2. Distribution of risk rating in general risk.

Of the 68 risks identified at the facility, only one was deemed to be insignificant, and this risk was observed to be present throughout the site. At the time of writing, the facility's open and closed areas are equipped with separate pedestrian and vehicle routes. Furthermore, the issue was communicated to facility personnel during occupational health and safety training sessions. It is evident that clear instructions are provided for employees and other individuals within the facility. It is prohibited for pedestrians to utilize other routes or traverse the facility except within the boundaries delineated on the accompanying map. Furthermore, the facility has developed a safety guide for third parties who will be entering the premises.

The guide contains a set of regulations and an explanation of the required standards of conduct within the facility. The speed limit within the facility is set at 20 km h^{-1}, and vehicles are positioned in a manner that does not impede emergency egress. A risk assessment of the facility identified that 25% of the risks that could potentially pose a hazard were associated with the acceptance of waste, 17% were linked to the hazardous waste incineration plant, 9% were related to the biogas plant, 8% were associated with the laboratory, 8% were connected to the wastewater treatment plant, 8% were linked to the medical waste plant, and 8% were associated with the interim storage facility. Upon examination of the risks identified through the risk assessment conducted at the facility, it becomes evident that there are potential hazards associated with vehicle accidents or the overloading of waste to vehicles during the acceptance of waste at the facility. In the present circumstances, it is evident that technical measures, such as protective railings against vehicle overturning, have been implemented to mitigate the risks associated with vehicle accidents at the facility.

Prior to entering the facility, vehicles loaded with waste are subject to inspection and weighing in accordance with the relevant licensing requirements. Only vehicles that meet the requisite standards are permitted to enter the facility. The studies conducted throughout the facility have revealed that landfill gases are kept to an acceptable level through the implementation of continuous monitoring for potential health risks, including eye irritation, respiratory tract irritation, and odor disturbances caused by ventilation conditions. Similarly, as gas is generated as a combustion byproduct in the hazardous waste incineration facility, the output gas is monitored and regulated through secondary measurements. The facility employs electrostatic filter units (ESF), which collect and remove particulate matter in the plant air and other particulate matter (PM) that may pose a health risk to employees and contribute to a pneumatic atmosphere due to the magnetic field within the units. Additionally, these ESF units regulate excessive heat and humidity. The personnel have undergone training on the potential hazards associated with chemical exposure and have been furnished with the requisite personal protective equipment. Furthermore, the facility is equipped with Material Safety Data Sheets (MSDSs) and emergency eye and body showers. Considering the aforementioned factors, while the potential consequences of the identified risks are deemed to be significant, the probability and frequency values are determined to be relatively low.

Consequently, these risks are classified within the probable risk group. A total of 22% of the risks identified at the facility are classified as significant and are distributed across the site, with 18% located in the hazardous waste incineration plant, 13% in the biogas plant, 13% in the bunker section, 9% in the waste leachate treatment plant, 9% in the medical waste plant, 4% in the acceptance of waste to the facility, 4% in the landfill facility, 4% in the laboratory and 4% in the sterilization section. Upon examination of the results of the risk assessment, it becomes evident that several significant risks are associated with chemical exposures and the performance of tasks in dusty environments during the processes of waste analysis and

sampling. The frequency values were determined to be medium level since the employees in question perform these operations with greater frequency than other processes in the facility, namely individually. As a result, the risk scores were determined to be "significant risk," but not "substantial risk" or "intolerable risk." In the plant, the burner is designed to operate on fuels that do not result in higher emissions than natural gas. If the burner exceeds the range of 850°C or 1100°C at start-up or shutdown, it will not engage in combustion or chemical reactions that may occur due thigh-temperature operation during waste feeding in the hazardous waste incineration plant. In addition, unwanted combustion reactions are prevented by flap covers that are opened and closed during and after waste feeding in the bunker.

Therefore, although the severity of the risks arising from these processes is high, the probability and frequency values were determined at a low-medium level and a "significant risk" conclusion was reached. It was determined that 30% of the risks at the facility at the level of major risk were at the hazardous waste incineration plant, 19% at the landfill, 15% at the site, 7% at the biogas plant, 7% at the laboratory, 7% at the landfill leachate treatment plant, 7% at the bunker section, 4% at the medical waste facility and 4% at the interim storage facility. It was observed that the major risks were mostly due to the operations at the hazardous waste incineration plant, followed by the landfills and the site in general. The fact that the waste accepted before the waste feeding in the facility is in a mixture or that the content information is not clear, and the waste gives an unexpected reaction poses a threat to worker health. It is anticipated that this will result in an increase in the probability parameter in the risk rating. However, the facility currently categorizes mixed wastes with unclear content information as the most hazardous waste. Furthermore, it was observed that separate pools had been created for different waste types in order to prevent any potential reactions in the bunker area. Therefore, although the frequency of occurrence of this process and the potential damage that could result from an adverse event is classified as medium-high, the probability was determined to be low-medium, and the risk was determined to be a level that can be improved upon within a period of 1-3 months.

The incineration of hazardous waste is subject to continuous monitoring in the facility's control room, with subsequent measurements taken. The potential for explosive methane gas accumulation in the landfill areas within the facility, if uncontrolled, could result in significant adverse reactions. To mitigate this risk, methane drainage pipes have been installed between the waste mounds. This process prevents the accumulation of methane and allows the release of the gas into the atmosphere under favorable conditions. Ultimately, it has been observed that the facility is equipped with the necessary infrastructure to mitigate the risk of injuries that may arise from medical waste, particularly sharp or piercing medical waste. This includes the provision of appropriate-thickness bags to contain the waste and prevent injury.

Additionally, the facility has the necessary measures in place to protect against exposure to biological agents, which could result from such injuries. Furthermore, the necessary personal protective equipment has been made available for personnel working in this section. The existing protection measures have reduced the probability of occurrence of the risk in question. Physical risk factors, including noise, vibration, and ergonomic conditions, as well as risks associated with working at heights during maintenance and cleaning of machinery and equipment, are prevalent in the facility.

The facility has implemented personal protective equipment for the aforementioned risks, handrails to prevent falls, and areas made of noise-absorbing material. However, the ergonomic appropriateness of the office workers in the control room, who are constantly sitting and working with multiple screens, has been identified as a concern. It is therefore recommended that improvements be made within a period of 1-3 months.

5. Discussion

In this study, similarly, a risk analysis study was carried out in a hazardous waste disposal facility with a biogas plant, and the risk of exposure to biological and chemical agents arising from working with plant and animal wastes was determined in this facility, and in addition, solutions were offered for the risk of infection.

Risk assessment was carried out at three different hazardous waste disposal facilities using the SLRA method (Ercan, 2016). In the selection of the risk assessment method used in the study, it was stated that the SLRA method was preferred because it is simple and understandable, and it has the opportunity to evaluate the consequences of hazardous events, employee health and safety, and financial losses. In the study, risk assessments were made separately for the three facilities, and the existence of different risk types in facilities with similar operations was emphasized. 96 risks were identified in the first facility, 84 risks in the second facility, and 74 risks in the third facility. In the first facility, 30% of the total risks were due to hazardous waste disposal operations, 28% were due to site-wide risks, and interim storage of hazardous waste accounted for 17%, followed by waste analysis, waste entry, and other risks. It is stated that the significant risk difference of the risks in the first facility compared to the other facilities is due to the fact that energy production with biogas is also carried out in this facility.

In the second facility, the risks arising from the disposal of hazardous wastes are 33%, and general risks are 32%. Interim storage, analyzing the waste, entry of the waste to the facility, and risks in other facilities constitute the remaining risks. In the third facility, the number of risks is lower compared to the other facilities since there is no disposal by incineration. In this facility, general risks constitute 38%, followed by interim storage of hazardous waste, disposal, analysis, and other operations. For all three facilities, hazardous events that may occur, risk levels, and urgency definitions were specified, and solution suggestions were presented.

Yapici (2012) addressed the current situation of hazardous waste recovery and disposal facilities in terms of occupational health and safety by conducting field studies in a total of 15 facilities in Turkey. Within the scope of the research, exposure measurements, surveys, and general risk assessment of the facilities were carried out. Of the 15 facilities in the study, 2 are hazardous waste interim storage facilities, 5 are hazardous waste recovery facilities, 3 are packaging waste recovery facilities, 1 is end-of-life tire recovery facility, and 4 are cement factories. In the survey study, a questionnaire consisting of 10 questions was directed to 15 employees in each facility. The survey results were handled on a question basis, and the participation rate and answers for each question were expressed in graphs. According to the observations and survey results, it has been emphasized that the level of awareness of the workers working in these facilities is low in terms of occupational health and safety, the company management is insufficient to provide the necessary

conditions for ensuring occupational health and safety, and occupational health and safety deficits in such facilities will indirectly cause environmental problems.

The previous study (Olcucu Sensoy, 2019) conducted a risk assessment of the potential hazards that may arise throughout the process, from the acceptance of waste to its disposal in a hazardous waste facility. It encompassed a comprehensive risk assessment across the entire facility, encompassing an in-depth evaluation of potential risk factors, occupational diseases, and biological factors. Furthermore, the significance of each risk factor was evaluated through a comparison of the 5x5 L-type matrix risk assessment method and the Fine Kinney method in risk assessment. Furthermore, the study revealed that a risk initially classified as category 1 in terms of importance according to the 5x5 L-type matrix method was subsequently categorized as category 3 when evaluated using the Fine Kinney method. It was highlighted that the incorporation of the frequency factor into the Fine Kinney method serves to reduce the degree of risk, contingent on the frequency with which the worker is exposed to the hazard in question.

Similarly, the Fine Kinney risk assessment method was selected for use in this study due to its incorporation of the frequency factor. Furthermore, it was emphasized in this study that the frequency of employee and work-related hazard exposure contact is included in the risk rating compared to other risk assessment methods, which will result in more accurate and healthier results.

A study (Mogan and Gungor, 2023) conducted an analysis of asbestos, a substance that is commonly found in residential, occupational, and industrial settings, including power plants, mines, and other structures. Asbestos is classified as a hazardous waste material. The potential risks associated with asbestos removal were evaluated using the 5x5 L-type matrix method. The evaluation yielded the following results: quarantine installation and dismantling, asbestos dismantling, post-dismantling hygiene works, and waste storage, and works were classified as high-risk. Ultimately, the engineering measures proposed were determined through the application of the risk control hierarchy. It was concluded that the most effective protection method is personal protective equipment.

A risk assessment was conducted by Demirel and Sert (2018) using the Preliminary Hazard Analysis (PHA) method in a plastic recycling plant that accepts waste from a variety of industrial sources, including the textile, automotive, machinery, and chemical industries. The plant handles a range of materials, including hazardous chemicals contaminated with plastic packaging and raw materials obtained from high-density polyethylene preparations. The PHA method was selected due to its ability to predict potential occupational safety deficiencies in a system prior to the occurrence of an accident. The risk assessment of the facility resulted in the categorization of risks into three groups: A-B, B-C and C-D. It was concluded that 49% of the identified risks require a review of the protection measures and practical solutions, 43% require a review of the protection measures and their implementation in the facility, and 8% can be continued with the existing protection measures.

A study examines hazardous waste management (HWM) in Turkey and highlights significant shortcomings in current practices, such as the dominance of landfilling over recycling and incineration. It notes that while incineration can detoxify hazardous waste, it poses health risks due to emissions. The study emphasizes the importance of accurate data collection, facilitated by a web-based monitoring system for hazardous waste tracking. It identifies the Marmara Region as the highest generator of hazardous waste and calls for improved administrative capacities, industry training, additional disposal facilities, and heightened public awareness. The article concludes with recommendations for enhancing HWM practices in alignment with European Union standards (Akkoyunlu et al., 2017).

Yildirim (2023) compared different risk assessment methods to provide a suitable working environment in terms of occupational health and safety in biogas production facilities. The advantages, disadvantages, and success rates of the risk assessment methods were analyzed. The study sought to answer the question of which risk assessment method is most appropriate for biogas plants. As a result of the evaluations, it was found that the FMEA (Failure Mode and Effects Analysis) method had the highest success rate for biogas plants. The Fine Kinney risk assessment method was ranked second. Although the L-Matrix method appears to be analyst-friendly, it is recommended that it is used in combination with other methods as it carries the risk of making mistakes that can lead to major accidents and occupational illnesses, and this method ranked third in terms of success rate. The X Matrix method was considered to have the lowest success rate due to the need for 5 years of accident history data and low scores in other criteria.

Another recent study conducted that the occupational health and safety of a solid waste facility in Gumushane Province was analyzed using the Elmeri method (Kulekci and Guvendi, 2024). The solid waste management process includes the stages of waste collection, transport, sorting, recycling, and disposal. In the study, the hazards that may occur in these processes (cutting, poisoning, fire risk, etc.) were identified and the risk levels were assessed. A total of 494 observations were made, of which 239 showed correct safety behavior, and 255 showed incorrect behavior. The highest safety index was calculated for the waste collection day shift at 57.2% and the lowest for the waste sorting day shift at 29.3%. The study draws some conclusions and recommendations based on the findings. Safety indices were generally lower for night shifts, and additional safety measures were recommended in this area. It also highlighted the need to improve tidiness and cleanliness standards, provide training on vehicle safety, and raise awareness of industrial hygiene issues. It is stated that the study should be considered as part of the continuous improvement process and that the implementation of the recommendations will increase the safety of employees.

6. Conclusion and recommendations

The findings of the study indicate that the Fine Kinney method is well-suited to the operations of hazardous waste disposal facilities. Furthermore, the Fine Kinney method also evaluates the hazard exposure of the employee due to the frequency of the operation, thereby providing more reliable results than other common risk analyses. Autonomous machines with self-control processes in the facility are programmed to cease operation or to initiate emergency measures automatically when they exceed the predetermined range values for their operations. This situation confers advantages in terms of occupational health and safety, as it reduces human-machine contact and the risks associated with human error.

In the study, the risks identified for each department in the plant were analyzed based on the processes of that department. The highest-scoring risks identified throughout the facility were

those pertaining to the hazardous waste incineration plant and landfill areas. The list was subsequently followed by that of the medical waste disposal plant and the biogas plant. The principal risks identified in this study are as follows: exposure to biological and chemical agents resulting from the disposal of contaminated waste; work machine accidents caused by waste acceptance and stacking operations; noise and vibration exposure; ignition of waste in hazardous waste incineration plants due to reaction or ignition sources; pneumatic gas exposure; toxic effects of harmful gases released after incineration and disposal; and odor exposure. No level of risk has been identified that could be considered "intolerable" in any part of the plant. This is due to the fact that, even when the severity value is determined to be high in risk assessments, the probability or frequency values are given a low rating as a result of the existing protection measures that have been put in place.

In conclusion, the study entitled "Occupational Health and Safety in a Hazardous Waste Disposal Facility Using Industry 4.0 Technologies: Fine Kinney Risk Analysis", The current occupational health and safety standards of the facility have been revealed by the Fine Kinney risk assessment method. This method offers to handle many disciplines at the same time and can adapt to many industries. It is therefore able to obtain healthier results since the parameters that make up the method include the frequency parameter compared to other risk analysis methods based on probability and severity. Furthermore, the study has shown that industrial machines with self-managing processes within the scope of Industry 4.0 provide advantages in terms of occupational health and safety. It is anticipated that the study will make a substantial contribution to the existing body of literature on the interaction between occupational health and safety and Industry 4.0 in the context of hazardous waste disposal.

Conflict of interest: The authors declare that they have no conflict of interests.

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References

Adsoy, C. (2020) Is sagligi ve guvenliginde calisanlarin yukumlulukleri ve haklari. Yuksek Lisans Tezi, Baskent Universitesi, Sosyal Bilimler Enstitusu, Ankara.

Aker, A. (2020). Metal sektorunde 5x5 matris ve Fine-Kinney yontemi ile risk değerlendirmesi. *Karaelmas Is Sagligi ve Guvenligi Dergisi*, *4*(1), 65-75.

Akkoyunlu, A., Avşar, Y., & Erguven, G. O. (2017). Hazardous waste management in Turkey. *Journal of Hazardous, Toxic, and Radioactive Waste*, *21*(4), 04017018.

Aylanc, C. (2022). Hukuki acidan atiklarin sinir otesi dolasimi, Yuksek Lisans Tezi, Dokuz Eylul Universitesi, Sosyal Bilimler Enstitusu, Izmir.

Baskan Takaoglu, Z., Celenk Kaya, E., & Olmezoglu Iri, N. (2018). Is guvenligi uzmanlarinin yasadigi sorunlar. *Gumushane Universitesi Saglik Bilimleri Dergisi*, *7*(2), 1-9.

Birgoren, B. (2017). Calculation challenges and solution suggestions for risk factors in the risk analysis method in the fine kinney risk analysis method. *International Journal of Engineering Research and Development*, *9*(1), 19-25.

Caner, V. (2021). Fiziksel risk etmenleri maruziyetine bagli ıs kazasi ve meslek hastaliklarinin onlenmesinde endustri 4.0 yaklasiminin degerlendirilmesi. *OHS Academy*, *4*(1), 55-61.

Celik, N., & Can, E. (2019). Endustri 4.0 sisteminde is sagligi ve guvenligi yonetim sistemi muhtemel problemleri ve cozum onerileri. *OHS Academy*, *2*(3), 119-126.

Coban, S., & Ozdemir, V. (2020). Muzik egitimcilerinin mesleki doyum duzeylerinin, clayton alderferin vig (erg) teorisine gore degerlendirmesi "II. Uluslararasi Istanbul Kodaly Egitim Gunleri" ornegi. *Fine Arts*, *15*(4), 217-232.

Demirel, S., & Sert, N. (2018). Bir plastik geri donusum tesisinde on tehlike analizi (pha) ile risk degerlendirmesi. *Nigde Omer Halisdemir Universitesi Muhendislik Bilimleri Dergisi*, *7*(2), 572-580.

Durczak, K., Pyzalski, M., Brylewski, T., Juszczyk, M., Leśniak, A., Libura, M., ... & Vaišnoras, M. (2024). Modern methods of asbestos waste management as innovative solutions for recycling and sustainable cement production. *Sustainability*, *16*(20), 8798.

EPA, (2020). Best practices for solid waste management: a guide for decision-makers in developing countries environmental protection agency, US.

Ercan, M. (2016). Tehlikeli atik bertaraf tesislerinin is sagligi ve guvenligi yonunden incelenmesi. Is sagligi ve guvenligi uzmanlik tezi, Calisma ve Sosyal Guvenlik Bakanligi, Is Sagligi ve Guvenligi Genel Mudurlugu, Ankara.

Harikaran, M., Boopathi, S., Gokulakannan, S., & Poonguzhali, M. (2023). Study on the source of e-waste management and disposal methods. In *Sustainable Approaches and Strategies for E-Waste Management and Utilization* (pp. 39-60).

Harmanci, H. (2024). Endustri 4.0'in is sagligi ve guvenligi uzerindeki etkileri: Bir literatur incelemesi.

Kokangül, A., Polat, U., & Dagsuyu, C. (2017). A new approximation for risk assessment using the AHP and Fine Kinney methodologies. *Safety science*, *91*, 24-32.

Kulekci, G., & Guvendi, A. (2024). Cevresel tehlikelerin belirlenmesi ve saglik etkilerinin ıncelenmesi: gumushane kati atik tesisi ornegi. *Gumushane Universitesi Saglik Bilimleri Dergisi*, *13*(3), 1327-1347.

Mogan, O., & Gungor, C. (2023). Asbest sokum isleri risk degerlendirmesi. *Duzce Universitesi Bilim Ve Teknoloji Dergisi*, *11*(4), 1758-1770.

Olcucu Sensoy, H. (2019). Tehlikeli atik bertaraf tesislerinde meslek hastaligi ve biyolojik faktorler acisindan risk değerlendirmesi, Yuksek Lisans Tezi, Tarsus Universitesi, Lisansustu Egitim Enstitusu, Mersin.

Oluk, F., Demir, Y., Sahiner, E., Gokcan, A., Ozcan, H. K., & Demir, G. (2023). Endüstri 4.0 sisteminin çalışanlar üzerinde oluşturduğu psikososyal risk etmenlerinin incelenmesi; makine ve ekipman imalatı sektörü örneği. *Frontiers in Life Sciences and Related Technologies*, *4*(SI), 9-17.

Oluk, F., Gokcan, A., & Demir, G. (2022). Üretkenlik karşıtı iş davranışlarının iş kazalarına olan etkisinde güvenlik ikliminin düzenleyici rolü; makine ve ekipman imalatı sektörü örneği. *Frontiers in Life Sciences and Related Technologies*, *3*(3), 128-133.

Sahin, G., & Onder, H. G. (2021). Atik yönetimi, sera gazi emisyonlari ve Türkiye: avrupa yeşil mutabakati çerçevesinde bir değerlendirme. *The Journal of Academic Social Science*, *9*(112), 194-216.

Sikder, S., Toha, M., & Mostafizur Rahman, M. (2024). An overview on municipal solid waste characteristics and its impacts on environment and human health. *Technical Landfills and Waste Management: Volume 1: Landfill Impacts, Characterization and Valorisation*, 135-155.

Taser, A., & Erdogan, B. Z. (2010). Avrupa Birliği ve Türkiye'de tehlikeli atık yönetiminin yasal gelişimi. Eskisehir Osmangazi Universitesi *Iktisadi Ve Idari Bilimler Dergisi*, *5*(2), 67-84.

Tenikler, G. (2007). Turkiye'de tehlikeli atik yonetimi ve avrupa birligi ulkeleri ile karsilastirmali bir analiz, Doktora Tezi, Dokuz Eylul Universitesi, Sosyal Bilimler Enstitusu, Izmir.

Yapici, A. (2012). Tehlikeli atik geri kazanim/bertaraf tesislerinde ıs sagligi ve guvenliginin mevcut durumunun değerlendirilmesi, Yuksek Lisans Tezi, Selcuk Universitesi, Fen Bilimleri Enstitusu, Konya.

Yildirim, R. (2023). Biyogaz uretim sureclerinde kullanilacak en uygun risk degerlendirme metodolojisinin analitik hiyerarsi prosesi ile belirlenmesi. *Nigde Omer Halisdemir Universitesi Muhendislik Bilimleri Dergisi*, *12*(4), 1130-1140.

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Research article

Assessment of sustainable nutrition practices among individuals attending the gym

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Abstract

Sustainable diets refer to diets with low environmental impacts and positive impacts on food security and health. Considering resource depletion and environmental pollution, it is thought that foods with low environmental impact should be chosen instead of foods with high environmental impact. However, given the high protein consumption of individuals who go to the gym, it is thought that they pose a risk for a sustainable future. Therefore, the aim of this study is to determine the behaviors of gym-going individuals towards sustainable nutrition. A total of 203 individuals with a mean age of 28.57±9.97 years were included in this cross-sectional study. A questionnaire containing demographic information was prepared by the researchers. In addition, the Sustainable Nutrition Behavior Scale was administered to the participants. An overwhelming majority of the participants, specifically 97.5%, reported consuming meat, chicken, or fish at least once or twice a week. The mean total score of the sustainable dietary behavior scale was 97.65±21.59. There was no significant difference between the body mass index values of the participants and the total score of the sustainable nutrition behavior scale (p>0.05). The total score of the behavior scale for sustainable nutrition was lower in participants with active sports duration of less than 5 years, single marital status, and male participants (p<0.05). Studies on sustainable nutrition are mostly focused on the general population. However, it is important to reveal the sustainable nutrition knowledge levels of individuals practicing sports.

Keywords: *Dietary behavior; environmental impact; sports nutrition; sustainable nutrition*

1. Introduction

Nutrition is regarded as a key component of athletic performance, with post-exercise nutritional recommendations being crucial for effective recovery and adaptation processes. Consequently, an effective recovery strategy between workouts or during competition can enhance adaptive responses to various fatigue mechanisms, improve muscle function, and increase exercise tolerance (Kerksick et al., 2017). The adaptive response to exercise training is influenced by several factors, including the duration, intensity, type, and frequency of exercise, as well as the quality and quantity of pre- and post-exercise nutrition (Meyer et al., 2020). A healthy and balanced diet is of great importance for athletes and active people to improve sports performance and general health (Amawi et al., 2024). It is known that athletes have a health advantage and that engaging in sports promotes a sustainable lifestyle (Meyer et al., 2020). Sustainable eating behaviors aim to increase the consumption of plant foods such as vegetables, fruits, and legumes and reduce the consumption of animal foods (Pinarli Falakacilar and Yucecan, 2024). However, animal protein consumption is recommended because it contains amino acids traditionally considered important for muscle growth (Goldman et al., 2024).

However, the continued emphasis on animal protein consumption raises concerns that it could lead to environmental problems as the world population and demand for meat grows.

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The environmental impacts of animal proteins and diminishing resources necessitate the search for alternative protein sources (López-Martínez et al., 2022). While meeting protein requirements for sustainability in athletes through plant-based approaches has been proposed, mitigation options such as reducing food waste and prioritizing seasonal produce have also been presented. However, more research is needed on the effects of plant-based strategies on performance and health, packaging, and food waste (Meyer et al., 2020). All of this highlights the need for continued research and reflection to balance sports nutrition and sustainability.

2. Materials and methods

2.1. Study design

The study was conducted to determine the sustainable nutrition behaviors of individuals applying to the gym. Sustainable food consumption behaviors of individuals were determined with the Sustainable Nutrition Scale. The sample of this cross-sectional study consisted of 203 voluntary individuals aged 19-65 years who exercised for 150 minutes or more in a private gym in Istanbul. This cross-sectional study was conducted following the guidelines outlined in the Declaration of Helsinki. The data collection process of the study was carried out after obtaining permission from the Istanbul Bilgi University Human Research Ethics Committee (2024-04/03).

2.2. Questionnaire

A questionnaire was prepared by the researchers to determine the demographic characteristics, exercise routines, dietary habits, and sustainable eating behaviors of the participants. Participants' sustainable food consumption behaviors were evaluated with the Sustainable Dietary Behavior Scale. This Likert-type five-point scale consists of 29 items and 4 sub-dimensions: reducing food waste and buying seasonal and local food. All items in the scale are scored by giving a numerical value from 1 to 5, from "never" to "always." The highest score that can be obtained from the scale is 145, and the lowest score is 29. Sub-dimension scores are calculated by dividing the sum of the scores given by individuals to the questions within each sub-dimension by the number of questions in that sub-dimension. Higher overall and sub-dimension scores indicate that the individual exhibits more sustainable nutrition behaviors (Garipoglu et al., 2023).

2.3. Data analysis

The data obtained were evaluated in SPSS software (version 28.0) Inc., Chicago package program. Statistical significance was set at $p<0.05$ for all analyses. Descriptive statistics encompassed the percentage, mean, number, median, minimum, maximum values, and standard deviation. The Kolmogorov-Smirnov test was used to check the data for normal distribution. The relationship between continuous variables was determined by Spearman correlation analysis. Mann-Whitney U and Kruskal-Wallis tests were employed to evaluate sustainable nutrition scores across various parameters. Multiple linear regression was utilized to estimate the effects of independent variables on the dependent variable, sustainable nutrition behavior. The variables of sport duration, sport branch, gender, and marital status were added to the model.

3. Results

Table 1 presents the general characteristics of the individuals. The average age of the participants was 28.57±9.97 years, and the average body weight was 71.35±15.45 kg. 50.7% of the participants were female and 82.8% were single. The education level of 74.4% of the participants was a bachelor's degree and above (Table 1).

Table 1
General characteristics of individuals applying to the gym (n=203).

Characteristics	Mean±SS	Med. (Min.-Maks.)
Age (year)	28.57±9.97	24 (18-59)
Height (cm)	171.82±9.16	170 (155-195)
Body weight (kg)	71.35±15.45	70 (42-125)
BMI (kg/m²)	23.88±4.29	23.66 (0-37.87)
Characteristics	**n**	**%**
Gender		
Men	100	49.3
Female	103	50.7
Marital status		
Single	168	82.8
Married	35	17.2
Educational background		
Bachelor's degree and higher	151	74.4
High School	34	16.7
Associate Degree	18	8.9

*BMI: body mass index

Table 2
Sports and nutrition habits of individuals.

Characteristics		n	%
Duration of active sport	0-6 months	52	25.6
	6-12 months	29	14.3
	1-2 years	32	15.8
	3-4 years	29	14.3
	5 years and above	61	30.0
Sport branch	Endurance	72	35.5
	Power/Strength	37	18.2
	Both	94	46.3
Sports nutrition knowledge level	No Information	17	8.4
	I know more than enough	31	15.3
	I have enough information	86	42.4
	I don't know enough	69	34.0
Meal skipping	No	60	29.6
	Yes	143	70.4
Frequency of meat/chicken/fish consumption per week	1-2 days	56	27.6
	3-4 days	78	38.4
	5-6 days	34	16.7
	Every day	30	14.8
	Never	5	2.5

Table 3
Behavior scale scores for sustainable nutrition.

	Mean±SS	Med. (Min.-Maks.)
Seasonal local nutrition	27.35±7.2	28 (10-40)
Food preference	17.84±5.53	18 (6-30)
Food purchasing	20.15±5.65	20 (6-30)
Reducing food waste	31.93±7.38	32 (13-45)
Total	97.65±21.59	97 (40-145)

The sports and nutrition habits of the individuals who applied to the gym are given in Table 2. Thirty percent of the participants have been practicing sports for 5 years or more, and

Table 4
The relationship between age and anthropometric measurements and the total score and subscale scores of the sustainable nutrition behavior scale.

		Food preference	Reducing food waste	Seasonal local nutrition	Food purchasing	Total
Age	r	0.361	0.302	0.252	0.314	0.387
	p*	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Body weight	r	-0.275	-0.093	-0.111	-0.169	-0.156
	p*	**<0.001**	0.189	0.119	**0.018**	**0.032**
Height	r	-0.336	-0.207	-0.214	-0.238	-0.276
	p*	**<0.001**	**0.003**	**0.002**	**0.001**	**<0.001**
BMI	r	-0.138	0.020	-0.012	-0.064	-0.022
	p*	0.052	0.779	0.871	0.373	0.768

**Spearman's correlation; BMI: body mass index

Table 5
The relationship between the sustainable nutrition behavior scale and demographic characteristics, sports and eating habits.

Characteristics		Food preference Mean±SS Med. (Min.Maks.)	Reducing food waste Mean±SS Med. (Min.Maks.)	Seasonal local nutrition Mean±SS Med. (Min.Maks.)	Food purchasing Mean±SS Med. (Min.Maks.)	Total Mean±SS Med. (Min.Maks.)
Gender	Men	15.64±5.56 16 (6-30)	30.15±7.4 30 (13-45)	25.23±7.18 25 (10-40)	18.47±5.6 18 (6-30)	90.06±21.23 90 (40-133)
	Female	19.92±4.64 20 (9-30)	33.64±6.99 34 (17-45)	29.43±6.64 29 (11-40)	21.77±5.23 21 (7-30)	104.92±19.4 101 (50-145)
	p[1]	**<0.001**	**0.001**	**<0.001**	**<0.001**	**<0.001**
Marital status	Single	17.36±5.34 18 (6-30)	31.29±7.41 31 (13-45)	26.56±7.07 27 (10-40)	19.82±5.52 20 (6-30)	95.37±21.5 95.5 (40-145)
	Married	20.18±5.89 19.5 (8-30)	34.94±6.56 36 (21-44)	31.06±6.77 31 (10-40)	21.79±6.1 21 (7-30)	108.91±18.51 108 (71-141)
	p[1]	**0.009**	**0.008**	**<0.001**	0.076	**0.002**
Educational background	Bachelor's degree and higher	17.54±5.6 18 (6-30)	32.07±7.24 32 (14-45)	26.99±7.09 27 (10-40)	19.81±5.69 20 (6-30)	96.82±21.59 96 (47-145)
	High School	18.61±5.36 19 (6-30)	30.58±7.73 31 (13-44)	29.0±7.42 30 (11-40)	21.85±4.95 22 (10-30)	100.31±21.8 102,5 (40-141)
	Associate Degree	19.57±5.4 18.5 (10-30)	32.63±8.29 34.5 (18-42)	26.33±7.47 26 (14-40)	19.43±5.98 20.5 (9-28)	98.42±22.17 95.5 (63-133)
	p[2]	0.625	0.448	0.294	0.263	0.715
Duration of active sport	0-6 months	16.76±4.98 18 (6-28)	30.9±7.44 30 (15-45)	26.92±7.14 26 (11-40)	19.73±4.8 19 (11-30)	95.27±20.01 94 (47-138)
	6-12 months	17.89±5.11 18 (9-30)	32.39±7.15 33.5 (14-44)	27.28±7.07 27 (14-40)	19.72±4.96 19 (9-28)	97.64±20.61 94.5 (48-141)
	1-2 years	16.66±6.09 17 (6-30)	30.94±7.49 30 (13-45)	26.97±8.26 27.5-(11-40)	18.45±6.36 18 (6-30)	92.03±24.69 89 (40-145)
	3-4 years	15.96±6.28 15,5 (6-29)	28.17±6.11 28 (18-41)	24.97±7.29 24 (10-37)	17.17±5.87 17 (7-28)	87.11±20.24 91 (50-133)
	5 years and above	20.29±4.75 21 (6-30)	35.0±6.95 37 (21-45)	29.14±6.44 30 (10-40)	23.12±4.92 24 (14-30)	108.25±18.21 110 (60-142)
	p[2]	**0.001**	**0.001**	0.104	**<0.001**	**<0.001**
Sport branch	Endurance	18.47±5.35 19- (6-30)	33.04±7.83 34- (14-45)	28.35±7.06 29- (10-40)	20.84±5.46 21- (9-30)	101.52±21.54 101- (48-145)
	Power/Strength	14.95±5.76 15- (6-25)	29.3±7.02 28- (13-44)	24.05±7.03 24- (10-39)	17.72±6.26 17- (6-30)	86.28±21.51 85- (40-138)
	Both	18.54±5.24 19 (6-30)	32.13±6.98 31.5 (15-45)	27.9±7.08 29 (10-40)	20.59±5.33 20.5 (9-30)	99.37±20.27 101 (47-142)
	p[2]	**0.006**	**0.034**	**0.006**	**0.023**	**0.001**
Meal skipping	No	19.71±5.15 20 (8-30)	34.22±7.21 36 (19-45)	28.35±7.23 29 (11-40)	21.96±5.62 22 (7-30)	104.63±20.05 103 (63-141)
	Yes	17.07±5.51 18 (6-30)	30.99±7.27 30.5 (13-45)	26.95±7.18 27 (10-40)	19.43±5.52 19 (6-30)	94.88±21.62 95.5 (40-145)
	p[1]	**0.004**	**0.007**	0.273	**0.004**	**0.007**

[1]Mann Whitney U test, [2]Kruskal Wallis test

46.3% of them stated that they practiced both endurance and power/strength sports. 42.4% of the participants stated that they had sufficient knowledge about sports nutrition. In addition, 70.4% of the participants skip meals. 14.8% of the participants consume meat/chicken/fish every day (Table 2).

Sustainable nutrition behavior scale scores are given in Table 3. The mean total score was 97.65±21.59. The mean score of the food preference subscale was 17.84±5.53, while the mean score of the food waste reduction subscale was 31.93±7.38 (Table 3).

The relationship between the total and subscale scores of the sustainable eating behavior scale and age and anthropometric measurements is shown in Table 4. When the relationship between the mean total sustainable nutrition behavior score of the participants and age and anthropometric measurements was evaluated, a significant negative correlation was found with height and body weight values and a significant positive correlation with age (p<0.05). However, no statistically significant difference was found between the participants' BMI values and the scale total score and subscale scores (p>0.05) (Table 4).

The relationship between the total score and sub-dimension scores of the sustainable nutrition behavior scale and demographic characteristics, sports, and eating habits is given in Table 5. Female participants had higher averages than male participants in seasonal local nutrition, food preference, food purchasing, food waste reduction, and total score (p≤0.001). Regarding marital status, married participants scored higher than single participants in food preference, reducing food waste, seasonal local nutrition, and total score (p<0.05). Participants who have been exercising for 5 years or more have more sustainable food preferences (p≤0.001). Those involved in endurance and endurance and power/strength sports had higher values in sustainable nutrition sub-dimensions food preference, food waste reduction, seasonal local diet, food purchasing, and total score than those involved in power/strength sports only (p<0.05). In addition, participants who reported not skipping meals had higher values in food preference, food waste reduction, food purchasing, and total score than participants who reported skipping meals (p<0.05) (Table 5).

Multiple linear regression analysis was performed with the variables found to be effective on the total score of the sustainable nutrition behavior scale (Table 6). According to the regression analysis, the variables affecting sustainable nutrition behaviors were duration of active sports, gender, and marital status (p<0.05). Consequently, having an active sport duration of less than 5 years negatively impacted the total score (p<0.05). Additionally, being male had a negative effect on the total score (p<0.001). Single participants also showed a negative effect on the total score (p<0.05) (Table 6).

4. Discussion

The concept of sustainable nutrition focuses on a health-promoting diet that is culturally acceptable, accessible, and environmentally friendly while reducing environmental costs for current and future generations (Gibas-Dorna and Żukiewicz-Sobczak, 2024). Individuals engaged in sports have varying nutrient requirements depending on their training intensity, performance goals, and health status, and therefore sustainability is often overlooked when planning athletes' diets (Meyer et al., 2020; Lim et al., 2021). In this context, this study aimed to determine the behaviors of individuals engaged in sports toward sustainable food consumption and to draw attention to the concept of sustainability in sports nutrition.

In a cross-sectional study published in 2020, 74.75% of 298 individuals who applied to 20 different gyms consumed red meat at least once a week (Bert et al., 2020). In our study, 97.5% of the participants consumed meat/chicken/fish 1-2 days a week or more. Increased demand for animal-based protein is expected to increase greenhouse gas emissions, require more water and land use, and thus have a negative impact on the environment (Henchion et al., 2017). Although the positive effects of some animal proteins on sports performance are available in the literature, increasing concerns about sustainability are expected to increase approaches to finding alternative sources (López-Martínez et al., 2022).

In our study, the participants who stated that they had information on sports nutrition were the majority (57.7%). In a study conducted by Calella et al. (2021), results contrary to our study were obtained. According to the study, gym members had a similar level of sports nutrition knowledge as individuals who were not actively involved in sports. However, the educational status of the participants was not specified in this study. In our study, 74.4% of the participants completed their undergraduate education. In one study, it was observed that those with a higher level of education had the best knowledge of sports nutrition (Finamore et al., 2022).

In this present study, the mean total score of the behavior scale for sustainable nutrition was 97.65±21.59. In previous studies, the sustainable nutrition compliance of individuals was generally found to be insufficient (Macit-Celebi et al., 2023; Oliveira Neta et al., 2023). Factors such as low awareness of healthy eating (Harray et al., 2022) and high animal protein consumption (Tepper et al., 2021) may be cited as the reason for this inadequacy. In our study, the sub-dimension of reducing food waste has the highest mean score. Similar to our study, in the study conducted by Żakowska-Biemans et al. (2019) with young adults, participants most frequently avoided food waste. In another study, students of the Department of Nutrition and Dietetics had a higher mean score on the food waste avoidance factor (Yolcuoglu and Kiziltan, 2022). Since food waste is a global problem, reducing food waste is essential to ensure sustainable food security for present and future generations (Palmisano et al., 2021).

In our study, 70.4% of individuals skipped meals and these individuals had a lower food preference subscale score (p=0.004). In a study conducted on university athletes, 51.2% of

Table 6
Multiple regression analysis of various variables on the total score of individuals' sustainable nutrition behavior scale.

		Adjust	Durbin Watson	Significance Level p	F Value	
Model	0.523	0.274	1.831	**<0.001**	9.791	
		Not standardized	**Standard Error**	**Standardized**	**Significance Level p**	**VIF Value**
Constant		122.066	3.919		**0.000**	
Sport duration 0-6 months		-14.611	3.771	-.295	**0.000**	1.451
Sport duration 6-12 months		-13.320	4.407	-.219	**0.003**	1.319
Sport duration 1-2 years		-12.142	4.389	-.208	**0.006**	1.422
Sport duration 3-4 years		-16.207	4.525	-.267	**0.000**	1.391
Sport: strength/strength		-7.355	3.754	-.134	0.052	1.170
Gender: male		-13.960	2.839	-.324	**0.000**	1.088
Marital status: single		-7.418	3.755	-.129	**0.048**	1.067

the participants skipped meals (Celik and Dagdeviren, 2022). Skipping meals is associated with a decrease in daily nutritional quality (Zeballos and Todd, 2020). For example, skipping meals is associated with low fruit and vegetable consumption (Pourrostami et al., 2020). In another study, when the food preferences of individuals were evaluated, it was observed that individuals with behaviors supportive of sustainable nutrition consumed more vegetables and fruits, while individuals with negative behaviors against sustainable nutrition consumed more processed and red meat (Irazusta-Garmendia et al., 2023). In another study with university students, positive perceptions of environmental sustainability and a desire to mitigate climate change were associated with lower red meat consumption (Slotnick et al., 2023).

According to the correlation analysis, a significant positive relationship was found between the total and all sub-dimension scores of the sustainable eating behavior scale and age. In a study, generational differences in sustainable food consumption behavior were evaluated and the organic food purchasing behavior of young individuals (Generation Z) was found to be the lowest (Kamenidou et al., 2020). This may be due to younger individuals' lower awareness of the environmental impacts of their dietary choices (Bogueva and Marinova, 2022). In addition, there was a significant negative correlation between participants' body weight and sustainable food preference and food purchasing sub-dimensions. In a prospective cohort study, participants in the first quartile reflecting the lowest sustainable dietary pattern were found to be at higher risk of obesity. In addition, higher consumption of vegetables and fruits, which contributes to reducing the energy density of the diet, is thought to reduce the risk of obesity in participants in the last quartile reflecting sustainable diets (Seconda et al., 2020).

According to the regression analysis, the variables affecting the total score of the sustainable nutrition behavior scale were gender, marital status, and duration of active sports. In our study, the average total sustainable nutrition behavior score of women was significantly (p<0.001) higher than that of men, indicating that women have higher sustainable nutrition behaviors. In a study published in 2022 with undergraduate students, similar to our study, sustainable nutrition behavior score was found to be significantly higher in women compared to men (Engin and Sevim, 2022). Women may tend to have more positive attitudes towards food and nutrition literacy compared to men (Mortas et al., 2023). Food and nutrition literacy not only improves the nutrition and health of individuals but also helps individuals understand the effects of their food choices on the environment (Teng and Chih, 2022).

The study shows that the average total sustainable dietary behavior score of married participants was higher than that of single participants. In a cross-sectional study investigating the change in individuals' food choices according to sustainability issues, it was observed that married participants took sustainability into account more than single participants when making food choices (Guiné et al., 2021). In another study, it was found that married participants were more likely to consume at least 5 servings of vegetables and fruits per day compared to never-married participants, and it was stated that this may be associated with the behavior of one of the spouses that would pave the way for a diet rich in fruits and vegetables (Kabwama et al., 2019). In addition to the consumption of animal-derived food due to the lifestyle of athletes, processed and packaged food consumption and food waste are critical points related to the environment (Meyer et al., 2020). However, in our study, the total score of the behavior scale for sustainable nutrition was found to be significantly higher in those who have been doing sports for 5 years or more. It is thought that the increase in the duration of active sports increases awareness and positively affects the behavior towards sustainable nutrition.

In the findings of our study, the mean total sustainable dietary behavior score of participants who practiced endurance sports or both endurance and strength/strength sports was higher than that of participants who practiced only strength/strength sports. In a study conducted by Jansen et al. (2024), outdoor sports athletes showed the highest values in terms of sustainable attitudes and behaviors but since the data of the study is based on self-report, larger studies with larger samples are needed. The result of this study may be related to the fact that outdoor athletes are more intertwined with nature than indoor athletes. For example, marathon runners may show more concern for the environment, as marathons are often held in places surrounded by nature (Konecke et al., 2021).

Nowadays, some recreational runners have adopted a flexitarian, vegetarian, or vegan diet due to better sports performance, ecological aspects, animal ethics, and current trends in sustainable nutrition (Tanous et al., 2024). However, the global number of vegetarian athletes is unknown (Baroni et al., 2023). According to a systematic review assessing the relationship between a vegetarian diet and sports performance, vegetarian athletes do not have a higher sports performance compared to omnivorous athletes (Hernández-Lougedo et al., 2023). However, another systematic review and meta-analysis showed that plant-based nutrition has the potential to aid aerobic performance and does not compromise strength/power performance. This result is especially valuable for athletes with plant-based diets, but more studies comparing the effects of omnivorous and plant-based diets are needed in the literature. (Damasceno et al., 2024).

5. Conclusion

Based on the results obtained from this study, we suggest that the sustainable nutrition behaviors of individuals applying to the gym should be improved. Sustainable nutrition is an approach that both takes into account the nutrient adequacy of the individual and optimizes global inequalities, food waste, and environmental impacts. The total score of the sustainable nutrition behavior scale was lower in those whose active sports duration was less than 5 years, those whose marital status was single, and male participants. Our study will be a reference for future studies that will evaluate the behaviors of individuals applying to the gym towards sustainable nutrition. In addition, dietitians should raise awareness about healthy and sustainable nutrition among individuals who are active in sports.

Conflict of interest: The authors declare that they have no conflict of interests.

Informed consent: The authors declare that this manuscript did not involve human or animal participants and informed consent was not collected.

References

Amawi, A., AlKasasbeh, W., Jaradat, M., Almasri, A., Alobaidi, S., Hammad, A. A., ... & Ghazzawi, H. (2024). Athletes' nutritional demands: a narrative review of nutritional requirements. *Frontiers in Nutrition*, *10*, 1331854.

Baroni, L., Pelosi, E., Giampieri, F., & Battino, M. (2023). The VegPlate for sports: a plant-based food guide for athletes. *Nutrients*, *15*(1746), 1-18.

Bert, F., Scaioli, G., Tolomeo, M., Lo Moro, G., Gualano, M. R., & Siliquini, R. (2020). Knowledge, attitudes and eating habits red and processed meat among gym users: a cross-sectional survey. *Perspectives in Public Health*, *140*(4), 203-213.

Bogueva, D., & Marinova, D. (2022). Australian Generation Z and the nexus between climate change and alternative proteins. *Animals*, *12*(19), 2512.

Calella, P., Gallè, F., Di Onofrio, V., Buono, P., Liguori, G., & Valerio, G. (2021). Gym members show lower nutrition knowledge than youth engaged in competitive sports. *Journal of the American College of Nutrition*, *40*(5), 465-471.

Celik, O. M., & Dagdeviren, N. (2022). Dietary intakes, nutritional habits, and nutritional supplement use of collegiate athletes: A sample from a university in Turkey. *Progress in Nutrition*, *24*(4).

Damasceno, Y. O., Leitão, C. V., de Oliveira, G. M., Andrade, F. A. B., Pereira, A. B., Viza, R. S., ... & Coimbra, C. C. (2024). Plant-based diets benefit aerobic performance and do not compromise strength/power performance: a systematic review and meta-analysis. *British Journal of Nutrition*, *131*(5), 829-840.

Engin, S., & Sevim, Y. (2022). The relationship between knowledge and behaviors on sustainable nutrition with food choices of undergraduate students: A single centre study. *European Journal of Science and Technology*, (38), 259-269.

Finamore, A., Benvenuti, L., De Santis, A., Cinti, S., & Rossi, L. (2022). Sportsmen's attitude towards dietary supplements and nutrition knowledge: an investigation in selected Roman area gyms. *Nutrients*, *14*(5), 945.

Garipoglu, G., Meral Koc, B., & Ozlu, T. (2023). Behaviors scale towards sustainable nutrition: development and validity-reliability analysis. *Nutrition & Food Science*, *53*(8), 1332-1343.

Gibas-Dorna, M., & Żukiewicz-Sobczak, W. (2024). Sustainable Nutrition and Human Health as Part of Sustainable Development. *Nutrients*, *16*(2), 225.

Goldman, D. M., Warbeck, C. B., & Karlsen, M. C. (2024). Protein Requirements for Maximal Muscle Mass and Athletic Performance Are Achieved with Completely Plant-Based Diets Scaled to Meet Energy Needs: A Modeling Study in Professional American Football Players. *Nutrients*, *16*(12), 1903.

Guiné, R. P., Bartkiene, E., Florença, S. G., Djekić, I., Bizjak, M. Č., Tarcea, M., ... & Cardoso, A. P. (2021). Environmental issues as drivers for food choice: study from a multinational framework. *Sustainability*, *13*(5), 2869.

Harray, A. J., Boushey, C. J., Pollard, C. M., Dhaliwal, S. S., Mukhtar, S. A., Delp, E. J., & Kerr, D. A. (2022). Healthy and Sustainable Diet Index: development, application and evaluation using image-based food records. *Nutrients*, *14*(18), 3838.

Henchion, M., Hayes, M., Mullen, A. M., Fenelon, M., & Tiwari, B. (2017). Future protein supply and demand: strategies and factors influencing a sustainable equilibrium. *Foods*, *6*(7), 53.

Hernández-Lougedo, J., Maté-Muñoz, J. L., García-Fernández, P., Úbeda-D'Ocasar, E., Hervás-Pérez, J. P., & Pedauyé-Rueda, B. (2023). The relationship between vegetarian diet and sports performance: a systematic review. *Nutrients*, *15*(21), 4703.

Irazusta-Garmendia, A., Orpí, E., Bach-Faig, A., & González Svatetz, C. A. (2023). Food sustainability knowledge, attitudes, and dietary habits among students and professionals of the health sciences. *Nutrients*, *15*(9), 2064.

Jansen, P., Hoja, S., & Rahe, M. (2024). Connectedness and sustainable attitudes and behavior in athletes. *German Journal of Exercise and Sport Research*, *54*(1), 55-63.

Kabwama, S. N., Bahendeka, S. K., Wesonga, R., Mutungi, G., & Guwatudde, D. (2019). Low consumption of fruits and vegetables among adults in Uganda: findings from a countrywide cross-sectional survey. *Archives of Public Health*, *77*, 1-8.

Kamenidou, I., Stavrianea, A., & Bara, E. Z. (2020). Generational differences toward organic food behavior: Insights from five generational cohorts. *Sustainability*, *12*(6), 2299.

Kerksick, C. M., Arent, S., Schoenfeld, B. J., Stout, J. R., Campbell, B., Wilborn, C. D., ... & Antonio, J. (2017). International Society of Sports Nutrition position stand: nutrient timing. *Journal of the International Society of Sports Nutrition*, *14*, 1-21.

Konecke, T., Schunk, H., Schappel, T., Hugaerts, I., Wagner, F., & Malchrowicz-Mośko, E. (2021). German marathon runners' opinions on and willingness to pay for environmental sustainability. *Sustainability*, *13*(18), 10337.

Lim, M. T., Pan, B. J., Toh, D. W. K., Sutanto, C. N., & Kim, J. E. (2021). Animal protein versus plant protein in supporting lean mass and muscle strength: a systematic review and meta-analysis of randomized controlled trials. *Nutrients*, *13*(2), 661.

López-Martínez, M. I., Miguel, M., & Garcés-Rimón, M. (2022). Protein and sport: Alternative sources and strategies for bioactive and sustainable sports nutrition. *Frontiers In Nutrition*, *9*, 926043.

Macit-Celebi, M. S., Bozkurt, O., Kocaadam-Bozkurt, B., & Koksal, E. (2023). Evaluation of sustainable and healthy eating behaviors and adherence to the planetary health diet index in Turkish adults: a cross-sectional study. *Frontiers in Nutrition*, *10*, 1180880.

Meyer, N. L., Reguant-Closa, A., & Nemecek, T. (2020). Sustainable diets for athletes. *Current Nutrition Reports*, *9*, 147-162.

Mortas, H., Navruz-Varli, S., Citar-Daziroglu, M. E., & Bilici, S. (2023). Can unveiling the relationship between nutritional literacy and sustainable eating behaviors survive our future?. *Sustainability*, *15*(18), 13925.

Oliveira Neta, R. S. D., Lima, S. C. V. C., Medeiros, M. F. A. D., Neta, A. D. C. P. D. A., Jacob, M. C. M., Marchioni, D. M. L., ... & Oliveira, A. G. R. D. C. (2023). Adherence to the EAT-Lancet dietary recommendations for a healthy and sustainable diet-the case of the brazuca natal study. *Sustainability*, *15*(23), 16526.

Palmisano, G. O., Bottalico, F., El Bilali, H., Cardone, G., & Capone, R. (2021). Food losses and waste in the context of sustainable food and nutrition security. In *Food Security and Nutrition* (pp. 235-255). Academic Press.

Pinarli Falakacilar, C., & Yucecan, S. (2024). The impact of sustainability courses: Are they effective in improving diet quality and anthropometric indices?. *Nutrients*, *16*(11), 1700.

Pourrostami, K., Heshmat, R., Hemati, Z., Heidari-Beni, M., Qorbani, M., Motlagh, M. E., ... & Kelishadi, R. (2020). Association of fruit and vegetable intake with meal skipping in children and adolescents: The CASPIAN-V study. *Eating and Weight Disorders-Studies on Anorexia, Bulimia and Obesity*, *25*, 903-910.

Seconda, L., Egnell, M., Julia, C., Touvier, M., Hercberg, S., Pointereau, P., ... & Kesse-Guyot, E. (2020). Association between sustainable dietary patterns and body weight, overweight, and obesity risk in the NutriNet-Santé prospective cohort. *The American Journal of Clinical Nutrition*, *112*(1), 138-149.

Slotnick, M. J., Falbe, J., Cohen, J. F., Gearhardt, A. N., Wolfson, J. A., & Leung, C. W. (2023). Environmental and climate impact perceptions in university students: sustainability motivations and perceptions correspond with lower red meat intake. *Journal of the Academy of Nutrition and Dietetics*, *123*(5), 740-750.

Tanous, D. R., Motevalli, M., Leitzmann, C., Wirnitzer, G., Rosemann, T., Knechtle, B., & Wirnitzer, K. (2024). Dietary habits and race day strategies among flexitarian, vegetarian, and vegan recreational endurance runners: A cross-sectional investigation from The NURMI study (Step 2). *Nutrients*, *16*(11), 1647.

Teng, C. C., & Chih, C. (2022). Sustainable food literacy: A measure to promote sustainable diet practices. *Sustainable Production and Consumption*, *30*, 776-786.

Tepper, S., Geva, D., Shahar, D. R., Shepon, A., Mendelsohn, O., Golan, M., ... & Golan, R. (2021). The SHED Index: a tool for assessing a Sustainable Healthy Diet. *European Journal of Nutrition*, *60*(7), 3897-3909.

Yolcuoglu, I. Z., & Kiziltan, G. (2022). Effect of nutrition education on diet quality, sustainable nutrition and eating behaviors among university students. *Journal of the American Nutrition Association*, *41*(7), 713-719.

Żakowska-Biemans, S., Pieniak, Z., Kostyra, E., & Gutkowska, K. (2019). Searching for a measure integrating sustainable and healthy eating behaviors. *Nutrients*, *11*(95), 1-17.

Zeballos, E., & Todd, J. E. (2020). The effects of skipping a meal on daily energy intake and diet quality. *Public Health Nutrition*, *23*(18), 3346-3355.

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Research article

Detection of advanced glycation end product precursors in chocolates enriched with lyophilized cornelian cherry (*Cornus mas* L.)

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Abstract

Advanced glycation end product (AGEs) precursors, glyoxal (GO) and methylglyoxal (MGO), are toxic compounds formed during food processing through the Maillard reaction and, protein and lipid oxidation. Chocolate, a widely consumed product, has been extensively studied for its health effects and contains AGEs and their precursors, which are associated with many chronic inflammatory diseases. Cornelian cherry (*Cornus mas* L.), naturally grown in Türkiye, is rich in antioxidants, vitamin C, anthocyanins, flavonoids, and phenolic compounds. Fruits with natural antioxidant content are known to reduce AGE formation. This study aimed to investigate changes in GO and MGO contents by adding various amounts (10 g, 15 g, and 20 g) of lyophilized *C. mas* powder to different types of chocolate (dark, milk, and white). AGE precursors analysis was performed using High-Performance Liquid Chromatography (HPLC). Additionally, sensory analysis was conducted to determine the consumption potential of the chocolates. Fourteen panelists aged 18-65 evaluated the chocolate samples using a single-blind method by tasting the samples and completing a sensory analysis questionnaire. Data were evaluated and reported using the SPSS 26.0 software package. GO contents of the samples ranged from 14.0 to 268.6 µg/100g, while MGO contents ranged from 122.3 to 284.0 µg/100g. It was observed that only in milk chocolate samples did the GO content decrease with increased amounts of *C. mas*. In the sensory analysis, among chocolate groups, the most preferred product after the control groups was white chocolate with 10 g (3.86 ± 0.86). Significant differences were found among chocolate types in terms of taste, bitterness, melting in the mouth, texture, hardness, sourness, and overall acceptance (p<0.05). Foods with high antioxidant content, such as *C. mas* L. affect the AGE precursors in products. More comprehensive studies examining the antioxidant capacity concerning GO and MGO determination in chocolate types are needed.

Keywords: *Advanced glycation end products; chocolate; cornelian cherry; Cornus mas; glyoxal; methylglyoxal*

1. Introduction

Advanced glycation end products (AGEs) are formed through a non-enzymatic reaction between reducing sugars and free amino groups of proteins, lipids, or nucleic acids (Uribarri et al., 2010). This non-enzymatic reaction is commonly known as Maillard or browning reaction (O'Brien and Morrissey, 1989; Uribarri et al., 2010; Zhu et al., 2024). The main AGE precursors formed in the process of Maillard reactions are glyoxal (GO), methylglyoxal (MGO), and 3-deoxyglucosone (Sharma et al., 2015; Nowotny et al., 2018). These precursors are called α-dicarbonyl compounds, and these compounds interact with the

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amino groups of proteins to form AGEs (Cengiz et al., 2020).

The formation of AGEs is a natural part of human metabolism, but the excessive contents of AGEs in tissues and circulation can lead to pathogenic effects in the body (Ulrich, 2001; Uribarri, 2010). In addition to the formation of AGEs within the human body, these compounds are also ingested through the consumption of food. Clinical studies show that dietary AGEs may increase risk factors associated with chronic diseases such as inflammation, oxidative stress, insulin resistance, diabetes, kidney diseases, and cardiovascular and cerebrovascular disorders (Goldberg et al., 2004; Uribarri et al., 2010; Wei et al., 2018; Tian et al., 2023; Zgutka et al., 2023). Almost all foods, such as chocolate, sugary products, bread, coffee, baby food, and breast milk, contain glycation products to a lesser or greater extent (Singh et al., 2001; Zhang et al., 2024).

According to the literature, it is seen that the determination of AGE precursors in major processed products such as chips, crackers, and packaged cakes has been carried out (Cengiz et al., 2020; Catak and Balci, 2022). Conversely, no study was identified in the existing literature that has determined and compared AGE precursors in different types of chocolate, which are consumed by individuals across a wide age range.

Chocolate is a widely consumed flavorful product and is the subject of many researchers due to its positive or negative effects on health. There is evidence suggesting that cocoa, the primary component of chocolate, has positive effects on the heart, is beneficial for the liver, aids digestion, and improves sleep (Dilinger et al., 2000; Comert and Merdol., 2018). Today, scientific studies on the effects of cocoa and chocolate on risk factors associated with Type 2 diabetes mellitus, cardiovascular diseases, blood lipid profile, antioxidant capacity, insulin resistance, blood pressure, inflammation, and obesity have become widespread (Bruinsma and Taren, 1999; Katz et al., 2011; Ellam and Williamson, 2013; Comert and Merdol, 2018). The adverse health effects of commercially available chocolates are generally analyzed in terms of the amount of sugar and additives they contain. It is known that these products contain AGEs and AGE precursors, which are known to have a negative effect on many chronic inflammatory diseases (Javed et al., 2021; Yan et al., 2023).

Two strategies have been proposed to reduce α-dicarbonyl compounds, which are one of the AGE precursors in foods. The first one is to use non-thermal technologies in food processing, and the second is to optimize the formulation of food components by adding phenolic compounds. However, it should be noted that these approaches may affect the organoleptic properties and nutritional value of foods (Javed et al., 2021; Yan et al., 2023; Kou et al., 2024).

There are studies supporting the hypothesis that red-purple fruits, known for their antioxidant properties, inhibit AGE formation (Chen et al., 2014; Yusufoglu et al., 2020; Hsiao et., 2024; Tan et al., 2024). Studies on Cornelian cherry (*C. mas* L.; CM) fruit, which is one of these red fruits, and its health benefits have increased in recent years (Celik et al., 2023; Bayram et al., 2024a; Bayram et al., 2024b; Szot et al., 2024). CM is thought to contribute to a healthy diet due to its phenolic components, organic acids, vitamin C, pectins, carotenoids, and essential minerals. Antioxidant, antimicrobial, antidiabetic, anti-atherosclerosis, anti-obesity, anti-glaucoma, cytoprotective, neuroprotective, cardioprotective, liver and kidney protective properties, hypolipidemic and hypotensive effects of CM have been noted (Bayram and Ozturkcan, 2020; Lidiková et al., 2024).

In the literature, studies on products enriched with CM mostly refer to the antioxidant content of the products and do not include the effects on the content of AGE precursors. For this reason, this study aimed to investigate the formation of AGE precursors in different types of chocolate (white, milk, dark) by adding lyophilized dried CM. Moreover, the organoleptic properties of the chocolates were also evaluated by sensory analysis (Lidiková et al., 2024; Bayram et al., 2024a).

2. Materials and methods

2.1. Preparation of samples

The CM fruits were collected from the Uzundere district of Erzurum province, pureed, and lyophilized by sublimation method in Ray 125 Freeze Dry machine with 300 tray capacities. Milk, dark (54.0% cocoa content), and white couverture chocolates of a brand that is widely used as chocolate and available on the market shelves were purchased. Declared macronutrient values of the chocolate samples are shown in Table 1.

Table 1
Declared macronutrient values of the chocolate samples.

Sample	Carbohydrate (g/100 g)	Protein (g/100 g)	Fat (g/100 g)
Milk Chocolate	61	6	27
Dark Chocolate	48	6.1	34
White Chocolate	58	3.9	36

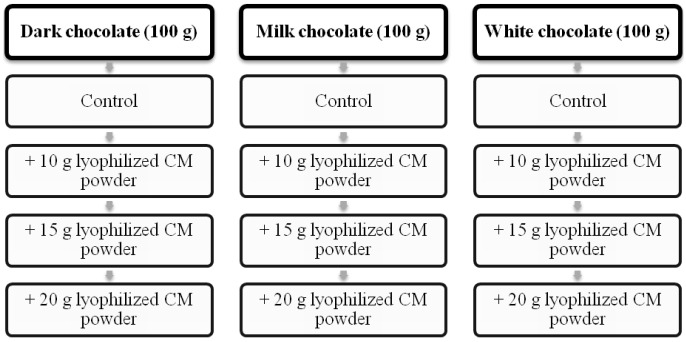

Fig. 1. Preparation of chocolate samples.

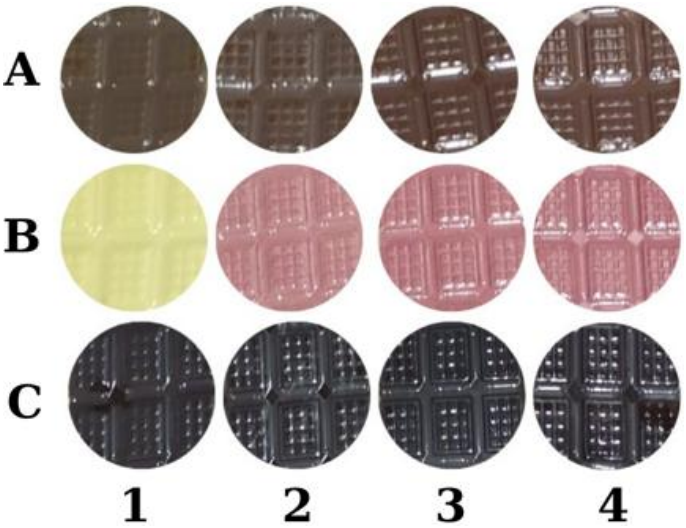

Fig. 2. Chocolate samples. (A) milk chocolate, (B) white chocolate, (C) dark chocolate, (1) control, (2) 10 g CM added, (3) 15 g CM added, (4) 20 g CM added.

All samples, with or without the addition of CM, were processed in the same way, melted in a bain-marie and then poured into chocolate molds and frozen. Determined amounts of CM were added to each chocolate flavor separately.

The chocolates were melted in 100 g samples in a bain-marie. Dark chocolate melted at 63°C, milk chocolate at 51°C, and white chocolate at 53.6°C. To preserve the vitamin C content of CM, the temperature of the melted chocolates was not high. Temperature control was done with a probe thermometer. Lyophilized CM powder in different amounts was added to the chocolates at 40°C and mixed (Fig. 1). The mixed chocolates were poured into silicone chocolate cups and placed in the freezer. The frozen chocolates were vacuumed and placed in cooler bags for analysis (Fig. 2).

2.2. Chemical analyses of AGE precursors in samples

AGE precursors of 12 different samples were analyzed by the HPLC method using 4-nitro-1,2-phenylenediamine as a pre-column derivatization reagent, a method used in the studies of Cengiz et al. (2020) and Catak and Balci (2022). GO and MGO contents were analyzed as AGE precursors in the samples.

2.2.1. Extraction and derivatization of GO and MGO

The extraction of GO and MGO in the samples was carried out according to the method described by Cengiz et al. (2020) with some modifications. All samples were homogenized and then 5 g of each sample was taken and placed in a 50 ml falcon tube. 5 ml of methanol was added. The samples were extracted for 2 min using an Ultra Turrax homogenizer (IKA, Staufen, Germany) and centrifuged at 8000 rpm for 5 min. After centrifugation, 0.5 ml of liquid samples were taken into 10 ml glass tubes, and 1 ml of sodium acetate buffer (0.1 M, pH: 3) was added to the samples. After adding 0.5 ml of derivatization solution (4-nitro-1,2-phenylenediamine in 1% methanol) to the samples, the resulting mixtures were incubated at 70°C for 20 min and filtered through cellulose acetate filter (pore size 0.45 µm) and injected into HPLC.

2.2.2. HPLC determination of GO and MGO

The HPLC conditions described by Cengiz et al. (2020) were modified. The HPLC system consisted of a Shimadzu SPD-20A UV/VIS detector (Shimadzu Corporation, Kyoto, Japan), and a Shimadzu LC 20AT pump was used. The mobile phase was methanol:water: acetonitrile (42:56:2, v/v/v). The wavelength was 255 nm. GO and MGO were separated by an Inertsil ODS-3, 250 x 4.6 mm, 5 µm column with a flow rate of 1 mL/min. The column oven temperature was set to 30°C.

2.3. Sensory Analysis of the Samples

For sensory analysis, the samples were evaluated by trained panelists using the single-blind method. During the sensory analysis, the sample names were not openly written, the samples were assigned random codes, and the panelists were presented with the samples in a random order. The panelists were blinded to the samples they tasted. The panelists were between the ages of 18-65 and included 14 participants. Analyses were performed according to the matched-comparison method. A questionnaire containing, color, smell, flavor, bitter taste, oily taste, texture, hardness, stickiness, melt in the mouth, sourness, and general acceptability criteria of the products were scored by 5-point Likert method (very good, good, neutral, bad and very bad). Panelists were asked to neutralize the aftertaste with water before tasting each sample. An informed consent form was signed by all participants before starting the sensory analysis. The sensory analysis of this study was approved ethically by the Marmara University Faculty of Health Sciences Non-Invasive Clinical Studies Ethics Committee (Protocol no: 2022/181), and the research was conducted following the principles stated in the Helsinki Declaration.

2.4. Statistical Analyses

All the AGE analyses were carried out in triplicate and data has been presented as mean±SD. For all data, univariate analysis of variance (ANOVA) with Tukey's post-hoc test was applied using the SPSS 26.0 package programme. Statistical significance was accepted as $p<0.05$ in all analyses.

3. Results

3.1. MGO and GO analyses of samples

As shown in Table 2, the MGO content of the samples varied between 122.3-284.0 µg/100 g. There is a significant difference between chocolate types and MGO content ($p<0.05$). The sample with the highest MGO content was white chocolate 10 g (284.0 µg/100 g) and the sample with the lowest MGO content was milk chocolate 10 g (122.3 µg/100 g). White chocolate was found to have the highest MGO content among the control samples (240.6±11.0 µg/100 g). Following white chocolate, the order was milk chocolate and dark chocolate (196.6±9.0 µg/100 g and 140.3±6.5 µg/100 g, respectively). Among the dark chocolate samples, the highest MGO content was found in the sample with 20 g CM addition (271.0 µg/100 g), while the lowest contents were found in the samples with 10 g CM addition (140.3 µg/100 g) and control (140.3 µg/100 g). The analysis revealed that among the milk chocolate samples, the sample with 10 g CM addition (122.3 µg/100 g) contained statistically significantly lower MGO than the other milk chocolate samples. Of the white chocolates, the MGO content was found to be statistically significantly highest in the white chocolate with 10 g CM addition (284.0 µg/100 g).

Table 2
MGO content of samples.

Chocolate Sample	Mean±SD (µg/100 g)	95% CI	*p* Value
Dark chocolate - control	$140.3^{a,b}$±6.5	124.1-156.4	< 0.001
Dark chocolate + 10 g CM	$140.3^{a,b}$±6.5	124.1-156.4	
Dark chocolate + 15 g CM	$167.3^{b,c}$±7.5	148.6-185.9	
Dark chocolate + 20 g CM	$271.0^{f,g}$±12.5	239.8-302.1	
Milk chocolate - control	196.6^{d}±9.0	174.2-219.0	
Milk chocolate + 10 g CM	122.3^{a}±5.5	108.6-136.0	
Milk chocolate + 15 g CM	$193.6^{c,d}$±9.0	171.2-216.0	
Milk chocolate + 20 g CM	211.0^{d}±9.5	187.3-234.6	
White chocolate - control	240.6^{e}±11.0	213.3-268.0	
White chocolate + 10 g CM	284.0^{g}±12.5	252.8-315.1	
White chocolate + 15 g CM	$251.0^{e,f}$±11.5	222.3-279.6	
White chocolate + 20 g CM	241.6^{e}±11.0	214.3-269.0	

CM: *Cornus mas*. L.; SD: Standard Deviation; CI: Confidence Interval. The one-way analysis of variance (ANOVA) was used. There is a significant difference between values with different letters in the superscripts in the column.

Table 3
GO content of samples.

Chocolate Sample	Mean±SD (µg/100 g)	95% CI	*p* Value
Dark chocolate - control	268.6^{h}±12.0	238.8-156.4	< 0.001
Dark chocolate + 10 g CM	51.3^{c,d}±2.5	45.0-156.4	
Dark chocolate + 15 g CM	63.0^{d,e}±3.0	55.5-185.9	
Dark chocolate + 20 g CM	61.3^{c,d,e}±2.5	55.0-302.1	
Milk chocolate - control	113.3^{g}±5.5	99.6-219.0	
Milk chocolate + 10 g CM	67.0^{e}±3.0	59.5-136.0	
Milk chocolate + 15 g CM	63.0^{d,e}±3.0	55.5-216.0	
Milk chocolate + 20 g CM	37.3^{b}±1.5	33.3-234.6	
White chocolate - control	14.0^{a}±1.0	11.5-268.0	
White chocolate + 10 g CM	48.0^{b,c}±2.0	43.0-315.1	
White chocolate + 15 g CM	86.3^{f}±3.5	77.6-279.6	
White chocolate + 20 g CM	85.3^{f}±3.5	76.6-269.0	

CM: *Cornus mas*. L.; SD: Standard Deviation; CI: Confidence Interval. The one-way analysis of variance (ANOVA) was used. There is a significant difference between values with different letters in the superscripts in the column.

The GO content of the samples ranged from 14.0-268.6 µg/100g (Table 3). There was a significant difference between chocolate types and GO contents (p<0.05). Among the control groups, dark chocolate had the highest GO content (268.6±12.0 µg/100 g). Milk chocolate and white chocolate followed by dark chocolate (113.3±5.5 µg/100 g and 14.0±1.0 µg/100 g, respectively). Among the dark chocolate samples, the highest GO content was found in the dark control (268.6 µg/ 100 g) and the GO content of all dark samples to which CM was added was statistically lower than that of the control sample. Among the milk chocolate samples, the highest amount of GO was found in the control milk chocolate (113.3 µg/100 g), and the lowest amount was found in the milk chocolate with 20 g CM (37.3 µg/100 g), and there was a statistically significant difference between them. Among the white chocolate samples, the highest GO content was found in the samples with 15 g CM (86.3 µg/100 g) and 20 g CM (85.3 µg/100 g), while the lowest GO content was found in the control chocolate (14.0 µg/100 g).

3.2. Sensory analysis results of samples

For sensory analysis, the samples were evaluated by trained panelists using the single-blind method. The panelists were between the ages of 18-65 and included 14 participants. The results of sensory analysis of dark, milk and white chocolate samples were presented as mean scores in Table 4. According to the general acceptance criteria, it was determined that the most liked sample after the control groups was 10 g CM added white chocolate (3.86±0.86). Taste, bitter taste, melt-in-the-mouth, texture, hardness, sour taste, and general acceptance criteria showed differences between the samples (p<0.05).

Among the dark chocolate samples, the most liked sample in terms of overall acceptance was the control sample (4.29±0.83), while the least liked sample was the 20 g CM added dark chocolate (3.07±1.07). In terms of the bitter taste criterion, the most bitter sample was the control dark chocolate (4.21±0.89). Among the dark chocolates, the dark chocolates with 10 g CM and 20 g CM (3.36±0.93 and 3.36±1.22, respectively) scored the lowest in terms of texture. When they were evaluated in terms of sourness, the control sample (4.36±1.08) received the highest appreciation, and the samples with 15 g CM added, and 20 g CM added (2.79±0.89 and 2.86±1.10, respectively) received the lowest score.

Table 4
Sensory analysis results of chocolate samples.

Criteria	Samples												*p*
	Dark chocolate				Milk chocolate				White chocolate				
	C	10 g	15 g	20 g	C	10 g	15 g	20 g	C	10 g	15 g	20 g	
Color	4.57±0.51	4.29±0.73	4.64±0.63	4.36±0.84	4.71±0.47	4.29±0.83	3.93±0.83	3.50±1.40	4.29±0.83	4.36±0.93	4.43±0.76	4.50±0.65	0.091
Smell	4.50±0.52	4.00±0.68	4.29±0.61	3.93±0.83	4.50±0.52	4.29±0.47	4.07±0.73	3.93±1.00	4.14±0.66	4.14±0.86	3.86±0.86	3.86±1.10	0.395
Taste	4.43^{b}±1.09	3.29^{a}±0.99	3.50^{a,c}±1.09	3.14^{a}±1.17	4.29^{b,c}±0.83	3.57^{a,c}±0.76	3.07^{a}±1.00	2.93^{a}±1.07	3.57^{a,c}±1.22	3.71^{a,b,c}±1.14	3.14^{a}±1.17	3.29^{a}±1.14	0.002
Bitter taste	4.21^{c}±0.89	3.29^{a}±1.38	3.50^{a,b}±1.22	3.43^{a,b}±1.09	4.36^{c}±0.84	3.43^{a}±0.65	3.21^{a}±0.97	3.21^{a}±1.12	4.07^{b,c}±0.62	4.21^{b,c}±0.70	3.29^{a}±1.07	3.50^{a,b}±0.94	0.002
Oily taste	4.14±1.03	3.64±1.15	3.50±1.09	3.57±1.02	3.64±1.15	3.57±0.85	3.07±1.00	2.93±1.33	3.21±1.31	3.43±1.09	3.29±1.20	3.50±1.09	0.316
Melting in the mouth	4.36^{b}±0.84	3.43^{a}±1.02	3.50^{a}±1.02	3.36^{a}±1.15	4.21^{b}±0.89	3.21^{a}±1.12	3.00^{a}±1.04	2.86^{a}±1.10	4.29^{b}±0.61	3.43^{a}±1.02	3.21^{a}±1.05	3.00^{a}±1.11	<0.001
Texture	4.43^{b}±0.85	3.36^{a}±0.93	3.64^{a}±1.08	3.36^{a}±1.22	4.50^{b}±0.65	3.36^{a}±0.93	3.14^{a}±0.95	3.07^{a}±0.92	4.43^{b}±0.65	3.71^{a}±0.91	3.14^{a}±1.03	3.14^{a}±1.17	<0.001
Hardness	4.50^{b}±0.65	3.79^{a}±0.89	3.79^{a}±1.19	3.86^{a}±1.03	4.14^{b}±0.86	3.93^{a}±0.83	3.36^{a}±1.01	3.14^{a}±1.03	4.29^{b}±0.83	4.14^{b}±0.77	3.64^{a}±0.93	3.36^{a}±0.84	<0.001
Stickiness	4.00±1.18	3.71±1.20	3.43±0.94	3.50±1.02	3.86±1.03	3.79±0.70	3.36±0.93	3.21±1.05	4.07±0.83	3.86±0.77	3.57±0.85	3.43±0.76	0.188
Sour taste	4.36^{b}±1.08	2.93^{a,c}±1.27	2.79^{a}±0.89	2.86^{a,c}±1.10	4.21^{b}±0.89	2.93^{a,c}±1.07	2.93^{a,c}±0.92	2.43^{a}±0.85	4.07^{b}±0.83	3.64^{b,c}±0.93	2.93^{a,c}±1.14	3.21^{a,c}±0.97	<0.001
Overall Acceptance	4.29^{b}±0.83	3.36^{a,c}±1.08	3.50^{a,c,d}±1.02	3.07^{a}±1.07	4.29^{b}±0.61	3.64^{a,b,c,d}±0.74	3.29^{a,c}±0.91	2.93^{a}±1.07	4.07^{b,d}±1.00	3.86^{b,c}±0.86	3.21^{a,c}±0.80	2.93^{a}±0.92	<0.001

C; control. Values are expressed as mean ± standard deviation. The one-way analysis of variance (ANOVA) was used. There is a significant difference between values with different letters in the superscripts in the rows (p < 0.05).

In terms of overall acceptance of the milk chocolate samples, the control chocolate had the highest score (4.29±0.61), while the milk chocolate with 20 g CM added had the lowest score (2.93±1.07). When the milk chocolate samples were evaluated in terms of taste and fatty taste criteria, it was found that the sample with 20 g CM added received the lowest score (2.93±1.07 and 2.93±1.33, respectively). The milk chocolate sample with the best melt-in-the-mouth score was the control chocolate (4.21±0.89). Among these chocolates, in terms of texture criterion, the milk chocolate with 20 g CM added had the lowest score (3.07±0.92). For sourness, the highest score was given to the control sample (4.21±0.89), and the lowest score was given to the sample with 20 g CM (2.43±0.85).

In terms of overall acceptance of the white chocolate samples, the control sample received the highest score, while the sample with 20 g CM added received the lowest score (4.07±1.00 and 2.93±0.92, respectively). Among the white chocolates, the sample that had the lowest score in terms of taste was the sample to which 15 g CM was added (3.14±1.17). According to the melting in the mouth and texture criteria, the white chocolate sample with the highest score was the control (4.29±0.61 and 4.43±0.65, respectively), while the sample with the lowest score was the sample with 20 g CM (3.00±1.11 and 3.14±1.17, respectively). In terms of sourness, it was determined that the sample with the highest score was the control (4.07±0.83) and the lowest score was the white chocolate with 15 g CM (2.93±1.14).

4. Discussion

This study investigated the GO and MGO contents and sensory parameters of dark, milk, and white chocolates to which different amounts of lyophilized cornelian cherry (*C. mas* L.) powder were added. Among the tested samples, milk chocolate showed a notable reduction in GO levels as the number of *C. mas* L. powder increased, highlighting its potential role in mitigating AGE formation. Sensory analysis revealed that while control samples were most favored, white chocolate with 10 g *C. mas* L. powder was the most acceptable among the enriched samples.

The main ingredients of chocolate are cocoa butter, cocoa powder, milk, and sugar. The formation of AGEs in chocolate is dependent on several factors, including lipid oxidation, sugar autoxidation, nutrient composition, moisture content, pH, and thermal processing conditions. Prolonged storage can also influence the formation of AGEs. Cintesun et al. (2022) found that the content of GO and MGO in chocolates varied between 20-258 µg/100 g and 50-274 µg/100 g, respectively. The contents of GO and MGO in the chocolates used in our study were found to vary between 14-268.6 µg/100 g and 122.3-284 µg/100 g, respectively. It is thought that these differences in the results are due to the type, content, and brands of chocolates used.

It has been reported that the MGO and GO contents of foods with high carbohydrate content are high. In addition to carbohydrates, it has been shown in the literature that there is a positive correlation between the fat and protein contents of foods and MGO and GO contents (Yilmaz and Karabudak, 2016; Maasen et al., 2021). Among the chocolates used in this study, although milk chocolate had the highest carbohydrate content, MGO and GO contents of milk chocolate were lower than dark and white chocolate. It is thought that this may be due to the fact that milk chocolate has the lowest fat content. It was observed that MGO contents were generally high in white chocolate samples, which had the highest fat content among the chocolate types used in this study. In addition, an increase in GO contents was detected in proportion to the amount of CM added. Since white chocolate contains cocoa butter rather than cocoa beans, it is thought to have very low polyphenols and therefore low antioxidant capacity. For this reason, CM-added chocolates prepared with white chocolate may have been insufficient to inhibit AGE precursors formed during production.

The reducing effect of polyphenols on AGE formation is based on increasing the antioxidant capacity of the added food and binding dicarbonyls (Yalcin and Rakicioglu, 2022). In a study by Ede-Cintesun et al. (2024), the GO and MGO contents of different types of chocolate available in the markets were analyzed and it was shown that the GO content of white chocolate containing raspberries was lower and the MGO content was higher than plain white chocolate. In the same study, it was reported that dark chocolate containing mulberry had higher GO and MGO content than plain dark chocolate (Ede-Cintesun et al., 2024). In a study conducted by Yusufoglu et al. (2020), it was reported that fruit-based heat-treated foods contain high amounts of AGE precursors and the reason for this was caramelization and sugar autooxidation occurring during production (Yusufoglu et al., 2020). In the study of Karatay (2022), it was observed that the highest GO content among the protein bars in the market was in the forest fruit protein bar and it was thought that the reason for this may be the fructose contents in the heat-treated fruit. Cintesun et al. (2022) also found that fruit cake had the highest GO and MGO contents among cakes in a study on snacks. In our study, the drying of CM by lyophilization method and the fact that the chocolates were below 40°C when added to the chocolates ensured that the heat treatment of CM was kept at a minimum level. It is thought that the decrease in GO contents in milk and dark chocolate samples to which CM was added may be related to this. However, despite antioxidant components like polyphenols in cocoa and CM, it was observed that MGO contents in the samples did not decrease and even showed an increase. These differences in the results may be due to the complex composition of the chocolates and the increased carbohydrate content with the added CM.

Although CM added to different chocolate samples has been shown to affect MGO and GO contents, it is also important to evaluate the consumability of these chocolates. In a study conducted by Uzumcu and Ozsisli (2023), varying quantities of blueberries and oatmeal were incorporated into milk chocolates. The results of the sensory analysis indicated that the control groups received the highest scores, while the chocolates containing 40% and 50% additives received the lowest scores. Furthermore, the impact of oatmeal and blueberry-added chocolates on overall acceptability was found to be statistically insignificant. In another study, pomegranate seeds and pomegranate jelly were added to white and milk chocolates to investigate the sensory properties of these chocolates, and the white chocolate sample with pomegranate jelly received the highest scores in terms of color, smell, and taste. However, in terms of consumption and consumer acceptability, it was noted that milk chocolate with pomegranate jelly was the most preferred chocolate sample overall (Yildirim et al., 2016). According to our results of the sensory analyses, it was found that milk chocolate with 10 g CM, which was one of the most successful samples in terms of reducing MGO and GO contents, was also similar to the milk control in terms of overall

acceptability. Among the chocolates to which CM was added, 10 g CM added milk chocolate had the highest score after 10 g CM added white chocolate. The reason for the 10 g CM added white chocolate having the highest overall score is that it has the highest score in terms of color. On the other hand, the sample with the lowest appreciation score was found to be dark chocolate with 20 g CM powder added. Nevertheless, in contrast to the findings of the study by Uzumcu and Ozsisli (2023), the impact of CM-added chocolate groups on overall acceptability was determined to be statistically significant. However, similar to the study by Yildirim et al. (2016), the addition of a red fruit increased the acceptability of white chocolates in terms of color criterion.

The inclusion of three different control groups in this study enabled comparisons with the test groups and increased the reliability of the data obtained. However, the analysis of AGE precursors (MGO and GO) instead of AGEs presents a limitation, as it does not directly quantify the content of AGEs in the products. Moreover, it does not allow for determining the extent to which these precursors are converted into AGEs within the organism. Another limitation is that only one brand of chocolate was analyzed, which restricts the generalizability of the findings. Future research could address this limitation by including chocolates from different brands and with varying cocoa ratios. It is also recommended that future studies analyze the total antioxidant capacity and anthocyanin content of chocolates to obtain more accurate and comprehensive results. Nonetheless, to the best of our knowledge, no previous studies have focused on determining AGE precursors in cornelian cherry (*C. mas* L.) powder-enriched chocolate samples. This study represents a novel approach, combining the determination of AGE precursors and sensory analysis for chocolates enriched with *C. mas* L. powder, providing a foundation for more extensive research in this area.

5. Conclusion

As a result of the analysis of the samples prepared in the study, it was observed that the MGO amounts were generally high, but the GO content decreased. It is known that foods with high antioxidant content, such as CM, affect the AGE content. For this reason, new studies are necessary to better understand the reasons for these changes in MGO and GO. When we look at the literature and the results of this study, one area requiring more detailed examination is the formation mechanism of MGO and GO. Upon examining the contents of these two AGE precursors, which share similar pathways, in chocolate samples, it is observed that there is no consistent decrease, particularly in dark and white chocolates, with MGO contents remaining elevated. However, assessing the total antioxidant capacity of chocolate and cornelian cherry may offer a more accurate interpretation on this subject.

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Conflict of interest: The authors declare that they have no conflict of interests.

Ethical Approval: Sensory analysis of this study was approved ethically by the Marmara University Faculty of Health Sciences Non-Invasive Clinical Studies Ethics Committee (Protocol no: 2022/181) and the research was conducted following the principles stated in the Helsinki Declaration.

References

Bayram, H. M., & Ozturkcan, S. A. (2020). Bioactive components and biological properties of cornelian cherry (*Cornus mas* L.): A comprehensive review. *Journal of Functional Foods, 75*, 104252.

Bayram, H. M., Iliaz, R., & Gunes, F. E. (2024a). Effects of *Cornus mas* L. on anthropometric and biochemical parameters among metabolic associated fatty liver disease patients: Randomized clinical trial. *Journal of Ethnopharmacology, 318*, 117068.

Bayram, H. M., Ozkan, K., Ozturkcan, A., Sagdic, O., Gunes, E., & Karadag, A. (2024b). Effect of drying methods on free and bound phenolic compounds, antioxidant capacities, and bioaccessibility of Cornelian cherry. *European Food Research and Technology*, 1-18.

Bruinsma, K., & Taren, D. L. (1999). Chocolate: food or drug?. *Journal of the American Dietetic Association, 99*(10), 1249-1256.

Catak, J., & Balci, E. (2022). Paketli keklerde ileri glikasyon son ürünleri öncüllerinin hplc ile tespiti ve değerlendirilmesi. *Journal of Characterization, 2*(1), 82-90.

Celik, Z. M., Sargin, M., Tamer, H. G., & Gunes, F. E. (2023). The effect of lyophilized dried cornelian cherry (*Cornus mas* L.) intake on anthropometric and biochemical parameters in women with insulin resistance: A randomized controlled trial. *Food Science & Nutrition, 11*(12), 8060-8071.

Cengiz, S., Kismiroglu, C., Cebi, N., Catak, J., & Yaman, M. (2020). Determination of the most potent precursors of advanced glycation end products (AGEs) in chips, crackers, and breakfast cereals by high performance liquid chromatography (HPLC) using precolumn derivatization with 4-nitro-1, 2-phenylenediamine. *Microchemical Journal, 158*, 105170.

Chen, X. Y., Huang, I. M., Hwang, L. S., Ho, C. T., Li, S., & Lo, C. Y. (2014). Anthocyanins in blackcurrant effectively prevent the formation of advanced glycation end products by trapping methylglyoxal. *Journal of Functional Foods, 8*, 259-268.

Cintesun E.E., Yaman, M., Tanyildiz, S.N., Yildirim, H., & Mizrak, Ö.F. (2022). Investigation of the α-Dicarbonyl compounds in some snack foods by HPLC using precolumn derivatization with 4-Nitro-1,2-Phenylenediamine. *Biointerface Research in Applied Chemistry, 12*(2), 2242-2250.

Comert, T. K., & Merdol, T. K. (2018). Çikolata ve sağlık beyanları. *Beslenme ve Diyet Dergisi, 46*(1), 56-65.

Dilinger, T. L., Barriga, P., Escárcega, S., Jimenez, M., Lowe, D. S., & Grivetti, L. E. (2000). Food of the gods: cure for humanity? A cultural history of the medicinal and ritual use of chocolate. *The Journal of nutrition, 130*(8), 2057S-2072S.

Ede-Cintesun, E., Catak, J., Ateş, E., & Yaman, M. (2024). Glyoxal and methylglyoxal formation in chocolate and their bioaccessibility. *Food Research International*, 114552.

Ellam, S., & Williamson, G. (2013). Cocoa and human health. *Annual Review of Nutrition, 33*(1), 105-128.

Goldberg, T., Cai, W., Peppa, M., Dardaine, V., Baliga, B. S., Uribarri, J., & Vlassara, H. (2004). Advanced glycoxidation end products in commonly consumed foods. *Journal of the American Dietetic Association, 104*(8), 1287-1291.

Hsiao, Y. W., Hsia, S. M., Pan, M. H., Ho, C. T., & Hung, W. L. (2024). Berry anthocyanins prevent α-dicarbonyls and advanced glycation end product formation in phosphate-buffered saline-based model systems, cookie and ground pork. *Journal of Food Science, 89*(6), 3745-3758.

Javed, F., Shahbaz, H. M., Nawaz, A., Olaimat, A. N., Stratakos, A. C., Wahyono, A., ... & Park, J. (2021). Formation of furan in baby food products: Identification and technical challenges. *Comprehensive Reviews in Food Science and Food Safety, 20*(3), 2699-2715.

Karatay, B. (2022). Piyasada proteinli veya vegan protein kaynağı olarak satışa sunulan bazı ürünlerin glioksal ve metilglioksal düzeylerinin belirlenmesi, Master's Thesis, İstanbul Sabahattin Zaim Üniversitesi, İstanbul, 1-81.

Katz, D. L., Doughty, K., & Ali, A. (2011). Cocoa and chocolate in human health and disease. *Antioxidants & Redox Signaling, 15*(10), 2779-2811.

Kou, Y., Song, Z., Jing, Y., Li, H., Wei, X., Xie, J., & Shen, M. (2024). Key Maillard intermediates-α-dicarbonyl compounds in foods: Occurrence, analysis, toxicity, and mitigation strategies. *Food Control*, 110652.

Lidiková, J., Čeryová, N., Grygorieva, O., Bobková, A., Bobko, M., Árvay, J., ... & Kňazovická, V. (2024). Cornelian cherry (*Cornus mas* L.) as a promising source of antioxidant phenolic substances and minerals. *European Food Research and Technology*, *250*(6), 1745-1754.

Maasen, K., Scheijen, J. L., Opperhuizen, A., Stehouwer, C. D., Van Greevenbroek, M. M., & Schalkwijk, C. G. (2021). Quantification of dicarbonyl compounds in commonly consumed foods and drinks; presentation of a food composition database for dicarbonyls. *Food Chemistry*, *339*, 128063.

Nowotny, K., Schröter, D., Schreiner, M., & Grune, T. (2018). Dietary advanced glycation end products and their relevance for human health. *Ageing Research Reviews*, *47*, 55-66.

O'Brien, J., Morrissey, P. A., & Ames, J. M. (1989). Nutritional and toxicological aspects of the Maillard browning reaction in foods. *Critical Reviews in Food Science & Nutrition*, *28*(3), 211-248.

Sharma, C., Kaur, A., Thind, S. S., Singh, B., & Raina, S. (2015). Advanced glycation end-products (AGEs): an emerging concern for processed food industries. *Journal of Food Science and Technology*, *52*(12), 7561-7576.

Singh, R. B. A. M., Barden, A., Mori, T., & Beilin, L. (2001). Advanced glycation end-products: a review. *Diabetologia*, *44*(2), 129-146.

Szot, I., Łysiak, G. P., Sosnowska, B., & Chojdak-Łukasiewicz, J. (2024). Health-promoting properties of anthocyanins from cornelian cherry (*Cornus mas* L.) fruits. *Molecules*, *29*(2), 449.

Tan, H., Cui, B., Zheng, K., Gao, N., An, X., Zhang, Y., ... & Li, B. (2024). Novel inhibitory effect of black chokeberry (*Aronia melanocarpa*) from selected eight berries extracts on advanced glycation end-products formation and corresponding mechanism study. *Food Chemistry: X*, *21*, 101032.

Tian, Z., Chen, S., Shi, Y., Wang, P., Wu, Y., & Li, G. (2023). Dietary advanced glycation end products (dAGEs): An insight between modern diet and health. *Food Chemistry*, *415*, 135735.

Ulrich, P., & Cerami, A. (2001). Protein glycation, diabetes, and aging. *Recent progress in hormone research*, *56*(1), 1-22.

Uribarri, J., Woodruff, S., Goodman, S., Cai, W., Chen, X. U. E., Pyzik, R., ... & Vlassara, H. (2010). Advanced glycation end products in foods and a practical guide to their reduction in the diet. *Journal of the American Dietetic Association*, *110*(6), 911-916.

Uzumcu, Z., & Ozsisli, B. (2023). Yulaf ezmeli ve yaban mersinli çikolatanın bazı özelliklerinin belirlenmesi. *Turkish Journal of Agriculture-Food Science and Technology*, *11*(3), 478-484.

Wei, Q., Liu, T., & Sun, D. W. (2018). Advanced glycation end-products (AGEs) in foods and their detecting techniques and methods: A review. *Trends in Food Science & Technology*, *82*, 32-45.

Yan, S., Wu, L., & Xue, X. (2023). α-Dicarbonyl compounds in food products: Comprehensively understanding their occurrence, analysis, and control. *Comprehensive Reviews in Food Science and Food Safety*, *22*(2), 1387-1417.

Yalcin, E., & Rakicioglu, N. (2022). Besinlerde oluşan ileri glikasyon son ürünlerine polifenollerin etkisi. *Beslenme ve Diyet Dergisi*, *50*(2), 66-75.

Yildirim, A., Cetin, S., Ogretmen, H., Sarı, P., & Hayoğlu, I. (2016). Narın çikolata üretiminde kullanımı. *Harran Tarım ve Gıda Bilimleri Dergisi*, *20*(1), 12-19.

Yilmaz, B., & Karabudak, E. (2016). Besinlerdeki ileri glikasyon son ürünleri ve azaltma yöntemleri. *Beslenme ve Diyet Dergisi*, *44*(3): 280–288.

Yusufoglu, B., Yaman, M., & Karakus, E. (2020). Determination of the most potent precursors of advanced glycation end products in some high-sugar containing traditional foods using high-performance liquid chromatography. *Journal of Food Processing and Preservation*, *44*(9), e14708.

Zhang, Q., Li, H., Zheng, R., Cao, L., Zhang, S., Zhang, S., ... & Fu, L. (2024). Comprehensive analysis of advanced glycation end-products in commonly consumed foods: presenting a database for dietary AGEs and associated exposure assessment. *Food Science and Human Wellness*, *13*(4), 1917-1928.

Zhu, J., Wang, Z., Lv, C., Li, M., Wang, K., & Chen, Z. (2024). Advanced Glycation End Products and Health: A Systematic Review. *Annals of Biomedical Engineering*, 1-12.

Zgutka, K., Tkacz, M., Tomasiak, P., & Tarnowski, M. (2023). A role for advanced glycation end products in molecular ageing. *International Journal of Molecular Scien*ces, *24*(12), 9881.

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Research article

Epiphytic bacterial community analysis of the macroalgae *Gongolaria barbata* collected from the Sinop region on the Black Sea coast

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Abstract

The purpose of this study was to ascertain the epiphytic bacterial community structure of macroalgae *Gongolaria barbata* (Stackhouse) Kuntze samples taken from seawater using Single Strand Conformation Polymorphism (SSCP) analysis. It also aims to quickly obtain information regarding the composition of communities and the quality of the seawater. *G. barbata* samples were subjected to total DNA extraction, SSCP analysis was conducted with a focus on the V4-V5 region of 16S rRNA, and the bacterial community structure was determined through sequence analysis of a few chosen bands. Upon analyzing the SSCP gel picture and dendrogram, it was seen that the bacterial community structure on the macroalgae varied based on the location as well as within the same species. It was noted that the *Gammaproteobacteria* class accounted for 84.375 percent of the bands that were acquired from the SSCP analysis. The fact that the sequencing data generated from the bands collected at various points largely resembled *Vibrio* and *Klebsiella* genera was notable. This situation highlights the strong link between harmful or opportunistic infectious organisms and macroalgae species, several of which have been suggested for ingestion as food. Furthermore, even if research in the literature suggests that the macroalgae and the microbial load of the nearby water sample do not significantly correlate, we can conclude that this data suggests the possibility of risk.

Keywords: *Gongolaria barbata; bacterial community; SSCP; water quality; infectious agents*

1. Introduction

Macroalgae are multicellular, sessile, photosynthetic eukaryotic organisms that function as primary producers in marine environments by giving a variety of creatures food and shelter (Florez et al., 2017; Xiao et al., 2024). Microbial communities have a place to live on the surfaces of organisms that inhabit the maritime environment. The region of close algae-bacteria interactions is termed the "phycosphere" (Lu et al., 2023). In this sense, microbial communities benefit greatly from the shelter provided by macroalgae. The ability of macroalgae to create oxygen and organic matter, together with the bacterium's availability of minerals and CO_2, determines the connection between macroalgae and bacteria. Additionally, it is known that certain bacteria release regulatory factors resembling auxin and cytokinin, which promote increased plant cell division (Singh and Reddy, 2014; Comba-González et al., 2016).

Exchange activities including waste products, secondary metabolites, and nutrient uptake and release are all carried out on algal surfaces. On these surfaces, bacteria create a biofilm layer and encounter the seaweeds. It impacts the host organism's resistance, performance, and general health in this way. Additionally, it controls the ingress of light, gas, nutrients, pathogens, consumers, and other epibionts that form biofilms in seaweed (Mancuso et al., 2016; Nahor et al., 2024). It is well recognized that the host conditions, in addition to space and time, affect the structure and composition of the microbiota linked to seaweed. For instance, it has been noted that distinct microbial communities are displayed by stressed versus healthy *Ecklonia radiata*. It has also been demonstrated that seasonal

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variations have an impact on the variations in microbial populations. Abiotic variables like water temperature and biotic parameters like the age of the algal tissue and seaweed growth cycle play significant roles in these seasonal fluctuations (Bengtsson et al., 2010; Serebryakova et al., 2018). Macroalgae are regarded as the ecosystem's engineers as they are crucial to the structure of coastal communities. Accordingly, a significant decline in biodiversity is thought to result from the loss of macroalgae in coastal environments (Burke et al., 2011). Bacteria known as the *Vibrio* genus can be found in a wide range of habitats, including aquatic and marine ones. Particularly in humans, these bacteria can lead to illnesses both inside and beyond the gastrointestinal tract. There are almost 200 serogroups of *Vibrio cholerae* in this genus of bacteria. Serogroups O1 and O139 have the potential to become epidemics. When the water is at its hottest, which is in late summer and early autumn, they increase illnesses (Araj, 2019). For thousands of years, wild macroalgae have been consumed by humans. Nowadays, aquaculture methods are used to generate the majority of macroalgae species. Although it has not been assessed, it has been suggested that macroalgae collected from coastal waters might be near a source of potentially harmful bacteria to humans. Fecal coliforms play a crucial role in the digestive system as they are naturally occurring bacterial colonizers and are most prevalent in coastal waters. One of the members of this group, *Escherichia coli*, is regarded as both a pathogen and a sign of fecal contamination. Fecal coliforms are therefore indicative of gastrointestinal pathogen infection in food and drink. In addition to fecal coliforms, nutritional safety is also correlated with the presence of *Vibrio*, a naturally occurring microbe found in coastal waters. This species has certain diseases that affect humans. According to Barberi et al. (2020), *Vibrio parahaemolyticus* is the primary cause of food-borne gastroenteritis in the United States.

To identify bacterial species from environmental samples, numerous techniques are employed. The culture and isolation approach are the most important of them. However, bacterial diversity and isolates that are not identified in that environment are difficult or impossible to detect with this technique. Rather, it was mentioned that molecular methods based on 16S rRNA amplification and sequence analysis would be used to tackle this issue (Agrawal et al., 2015; Franco-Duarte et al., 2019). In addition, many methods based on metagenomics, meta-transcriptomics, metabolomics, and other omics-based techniques have been applied to detect microbial communities in their natural environment (Vigil et al., 2024). Thus, the synergistic use of both culture-dependent and independent approaches will enable the identification of not only dominant microorganisms but also rare taxonomic members (Girão et al., 2024). Here, it is becoming increasingly important to investigate epiphytic bacterial communities in different macroalgae in more detail using new technologies, especially to enable the production of various metabolites through biotechnological applications (Kaur et al., 2023).

In the Black Sea region, edible brown seaweed called *Gongolaria barbata* is typically utilized as a functional food. Because of their high concentration of bioactive compounds, vitamins, minerals, polyphenols, fatty acids, and peptides, edible seaweeds have significant value as functional foods. They are effective against a variety of diseases, including cancer, heart disease, type 2 diabetes, and autoimmune diseases (Trica et al., 2019).

However, Barberi et al. (2020), there is little research on the contamination of sea vegetable products with harmful bacteria in the literature review. Once more, Barberi et al. (2020) revealed that several researchers in Europe found diseases in natural macroalgae. Specifically, they found *Listeria monocytogenes* (Blikra, 2019) in Norway, coliform, enterococci, and *Vibrio* in macroalgae in Japan (Mahmud, 2008). Our analysis of the literature indicates that no study has been conducted in Turkey that thoroughly screens for bacterial pathogens on macroalgae. The purpose of this study was to identify the epiphytic bacterial community of *G. barbata* samples that were taken from the Black Sea-coasting province of Sinop.

2. Materials and methods

2.1. Collection of samples and isolation of DNA

Samples were taken from Sinop's coast at a depth of 0.2-2 meters (Fig. 1) in July 2019. Following collection, the specimens were cleaned, and vouchers were created. DNA was extracted from the apical section of every thallus. A CTAB protocol modification was implemented (Wichachucherd et al., 2014). First, 500 µl of CTAB buffer was used to grind the tissue samples using a tissue grinder. The tissue was ground and then incubated for 20 minutes at 60°C. After thoroughly mixing the aqueous phase twice using CIA (chloroform: isoamyl alcohol, 24:1), the mixture was centrifuged at 15000 g for 10 minutes at +4°C. Lastly, an equivalent volume of cool isopropanol was added to the aqueous phase. At -20°C, the sample was incubated for an entire night. Following the incubation for the entire night, the tube was centrifuged at 15000 g for 20 minutes. After washing the DNA pellet in 70% ethanol, the sample was centrifuged at 15000 g for an additional 10 minutes. The DNA was removed from the ethanol, allowed to dry at room temperature, and then kept in Tris-EDTA buffer until needed.

Fig. 1. Station map where *G. barbata* specimens were collected. S1, Gerze; S2, Sinop inner port; S3, Akliman; S4, Inceburun; S5, Ayancik (Map taken from Google maps).

2.2. SSCP analysis of 16S rRNA gene regions for culture-independent community structure

In a prior study conducted in our lab, the steps for SSCP, DNA recovery from gel, and sequence data processing were thoroughly described (Avsar and Aras, 2020). The methods will be briefly discussed below. Com1 and modified com2-Ph primers specific for the 16S rRNA gene region were utilized for SSCP analysis. The techniques of Schwieger and Tebbe (2000)

and Smalla et al. (2007) were used to achieve the SSCP analysis. Using the Expin Combo GP (GeneAll) purification kit, PCR products were purified. To cleave the phosphorylated chain, 700 ng of the purified PCR product was incubated with 5U Lambda-exonuclease (Thermo Fisher Scientific, California) for 1 hour at 37°C. Using the Expin Combo GP (GeneAll) purification kit, single-chain DNA was purified. A 10 µL purified single-chain DNA sample was placed into four microliters of loading solution, which contained 95% formamide, 10 mmol/L NaOH, 0.025% bromophenol blue, and 0.025% xylene cyanol. After the samples were promptly chilled on ice after being denatured for two minutes at 95°C, 5 µL was put onto the gel. Using Hoofer (SE400, USA) equipment, a mixture of 0.6X MDE (Mutation Detection Enhancement, Thermo Fisher Scientific, Lonza) gel was produced for electrophoresis. For 36 hours, the gel was operated at 5 mA, 200 V, and 20°C. The silver staining approach was used after the DNA profiles from the gel were seen using Byun et al. (2009).

A sterile scalpel was used to cut any dominating or solitary bands found on the polyacrylamide gel after silver staining to prepare them for further examination. After the gel fragments were transferred, 100 µL of elution liquid (0.5 mol/L ammonium acetate, 10 mmol/L Mg^{2+}-acetate, 1 mmol/L EDTA [pH 8.0], and 0.1% SDS) was added to the microtubes. After three hours of incubation at 37°C, the tubes were centrifuged at 12,000×g for one minute at room temperature. Two volumes of cold ethanol (96%) were added to 80 µL of the supernatant, which was then transferred to a micro test tube to precipitate. Following a 7-minute centrifugation at 12,000×g, the DNA was allowed to dry at 30°C for 30 minutes before being dissolved in 10 mmol/L of Tris-HCl at pH 8.0. This solution's target DNA for PCR processing was two microliters (Schwieger and Tebbe, 1998). As previously mentioned, the Com1 and Com2 primer PCR procedure was carried out.

Sequencing was done on amplified products (BM Labs, Ankara). Technelysium Pty Ltd.'s Chromas version 2.24 software was used to alter the sequencing data. The alignment of these sequences was done with Clustal W (Version 2.1). The NCBI GenBank database's BLAST search was used to compare 16S rRNA sequences of bacteria that are phylogenetically related. PyElph version 1.4 was used to produce the UPGMA dendrogram analysis of the SSCP profiles of the bacterial populations. Using Molecular Evolution Genetic Analysis (MEGA), phylogenetic trees were created, and the sequences were corrected and aligned using the Clustal W tool.

3. Results and discussion

The bacterial population from our investigation, which was derived from SSCP analysis of *G. barbata* samples collected from 17 distinct locations, is displayed in Fig. 2. Based on the acquired results, it was noted that samples taken from the same and various sites had both comparable and dissimilar band profiles. Fig. 3 displays the dendrogram that was produced based on the band profiles. It was, therefore, observed that the samples obtained from the 17 sites were split into two fundamental groups (X and Y). These groupings were seen to be split into two subgroups within themselves once more. The distribution of samples 1, 2, and 3 from the closest station, for instance, shows that the band profiles acquired from various samples taken from the same region are included in groups that are separated from one another.

Based on samples collected from 17 distinct sites, as seen

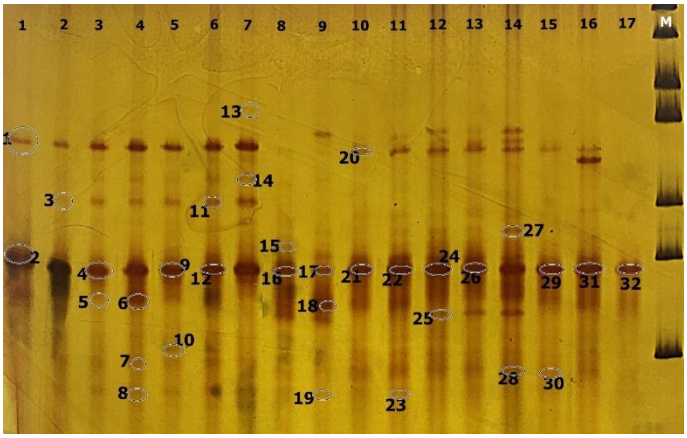

Fig. 2. SSCP band profiles of 17 different points (bands circled are those for which sequence analysis was performed). M, Marker.

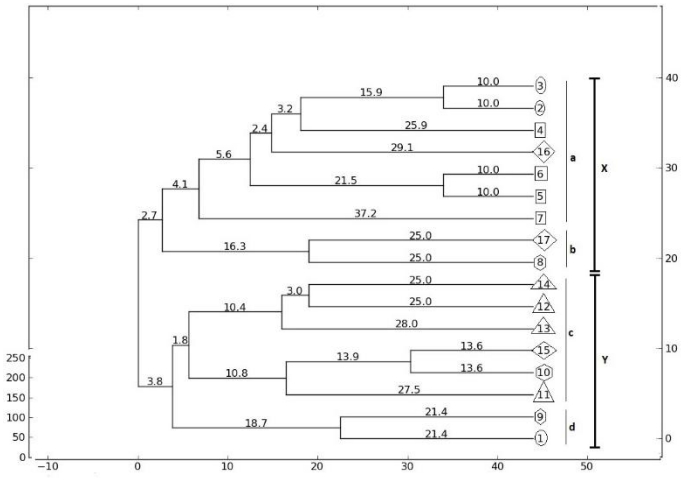

Fig. 3. UPGMA dendrogram analysis of SSCP profiles of bacterial communities from 17 different *G. barbata* samples.

in Fig. 2, 32 dominant and distinct bands were chosen, and the genus and/or species with the highest degree of similarity were identified using sequencing analysis and are listed in Table 1. A closer look at Fig. 2 reveals that certain bands, chosen for their distinctiveness rather than their dominance, represent unique types of microorganisms. Table 1 displays the sequence similarity data for this, which are displayed in bands 7, 8, 10, 19, 23, 25, 28, and 30. This demonstrates the significance of this method. Upon closer inspection of Table 1, it became evident that 84.375 percent of the bacterial groups identified were members of the *Gammaproteobacteria* class. It was discovered that two samples (6.25%) belonged to the phylum *Bacteroidetes*, three samples (9.375%) to the phylum *Cyanobacteria*, and only one sample (3.125%) to the class *Alphaproteobacteria*. Furthermore, it was notable that the sequence data revealed certain species of *Vibrio* and *Klebsiella* to be among the most prevalent opportunistic and marine infections. Furthermore, the phylogenetic tree based on the outgroups from NCBI shows the phylogenetic relationships of the results obtained from the band profiles tested (Fig. 4). In addition, many bacteria that were previously isolated from sediment and marine samples have been identified. Research has indicated that bacterial phylotypes in algae and the surrounding saltwater may differ from one another. In addition to this distinction, it has also been reported that bacteria and their host algae enter into antagonistic relationships with each other (Yang et al. 2023). They believed that the circumstances were connected to the antibacterial

qualities of macroalgae. For instance, whereas *E. coli* was found in the water and surrounding algae in certain investigations, it was not found on seaweed in other examinations. According to their ongoing research (Wiese et al., 2009; Michelou et al., 2013; Barberi et al., 2020), components of the algal bacterial community shown action against *E. coli*. In a prior work, we discovered that macroalgae extracts had microbiological activity against a variety of bacteria, including *E. coli* (Berber et al., 2015), which lends support to these investigations.

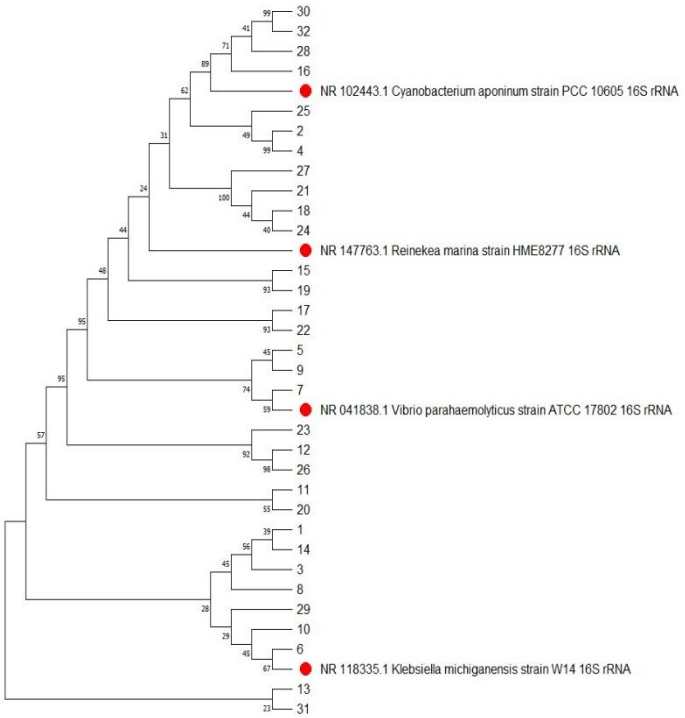

Fig. 4. Phylogenetic tree based on rRNA gene region sequence analysis. Red dots indicate strains outgroups from NCBI as understood from the "accession numbers" in front of their names.

The Neighbor-Joining approach was used to infer evolutionary history (Saitou and Nei, 1987). The ideal tree is shown. The percentage of replicate trees in which related taxa were grouped together in the bootstrap test (1000 replicates) is given next to the branches (Felsenstein, 1985). The tree is shown to scale with branch lengths expressed in the same units as the evolutionary distances used to estimate the phylogenetic tree. Evolutionary distances are shown as base changes per site and were calculated using the Maximum Composite Likelihood approach (Tamura et al., 2004). There were 36 nucleotide sequences in this study. For each sequence pair, all ambiguous sites were eliminated (pairwise deletion option). There was a total of 413 positions in the final dataset. Evolutionary analyses were performed in MEGA11 (Tamura et al., 2021).

According to Lachnit et al. (2011), heteroduplex formations, co-migrations on the gel, and potential variations within multiple copies of 16S rRNA genes can all have an impact on the band motif composition and density, therefore the DGGE technique they used to identify the epiphytic bacterial community only offers a rough overview. We can remark that the SSCP technique we utilize has similar drawbacks to those stated for this circumstance. In their investigation of the bacterial contamination of macroalgae, Barberi et al. (2020) used the membrane filtering approach to identify *E. coli*, *V. parahaemolyticus*, and *V. alginolyticus*. They claimed that the manner of counting was too low. Using molecular techniques, enterohemorrhagic *E. coli* O157:H7, *V. parahaemolyticus*, and *Salmonella enterica* ser. *typhimurium* were identified in the same samples. In their investigation on the microbiological quality of seaweeds, Blikra et al. (2019) found that spore-forming bacteria are present and that the microbial number is modest (1-3 log cfu g^{-1}). Additionally, they stated that while they identified *Bacillus* species, they were unable to find *Listeria monocytogenes*, pathogenic vibrio, coliforms, or enterococci. Burke et al. (2011) used 16S rRNA sequence sequencing in six *Ulva australis* and the surrounding waters to find that *Alphaproteobacteria* and *Bacteriodetes* were prominent in both settings. They did note that in both settings, the resemblance was not at the species level. The identical sponge samples from Lee et al. (2009) and brown algae from Staufenberg et al. (2008) revealed more diversity at the species level but more similarity at higher taxonomic levels in the microbial communities. In a planned investigation, Lachnit et al. (2011) used the DGGE technology to look at the bacterial population of three distinct macroalgae species at different dates. The results of 16S rRNA sequence analyses revealed strong species-level similarity between replicates, but also differences in bacterial communities between three algal species and between summer and winter samples of the same algal species. According to Mancuso et al. (2016), there is a dearth of knowledge regarding the interactions between epiphytic bacteria, despite the abundance of studies on the diverse macrofauna and flora connected to *Cyctoseria* (*sensu lato)*. They examined the interaction between the surrounding water and the bacterial communities in *Cystoseira compressa* in their investigation. The study's findings showed that the predominant sequences on algae and in water samples were those of *Proteobacteria* and *Bacteriodetes*. Furthermore, they discovered that only a few common species varied between the two ecosystems. A study on the connection between the organization of bacterial communities and two distinct *Labophora* macroalgae species was carried out by Vieira et al. (2016). They discovered that the two species' bacterial populations differed. They demonstrated that almost half of the OTU is shared by both species. They stated that *Planctomycetes*, *Proteobacteria*, and *Bacteriodetes* are the taxa to these species-specific OTUs belong. In addition, they identified sixteen culturable isolates from different algae species and discovered that these isolates were connected to corals nearby. The bacterial communities linked to *Ulva rigida* macroalgae, which Califano et al. (2020) gathered in aquaculture and coastal habitats, were shown to be distinct. Additionally, the *Proteobacteria* phylum in algae and the *Bacteriodetes* phylum in water samples were found to be prevalent by the researchers. In a planned investigation, Aires et al. (2016) investigated the association between two invasive seaweed species from the Atlantic Island coastal region and their bacterial communities. They discovered variations between the two species' bacterial communities. They also found that bacterial communities are host-specific and influenced by their surroundings in their investigation. Serebryakova et al. (2018) looked at the temporal and geographical alterations of the microbial community linked to the invasive brown seaweed *Sargassum muticum* in a different study. The researchers noticed that the seaweed displayed alterations based on the structural difference, even though they had anticipated microbial community differences based on the area and the sample month. They therefore show the significance of structural microscale variations in seaweeds, which are hosts connected to microbial populations. Tujula et al. (2010) examined the seasonal dynamics and diversity of bacterial

Table 1
Identify the bands with BLAST scanning in NCBI GenBank database.

Band	Blast top search - GenBank accession number	Phylum - Class	Similarity (%)	GenBank accession number
1	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	99.45	MN134438
2	*Labilibacter sediminis* strain CG51 - NR_169488.1	Bacteroidetes	89.10	MN134439
3	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	98.64	MN134440
4	*Labilibacter sediminis* strain CG51 - NR_169488.1	Bacteroidetes	89.37	MN134441
5	*Vibrio alginolyticus* strain ATCC 17749 - NR_118258.1	Gammaproteobacteria	94.86	MN134442
6	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	96.64	MN134443
7	*Vibrio alginolyticus* strain ATCC 17749 - NR_118258.1	Gammaproteobacteria	98.37	MN134444
8	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	98.66	MN134445
9	*Vibrio alginolyticus* strain ATCC 17749 - NR_118258.1	Gammaproteobacteria	92.06	MN134446
10	*Klebsiella oxytoca* strain NBRC 102593 - NR_114152.1	Gammaproteobacteria	98.91	MN134447
11	*Klebsiella oxytoca* strain NBRC 102593 - NR_114152.1	Gammaproteobacteria	97.84	MN134448
12	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	88.24	MN134449
13	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	98.67	MN134450
14	*Klebsiella oxytoca* strain NBRC 102593 - NR_114152.1	Gammaproteobacteria	98.66	MN134451
15	*Alcanivorax nanhaiticus* strain 19-m-6 - NR_152008.1	Gammaproteobacteria	91.30	MN134452
16	*Nodosilinea alaskaensis* strain L32 - NR_172588.1	Cyanobacteria	83.64	MN134453
17	*Vibrio alginolyticus* strain ATCC 17749 - NR_118258.1	Gammaproteobacteria	90.61	MN134454
18	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	94.91	MN134455
19	*Reinekea marina* strain HME8277 - NR_147763.1	Gammaproteobacteria	90.91	MN134456
20	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	95.04	MN134457
21	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	95.40	MN134458
22	*Vibrio ichthyoenteri* ATCC 700023 - NR_113813.1	Gammaproteobacteria	90.53	MN134459
23	*Kosakonia oryziphila* strain REICA_142 - NR_125587.1	Gammaproteobacteria	83.46	MN134460
24	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	94.01	MN134461
25	*Litoreibacter ponti* strain GJSW-31 - NR_134069.1	Alphaproteobacteria	88.89	MN134462
26	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	88.56	MN134463
27	*Klebsiella oxytoca* strain NBRC 102593 - NR_114152.1	Gammaproteobacteria	90.44	MN134464
28	*Thalassotalea insulae* strain JDTF-40 - NR_163662.1	Gammaproteobacteria	81.74	MN134465
29	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	98.93	MN134466
30	*Mamoreocelis xerophila* strain CATCB5 - NR_172610.1	Cyanobacteria	84.52	MN134467
31	*Klebsiella michiganensis* strain W14 - NR_118335.1	Gammaproteobacteria	98.39	MN134468
32	*Metis fasciculata* strain TAU-MAC 1415 - NR_172573.1	Cyanobacteria	85.32	MN134469

communities based on DGGE in *Ulva australis* macroalgae collected at various periods and places. They found that there are temporal and regional variations in bacterial communities. Using the DGGE approach, they also discovered that *Bacteriodetes* and *Alphaproteobacteria* are significant components of this alga.

Bengtsson et al. (2010) looked at the seasonal changes in the bacterial community in the biofilm that grows on the surface of *Laminaria hyperborea* seaweed in another study. Depending on the outcome they achieved with the DGGE technique, they observed that seasonal variations and water temperature had an impact on the bacterial community. Additionally, they demonstrated that there was little overlap between the bacterial community in the biofilm and the seawater nearby. We find it challenging to assess the water quality of bacterial populations isolated from macroalgae samples considering this reasoning. Even while newer sequencing technologies have nearly entirely replaced approaches like as Sanger sequencing and showing the structure of classical bacterial communities, these techniques are still useful in low-budget labs. Nonetheless, we believe that it should be valued equally regardless of the approach that discloses the structure of the bacterial population without relying on macroalgae growth, which has been increasingly popular for human use in recent years. We do not hold back when highlighting the significance of this work in this regard. Even so, we can still claim that this study is a first for us and serves as the foundation for our more thorough study preparation.

4. Conclusion

According to the bacterial community investigated by a culture-independent technique based on the total DNA sample obtained from the *G. barbata* macroalgae species collected from different stations, it was observed that the majority of the bacteria belonged to the *Gammaproteobacteria* class. Among these, it was noteworthy that members of the potentially dangerous *Klebsiella* and *Vibrio* genera were found. Another important finding was that the bacterial community structure was different in macroalgae collected from different stations. In light of all these and the information given above in the literature, it will be inevitable to plan detailed research on the detection of epiphytic bacteria with more comprehensive techniques, elucidation of their relationships with macroalgae, and what kind of biotechnological benefits/products can be obtained in the context of these relationships.

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References

Agrawal, P. K., Agrawal, S., & Shrivastava, R. (2015). Modern molecular approaches for analyzing microbial diversity from mushroom compost ecosystem. *3 Biotech, 5*(6), 853-866.

Aires, T., Serrão, E. A., & Engelen, A. H. (2016). Host and environmental specificity in bacterial communities associated with two highly invasive marine species (genus *Asparagopsis*). *Frontiers in Microbiology, 7*, 559.

Araj, G. F., Taleb, R., El Beayni, N. K., & Goksu, E. (2019). *Vibrio albensis*: An unusual urinary tract infection in a healthy male. *Journal of Infection and Public Health, 12*(5), 712-713.

Avsar, C., & Aras, E. S. (2020). Community structures and comparison of *nosZ* and 16S rRNA genes from culturable denitrifying bacteria. *Folia Microbiologica, 65*(3), 497-510.

Barberi, O. N., Byron, C. J., Burkholder, K. M., St. Gelais, A. T., & Williams, A. K. (2020). Assessment of bacterial pathogens on edible macroalgae in coastal waters. *Journal of Applied Phycology, 32*(1), 683-696.

Bengtsson, M. M., Sjøtun, K., & Øvreås, L. (2010). Seasonal dynamics of bacterial biofilms on the kelp *Laminaria hyperborea*. *Aquatic Microbial Ecology, 60*(1), 71-83.

Berber, I., Avşar, C., & Koyuncu, H. (2015). Antimicrobial and antioxidant activities of *Cystoseira crinita* Duby and *Ulva intestinalis* Linnaeus from the coastal region of Sinop, Turkey. *Journal of Coastal Life Medicine, 3*(6), 441-445.

Blikra, M., Løvda, T., Vaka, M., Roiha, I. S., & Lunestad, B. T. (2019). Assessment of food quality and microbial safety of brown macroalgae (*Alaria esculenta* and *Saccharina latissima*). *Journal of Science Food Agriculture, 99*, 1198-1206.

Burke, C., Thomas, T., Lewis, M., Steinberg, P., & Kjelleberg, S. (2011). Composition, uniqueness, and variability of the epiphytic bacterial community of the green alga *Ulva australis*. *The ISME Journal, 5*(4), 590-600.

Byun, S. O., Fang, Q., Zhou, H., & Hickford, J. G. H. (2009). An effective method for silver-staining DNA in large numbers of polyacrylamide gels. *Analytical Biochemistry, 385*(1), 174-175.

Califano, G., Kwantes, M., Abreu, M. H., Costa, R., & Wichard, T. (2020). Cultivating the macroalgal holobiont: Effects of integrated multi-trophic aquaculture on the microbiome of *Ulva rigida* (Chlorophyta). *Frontiers in Marine Science, 7*, 52.

Comba-González, N. B., Ruiz-Toquica, J. S., Lopez-Kleine, L., & Montoya-Castano, D. (2016). Epiphytic bacteria of macroalgae of the genus *Ulva* and their potential in producing enzymes having biotechnological interest. *Journal of Marine Biology, 2016*, 2-9.

Felsenstein, J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. *Evolution, 39*(4), 783-791.

Florez, J. Z., Camus, C., Hengst, M. B., & Buschmann, A. H. (2017). A functional perspective analysis of macroalgae and epiphytic bacterial community interaction. *Frontiers in Microbiology, 8*, 2561.

Franco-Duarte, R., Černáková, L., Kadam, S., S. Kaushik, K., Salehi, B., Bevilacqua, A., & Rodrigues, C. F. (2019). Advances in chemical and biological methods to identify microorganisms from past to present. *Microorganisms, 7*(5), 130.

Girão, M., Alexandrino, D. A., Cao, W., Costa, I., Jia, Z., & Carvalho, M. F. (2024). Unveiling the culturable and non-culturable actinobacterial diversity in two macroalgae species from the northern Portuguese coast. *Environmental Microbiology, 26*(4), e16620.

Kaur, M., Saini, K. C., Mallick, A., & Bast, F. (2023). Seaweed-associated epiphytic bacteria: Diversity, ecological and economic implications. *Aquatic Botany, 189*, 103698.

Lachnit, T., Meske, D., Wahl, M., Harder, T., & Schmitz, R. (2011). Epibacterial community patterns on marine macroalgae are host-specific but temporally variable. *Environmental Microbiology, 13*(3), 655-665.

Lee, O. O., Wong, Y. H., & Qian, P. Y. (2009). Inter-and intraspecific variations of bacterial communities associated with marine sponges from San Juan Island, Washington. *Applied and Environmental Microbiology, 75*(11), 3513-3521.

Lu, D. C., Wang, F. Q., Amann, R. I., Teeling, H., & Du, Z. J. (2023). Epiphytic common core bacteria in the microbiomes of co-located green (*Ulva*), brown (*Saccharina*) and red (*Grateloupia*, *Gelidium*) macroalgae. *Microbiome, 11*(1), 126.

Mahmud, Z. H., Neogi, S. B., Kassu, A., Huong, B. T., Jahid, I. K., Iqbal, K. J., Islam, M. S., & Ota, F. (2008). Occurrence, seasonality and genetic diversity of *Vibrio vulnificus* in coastal seaweeds and water along the Kii Channel, Japan. *FEMS Microbiology Ecology, 64*(2), 209-218.

Mancuso, F. P., D'hondt, S., Willems, A., Airoldi, L., & De Clerck, O. (2016). Diversity and temporal dynamics of the epiphytic bacterial communities associated with the canopy-forming seaweed *Cystoseira compressa* (Esper) Gerloff and Nizamuddin. *Frontiers in Microbiology, 7*, 476.

Michelou, V. K., Caporaso, J. G., Knight, R., & Palumbi, S. R. (2013). The ecology of microbial communities associated with *Macrocystis pyrifera*. *PLoS One, 8*(6), e67480.

Nahor, O., Israel, Á., Barger, N., Rubin-Blum, M., & Luzzatto-Knaan, T. (2024). Epiphytic microbiome associated with intertidal seaweeds in the Mediterranean Sea: Comparative analysis of bacterial communities across seaweed phyla. *Scientific Reports, 14*(1), 18631.

Saitou, N., & Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution, 4*(4), 406-425.

Schwieger, F., & Tebbe, C. C. (1998). A new approach to utilize PCR–single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. *Applied and Environmental Microbiology, 64*(12), 4870-4876.

Schwieger, F., & Tebbe, C. C. (2000). Effect of field inoculation with *Sinorhizobium meliloti* L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*)-Linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. *Applied and Environmental Microbiology, 66*(8), 3556-3565.

Serebryakova, A., Aires, T., Viard, F., Serrão, E. A., & Engelen, A. H. (2018). Summer shifts of bacterial communities associated with the invasive brown seaweed *Sargassum muticum* are location and tissue dependent. *PLoS One, 13*(12), e0206734.

Smalla, K., Oros-Sichler, M., Milling, A., Heuer, H., Baumgarte, S., Becker, R., ... & Tebbe, C. C. (2007). Bacterial diversity of soils assessed by DGGE, T-RFLP and SSCP fingerprints of PCR-amplified 16S rRNA gene fragments: Do the different methods provide similar results? *Journal of Microbiological Methods, 69*(3), 470-479.

Singh, R. P., & Reddy, C. R. K. (2014). Seaweed–microbial interactions: Key functions of seaweed-associated bacteria. *FEMS Microbiology Ecology, 88*(2), 213-230.

Staufenberger, T., Thiel, V., Wiese, J., & Imhoff, J. F. (2008). Phylogenetic analysis of bacteria associated with *Laminaria saccharina*. *FEMS Microbiology Ecology, 64*(1), 65-77.

Tamura, K., Nei, M., & Kumar, S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences (USA), 101*, 11030-11035.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*.

Tujula, N. A., Crocetti, G. R., Burke, C., Thomas, T., Holmström, C., & Kjelleberg, S. (2010). Variability and abundance of the epiphytic bacterial community associated with a green marine *Ulvacean* alga. *The ISME Journal, 4*(2), 301-311.

Trica, B., Delattre, C., Gros, F., Ursu, A. V., Dobre, T., Djelveh, G., ... & Oancea, F. (2019). Extraction and characterization of alginate from an edible brown seaweed (*Cystoseira barbata*) harvested in the Romanian Black Sea. *Marine Drugs, 17*(7), 405.

Wichachucherd, B., Prathep, A., & Zuccarello, G. C. (2014). Phylogeography of *Padina boryana* (Dictyotales, Phaeophyceae) around the Thai-Malay Peninsula. *European Journal of Phycology, 49*(3), 313-323.

Wiese, J., Thiel, V., Nagel, K., Staufenberger, T., & Imhoff, J. F. (2009). Diversity of antibiotic-active bacteria associated with the brown alga *Laminaria saccharina* from the Baltic Sea. *Marine Biotechnology, 11*(2), 287-300.

Xiao, Z., Feng, C., Gao, B., Huang, Y., Long, L., & Yang, F. (2024). Marine macroalgae and their associated bacterial communities affect larval settlement and survivorship of the coral *Pocillopora damicornis*. *Marine Environmental Research, 199*, 106597.

Vieira, C., Thomas, O. P., Culioli, G., Genta-Jouve, G., Houlbreque, F., Gaubert, J., ... & Payri, C. E. (2016). Allelopathic interactions between the brown algal genus *Lobophora* (Dictyotales, Phaeophyceae) and scleractinian corals. *Sci. Rep., 6*(1), 1-11.

Vigil, B. E., Ascue, F., Ayala, R. Y., Murúa, P., Calderon, M. S., & Bustamante, D. E. (2024). Functional prediction based on 16S rRNA metagenome data from bacterial microbiota associated with macroalgae from the Peruvian coast. *Scientific Reports*, *14*(1), 18577.

Yang, Z., Sun, T., Chen, J., Wang, J., Yu, X., Zhao, Y., ... & Xiao, H. (2023). The contribution of host tissue location and sex to epiphytic bacterial community assembly of *Sargassum thunbergii*. *Ecological Indicators*, *154*, 110871.

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Review article

The importance of 3D cell culture in drug discovery and development

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Abstract

Three-dimensional (3D) cell culture techniques represent a transformative advancement in biomedical research, particularly in drug discovery and development. By more closely replicating the physiological and microenvironmental conditions of *in vivo* tissues, 3D cell cultures enable more accurate assessments of drug efficacy, toxicity, and therapeutic potential compared to traditional two-dimensional (2D) cultures. These systems not only provide a more realistic model for preclinical testing but also allow for the study of complex cell-cell and cell-matrix interactions, which are often overlooked in 2D systems. This review provides a comprehensive examination of studies utilizing spheroids and organoids in 3D culture systems for drug screening and development. Furthermore, it highlights the critical role of these models in uncovering novel therapeutic targets, understanding disease mechanisms, and optimizing drug delivery strategies. Key challenges, such as scalability, standardization, and integration with high-throughput screening platforms, are also discussed. In conclusion, 3D cell culture techniques hold immense promise for revolutionizing the drug discovery pipeline, offering a more predictive and ethical approach to preclinical research while bridging the gap between laboratory findings and clinical outcomes.

Keywords: *3D cell culture; drug discovery; organoids; spheroid*

1. Introduction

The process of drug discovery and development is notoriously challenging, characterized by low success rates in clinical trials. This is largely due to the slow progression of the process, the high costs involved, and the complexities of translating preclinical results into successful treatments. In addition to these challenges, there is a significant gap in the availability of effective and safe treatment options for a variety of diseases, particularly complex conditions like cancer, neurodegenerative disorders, and cardiovascular diseases (Arrowsmith and Miller, 2013, Jordan et al., 2024). Furthermore, safety concerns arising from the inability to accurately predict human responses to drugs in preclinical models exacerbate the situation. As a result, there is a critical need to find innovative technologies that can improve the predictability of drug efficacy and safety in clinical trials. This would, in turn, enhance the success rates of drug discovery and development, providing new hope for the treatment of currently unmet medical needs.

One of the most promising areas for improving drug discovery outcomes is the advancement of 3D cell culture technology. 2D cell cultures have been widely used in drug discovery for decades; however, these cultures fail to adequately replicate the complex *in vivo* environment. Unlike 2D cultures, 3D cell cultures offer a more realistic representation of human tissues by allowing cells to grow and interact in a three-dimensional space, similar to how they would behave in the body. This approach has the potential to significantly improve the accuracy of drug testing and allow for better prediction of drug responses in humans (Biju et al., 2023). The initial studies that demonstrated the potential of 3D cell cultures to mimic the

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fundamental factors of tissues and replicate the extracellular matrix (ECM) in cell behavior were conducted in the 1980s by Mina Bissell and her team (Ravi et al., 2015; Langhans, 2018). These early studies laid the foundation for the development of 3D cell culture models that could more effectively simulate the natural environment of human tissues, which is essential for understanding cellular responses to drugs. Since then, advances in 3D culture technologies have led to the creation of more sophisticated models that enable the study of various diseases, including cancer, and the testing of new drugs in a more relevant setting. Despite the rapid advancements in 3D cell culture technologies, traditional single-layer 2D cell cultures are still commonly used in drug studies due to their simplicity, lower cost, and long-standing familiarity. However, as the limitations of 2D cultures in predicting drug responses become more apparent, the advantages of 3D cell cultures are becoming increasingly evident. In particular, 3D cell cultures are now being integrated into high-throughput screening (HTS) platforms, where they provide more accurate and reliable results in drug discovery applications (Wang and Jeon, 2022). This shift towards 3D culture-based screening has resulted in the identification of new drug candidates with better efficacy and fewer side effects, ultimately improving the chances of success in clinical trials (Sittampalam et al., 2015; Langhans, 2018).

In this review, we will explore the growing importance of 3D cell culture technologies in enhancing drug utilization and discovery. We will focus on the various types of 3D culture systems currently being employed, including spheroids, organoids, and bio-printed tissues, and their respective applications in drug testing. Additionally, we will discuss the key advantages of using 3D cell cultures in preclinical research, including their ability to mimic complex tissue structures, better represent disease models, and predict drug responses more accurately. However, despite their potential, the adoption of 3D cell cultures still faces challenges, such as the scalability of these systems, their integration into existing drug screening pipelines, and the need for standardized protocols. These challenges must be addressed to fully realize the potential of 3D cell culture technology in revolutionizing the drug discovery process. Ultimately, 3D cell culture systems hold the promise of improving the drug discovery pipeline by providing more predictive, accurate, and ethical alternatives to traditional preclinical models. The adoption of these advanced technologies could bridge the gap between laboratory findings and clinical outcomes, leading to the development of more effective and safer drugs for patients in the near future.

2. 3D cell culture

3D cell culture is a technique that simulates the environment where cells grow, mimicking cell-cell and cell-matrix interactions similar to those found in natural tissues. The aim of this technique is to model cellular hierarchy progression, investigate cell-cell interactions, observe cellular behavior, and develop treatment strategies. Using this technique, 3D models such as spheroids and organoids are created (Ajjarapu et al., 2023).

Spheroids are spherical or round-shaped cell clusters formed by the self-organization of cells. Organoids, on the other hand, are 3D models that resemble human tissues and organs in structure and function, developed from stem cells or progenitor cells with differentiation potential (Ravi et al., 2015; Simian and Bissell, 2017; Temple et al., 2022; Ajjarapu et al., 2023). Spheroids establish an architecture involving cell-cell and cell-matrix (ECM) interactions. Cell-surface integrins and cadherins play a critical role in activating signaling pathways that regulate several biological processes, including adhesion, organization, and maintaining structural integrity among cells (Białkowska et al., 2020; Sara Biju et al., 2023). Some of these techniques are illustrated in Fig. 1.

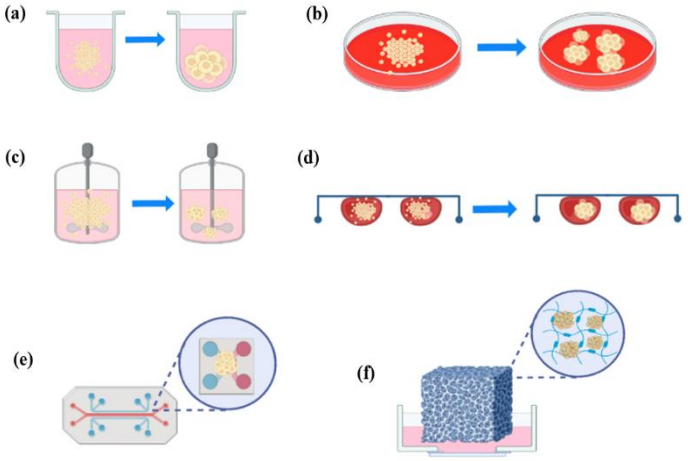

Fig. 1. The techniques used to create spheroids include: (a) Pellet culture, (b) surface coating, (c) bioreactor, (d) hanging drop method, (e) microfluidics, and (f) embedding onto or into a matrix (Demirel, 2023).

All these techniques enable the production of 3D cellular aggregates, spheroids, in a rapid and reproducible manner. However, spheroids produced using these techniques often display a low percentage of some ECM components, alongside cell-ECM interactions. Therefore, the dynamics of ECM components are crucial for mechanisms regulating cancer cell metabolism and responses to therapeutic molecules (Lu et al., 2012).

The pellet culture technique involves cells concentrating at the bottom of a tube under the influence of centrifugal force, leading to the formation of spheroids (Fig. 1-a). The adhesion between cells at the bottom of the tube plays a crucial role in forming spheroid cultures. To culture the formed cell aggregates, supernatants are removed, and the spheroids formed in the supernatant are resuspended in the cell culture medium. However, a disadvantage of this technique is that changing the environment in this way can damage spheroids, potentially disrupting their structure (Achilli et al., 2012; Maritan et al., 2017).

Spheroid formation using the surface coating technique (Fig. 1-b) is a widely used and straightforward method. Among spheroid formation techniques, the simplest approach involves seeding cells onto a surface where they cannot adhere. In this method, the surface is coated with materials such as agarose, poly-HEMA, polyethylene glycol (PEG), galactose, or polyvinyl alcohol (PVA) to create a low-adhesion surface (Liu et al., 2021).

The disadvantages of this technique include the inability to adequately support spheroids, its chemically sensitive structure, and weaker cell-cell interactions compared to other methods (Raghavan et al., 2016). For creating a low-adhesion surface, agar or agarose gel is commonly used. Agarose is a preferred material for adhesion inhibition and demonstrates lower adhesion properties compared to agar. On this low-adhesion surface, cells stimulate cell-cell adhesive molecules, leading to the formation of spheroids (Costa et al., 2018). However,

agarose struggles to interact with tumor cells and cannot activate signaling pathways (Carvalho et al., 2016). Recently, hyaluronic acid has emerged as the most suitable biomaterial to overcome these disadvantages of agarose (Demirel et al., 2024). This is due to hyaluronic acid's ability to interact with surface receptors of cancer cells, which facilitates the transmission of cellular signals related to proliferation, angiogenesis, survival, and differentiation, as well as increasing resistance to therapeutics (Carvalho et al., 2017).

In the technique of spheroid formation through a bioreactor, as depicted in Fig. 1-c, the cultured cells are subjected to a continuous rotating force that prevents them from statically adhering to a surface. Under these conditions, cells tend to cluster and form spheroids (Tostões et al., 2012). The bioreactor technique is suitable for long-term cultures and can be used to produce a larger quantity of spheroids. However, it produces heterogeneous spheroids, and it is impossible to perform simultaneous monitoring and tracking. Moreover, transferring the resulting spheroids to another culture environment is necessary for their examination (Kahn-Krell et al., 2021).

The hanging drop technique relies on cell sedimentation, or in other words, the settling of cells, to form cell clusters (Fig. 1-d). In this technique, cells are cultured in droplet form suspended on the lid of a petri dish. The foundation of the commonly encountered hanging drop technique lies in the simultaneous effect of surface tension and gravity, leading to the formation of droplets. At the apex of these droplets, 3D spheroids form (Achilli et al., 2012). In addition, due to the small volumes used, implementing this technique can be challenging, and spheroids can be easily lost. Furthermore, changing the culture medium in such a technique is not easy (Timmins and Nielsen, 2007). Unlike other techniques, the microfluidic technique, different from non-microfluidic methods, plays a significant role in spheroid formation in 3D cell culture. However, it has some disadvantages. The limitations in other techniques can include variations in spheroid diameters, low throughput, or difficulty of use, as well as issues like the reduction of oxygen and nutrients and the increase in osmolality and metabolite levels (Whitesides, 2006).

In Fig. 1-e, the microfluidic technique involves the creation of controlled concentration gradients, lower reagent consumption, and the application of pressure on cells and a regular perfusion system, which are among its most significant advantages (Moshksayan et al., 2018). Additionally, microfluidic chips provide a dynamic environment to better mimic the *in vivo* conditions. Spheroids cultured in a continuously dynamic environment within a microwell plate exhibit higher resistance to drugs compared to other techniques (Ruppen et al., 2014). Among the disadvantages of this technique are the occurrence of clogging problems in microchannels, the relatively low permeability and transparency of silicone and PDMS (polydimethylsiloxane), which can pose challenges during microscopy, and the possibility of the system being multi-channeled, leading to the emergence of a microenvironment that could cause contamination (Chueh et al., 2010; Ruppen et al., 2014).

The final spheroid formation technique is the 3D cell culture method created by embedding cells onto or into a matrix (Fig. 1-f). Hydrogels are used in this technique for spheroid formation. Tissue scaffolds created with hydrogels not only support the 3D structure of cells but also provide a microenvironment that supports cell-cell and cell-matrix interactions, influencing tumor cell functions (Nath and Devi, 2016; Li and Kumacheva, 2018; Ozkan and Ozturk, 2024). 3D culture systems commonly utilize tissue scaffolds, which include ECM-based natural hydrogels, synthetic hydrogels, and commercial hydrogels that attempt to mimic the natural ECM (Li and Kumacheva, 2018).

Organoids, also known as mini-organs, are complex clusters of cells that develop from stem cells or organ progenitors, capable of self-renewal and exhibiting organ-specific properties (Fang and Eglen, 2017; Lee et al., 2023). The source of organoid formation can be embryonic stem cells, induced pluripotent stem cells, or adult stem cells. The most significant difference from spheroids is that due to their stem cell potential and ability to differentiate in a complex manner similar to *in vivo* organogenesis, organoids can exhibit organ-like properties (Spence et al., 2010; McCauley and Wells, 2017). Organoids summarize the *in vitro* development of organs due to their *in vivo*-like architecture; therefore, they are highly useful tools in organogenesis, genetics, and pathology studies (Takebe et al., 2013; Dutta et al., 2017). They are typically used for modeling and investigating the functionality of a specific organ, while spheroids are employed for studying more general cellular behaviors and interactions (Table 1). Especially in cancer research, the complex architecture of organoids can better reflect the histological and genetic characteristics of cancerous tissue (Sato et al., 2009; Baillargeon et al., 2019; Lee et al., 2023).

Table 1
Representation of different characteristic features of spheroids and organoids.

Feature	Spheroids	Organoids
Definition	Three-dimensional structures formed by cells coming together in a spherical or round shape	Three-dimensional structures where cells mimic the architecture and functionality of a specific organ
Production Process	Typically involve simple production processes	Production process is generally more complex
Applications	Studying cell-cell interactions, understanding general cellular behaviors	Modeling specific organs, investigating organ-specific functionality, creating disease models, drug discovery, and development of treatment strategies
Cell Behavior	Do not mimic the complex architecture of a specific organ	Mimic the complex architecture of specific organs
Advantages	- Simple production process - Suitable for studying cell-cell interactions - Suitable for understanding general cellular behaviors	- Investigation of organ-specific functionality - Creation of disease models - Drug discovery and development of treatment strategies
Disadvantages	- Do not mimic the complex architecture of a specific organ - Do not reflect organ-specific functionality - Not suitable for modeling specific organs	- Production process is generally more complex - May require more resources and time - May require a long time for tissue maturation

3. The importance of spheroids and organoids in efficient drug discovery

The beneficial use of spheroids as a 3D model was first explored in 1970 to understand the phenotype of *in vitro* tumors and their responses to chemotherapy during radiotherapy applications (Sutherland et al., 1970). Since then, spheroids have been widely used in various cell types (Fig. 2).

3D cell cultures have emerged as a promising alternative in drug discovery and development, providing more accurate and faster results compared to *in vivo* models. These innovative cell culture models significantly contribute to the advancement of drug research and personalized medicine. It has been reported that mathematical models have been exemplified for the use of Leedale et al. (2020) in simulating drug transport and activity in hepatic spheroids. Through this study, it is emphasized that 3D spheroids can guide researchers on how dosage and culture conditions can be regulated to optimize drug distribution. Using 3D tumor spheroids, drug delivery system associated with Aluminum chloride phthalocyanine, for early-stage diagnosis and treatment of breast cancer, has been employed (Jayme et al., 2022). This system demonstrates the compatibility of DNA polymeric films with cells and increases cell death through visible light photoactivation, targeting breast cancer cells with photodynamic therapy. In a study conducted on PDAC, 3D spheroids and organoids were utilized (Shah et al., 2022).

Pisheh et al. (2024) conducted comparative experiments with 2D and 3D cell cultures to demonstrate that antibody-drug conjugates (ADCs) yield more accurate responses in 3D cell culture. This study aimed to directly destroy tumor cells through a monoclonal antibody conjugated with a cytotoxic agent. It was reported that ADCs targeting the epidermal growth factor receptor (EGFR) were combined with aminobisphosphonates, which bind to drugs like cetuximab administered to colorectal cancer patients. The results showed that the outcomes obtained in 3D cell culture closely resemble those observed *in vivo*. Nakazawa (2024) discovered an ADC through organoids, named EBET, which induces protein cleavage. This ADC consists of bromodomain and extra-terminal (BET) proteins. The cytotoxic effects of EBET on various PDAC organoids demonstrated its potential efficacy when delivered via EBET. A comparative study was conducted between 3D and 2D cell cultures to demonstrate that more accurate results can be obtained using 3D cell cultures. The study proposed that liposomes could be used as a new drug delivery system for photodynamic therapy (PDT) (De Leo et al., 2024). It was noted that the therapeutic efficacy of second-generation photosensitizers (PS) is enhanced by loading them onto nanocarriers, with methylene blue (MB) dye being considered a potential PS. MB, which is thought to exhibit photodynamic activity, was loaded onto liposomes and demonstrated higher photodynamic potency in 3D cell cultures. These findings suggest that MB-based PDT delivered via liposomes could be an effective drug delivery system, as demonstrated through the *in vivo* similarity of 3D cell cultures. However, organoid production is quite challenging with automated microfluidics and is not feasible. An important study in the field of personalized therapy also utilized 3D cell culture. Schuster et al. (2020) study an automatic microfluidic platform was developed to facilitate organoid formation and enable rapid and combinatorial drug screening. To validate this system, drug screenings were conducted on human-derived pancreatic cancer

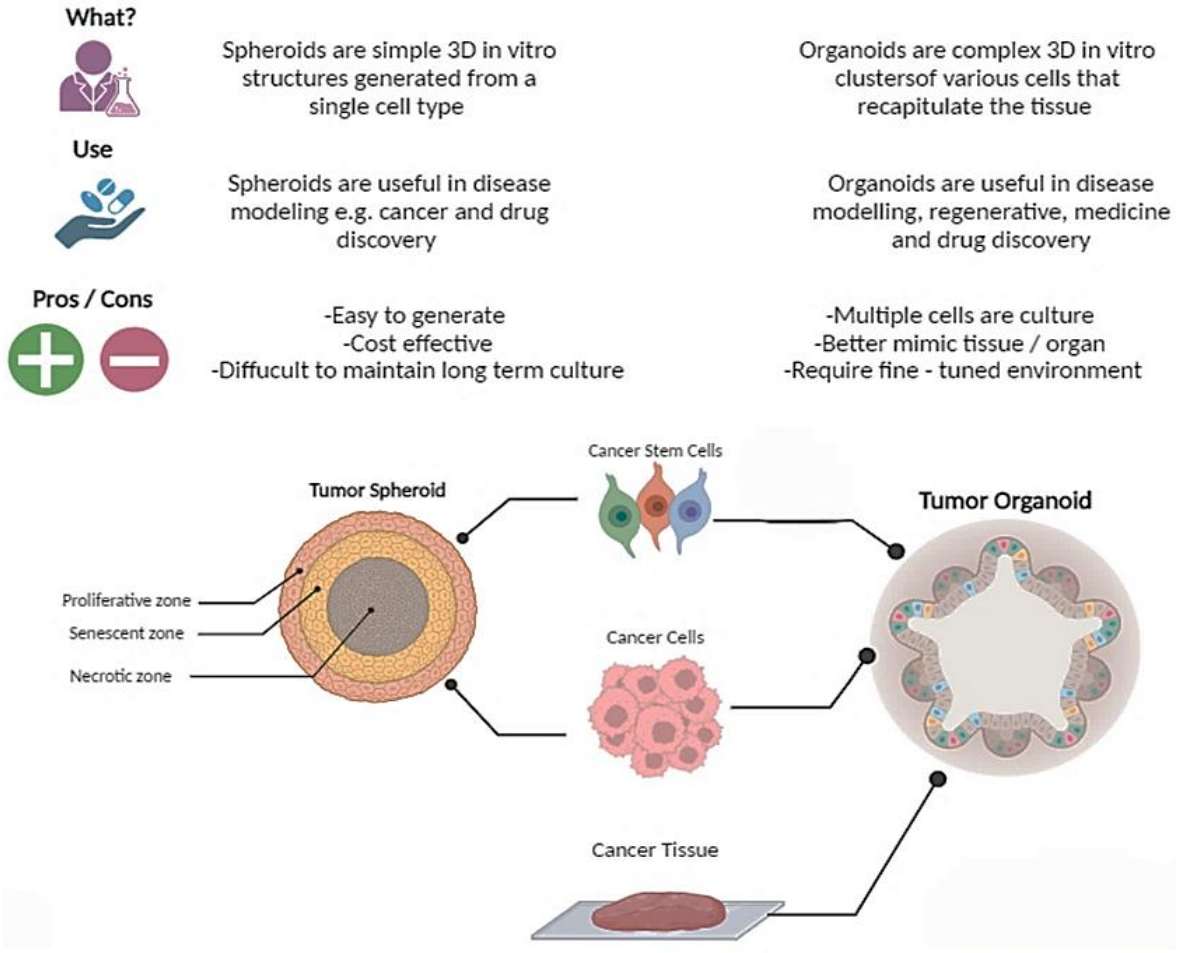

Fig. 2. Comparison and schematic representation between spheroids and organoids. (Created with Biorender).

organoids. Significant differences were observed in the responses of individual patient-based organoids to drug treatments, and it was reported that this represents an important advancement in personalized therapy for decision-making in treatment strategies for patients.

In a study conducted by a research team that hypothesized that organoids could show drug sensitivity and resistance similar to an *in vivo* environment, ovarian cancer organoids were specifically studied (Nanki et al., 2020). Within three weeks, ovarian cancer cells formed organoids, with each subtype showing different responses. For example, an organoid harboring a pathogenic variant in BRCA1 showed increased sensitivity to the PARP inhibitor olaparib, while an organoid derived from clear-cell ovarian cancer exhibited resistance to conventional drugs. According to the results, patient-derived organoids could serve as suitable *ex vivo* models for screening effective personalized ovarian cancer treatments.

Olijnik et al. (2024) developed bone marrow organoids derived from human induced pluripotent stem cells (hiPSCs). These organoids provide a powerful model for modeling disease phenotypes and evaluating the efficacy of pharmacological inhibitors by reflecting the natural architecture and functions of hematopoietic and stromal microenvironments. This approach preserves the characteristics of hematological cancer cells, which are difficult to maintain in 2D cell cultures, thereby reducing failure rates by more accurately predicting drug efficacy during the clinical transition process. One of the key advantages of 3D models is their ability to replicate the vascularization system. Since tumors *in vivo* are nourished through blood vessels, mimicking this system is highly beneficial for evaluating drug efficacy. For instance, Ascheid et al. (2024) developed vascularized tumor spheroids (VTS). These spheroids can be used to assess drug efficacy, such as in anti-angiogenic treatments, and enable a more accurate selection of drug candidates.

4. Challenges in 3D cell culture

3D cell culture models have gained significant attention as an important technique in drug efficacy evaluations. However, there are some limitations to the success of this technique. One of the most crucial factors is the determination and optimization of the culture medium components (Langhans, 2018). The components of the culture medium and substrate play a vital role in sustaining the culture. The limiting factor in this technique is the effect of the components in the culture medium on cellular growth and function (Lin and Chang, 2008). Moreover, the cells' ability to attach to the substrate, morphology, proliferation, and differentiation are affected by this. However, the balance and stability of the substrate interaction determine the success rate of the culture. Therefore, regulating and optimizing this balance is critical (Verma et al., 2020). The management of these components and the resulting cellular waste is a crucial detail. It has been emphasized that the management of components and waste is a limiting factor for the sustainability of this culture technique (Chaicharoenaudomrung et al., 2019). The bioactivity and potential toxicity of the materials used in 3D cell cultures are decisive factors for success. While the effect of each material may not be equally important, the combined effect of these components on the cells in the culture medium is expected to be positive (Moroni et al., 2008). One limiting factor in this technique is the presence of nutrients and oxygen in the inner parts of the cells, which negatively affects cell development. To address this issue, methods to increase the circulation of the culture medium are crucial (Demirel, 2021). The circular diffusion rate of the environment changes the interaction and organization level between cells (Lancaster and Knoblich, 2014). Cellular conditions vary with chemical and biomechanical signals. Therefore, the culture medium must be adjusted according to biomechanical signal limitations (Fennema et al., 2013). The formation of organoids and spheroids takes longer than in 2D cell cultures (Anton et al., 2015). The short lifespan of the obtained tissues and their inability to maintain viability after cell implantation require the engineering of biological materials (Murphy and Atala, 2014). One of the limitations of this technique is productivity and cost. The complexity and cost of long-term production processes are barriers to industrial-scale production (Lin and Chang, 2008). Standardization and reproducibility of this technique are essential for its reliability and safety (Handschel et al., 2007).

In 3D cell culture applications, the cell source and donor diversity are significant limitations. Genetic and phenotypic differences between different cell sources and donor diversity affect the results. Therefore, more studies are required to eliminate the lack of analysis and modeling (Knowlton et al., 2015; Gu et al., 2018). This lack of data validation and interpretation limits the applicability and validity of findings in clinical settings. Further research and validation are necessary for the application of clinical findings in studies (Tanner and Gottesman, 2015). 3D cell cultures are slightly more costly than 2D cell cultures, with the main reason being the biomaterials used to create the 3D structure. These biomaterials, which are a crucial parameter for mimicking the *in vivo* environment, can be commercially obtained or derived from natural polymers. However, researchers often have limited knowledge and expertise in using this technique (Ingber et al., 2006). There are ethical restrictions when using primary cells directly taken from patients, but these processes are crucial for testing drug efficacy for personalized treatments (Cvetkovic et al., 2014).

5. Conclusion

Significant progress has been made in drug discovery and development through the use of 3D cell culture models. These models, which reflect both the genomic and phenotypic characteristics of cancer and other diseases, provide highly reliable results. This system allows us to observe drug interactions in conditions similar to the *vivo* environment, offering a more accurate representation compared to traditional 2D models. Furthermore, 3D models play a critical role in the advancement of personalized treatments. The unique cellular characteristics of each patient enable the creation of accurate and effective personalized treatment protocols, allowing for more efficient application of the treatment process. However, challenges such as standardization and automation remain for the widespread adoption of 3D models in drug development. Despite these hurdles, the continuous advancements in drug efficacy testing through 3D cell cultures are expected to significantly contribute to the future of personalized medicine and drug discovery, ultimately improving treatment strategies.

Conflict of interest: The authors declare that they have no conflict of interests.

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References

Achilli, T. M., Meyer, J., & Morgan, J. R. (2012). Advances in the formation, use and understanding of multi-cellular spheroids. *Expert Opinion on Biological Therapy*, *12*(10), 1347.

Ajjarapu, S. M., Tiwari, A., & Kumar, S. (2023). Applications and utility of three-dimensional in vitro cell culture for therapeutics. *Future Pharmacology*, *3*(1), 213-228.

Anton, D., Burckel, H., Josset, E., & Noel, G. (2015). Three-dimensional cell culture: a breakthrough *in vivo*. *International Journal of Molecular Sciences*, 16(3), 5517-5527.

Arrowsmith, J., & Miller, P. (2013). Phase II and phase III attrition rates 2011-2012. N*ature Reviews Drug Discovery*, *12*(8), 569-570.

Ascheid, D., Baumann, M., Pinnecker, J., Friedrich, M., Szi-Marton, D., Medved, C., ... & Henke, E. (2024). A vascularized breast cancer spheroid platform for the ranked evaluation of tumor microenvironment-targeted drugs by light sheet fluorescence microscopy. *Nature Communications*, *15*(1), 3599.

Baillargeon, P., Shumate, J., Hou, S., Fernandez-Vega, V., Marques, N., Souza, G., ... & Scampavia, L. (2019). Automating a magnetic 3D spheroid model technology for high-throughput screening. *SLAS TECHNOLOGY: Translating Life Sciences Innovation*, *24*(4), 420-428.

Białkowska, K., Komorowski, P., Bryszewska, M., & Miłowska, K. (2020). Spheroids as a type of three-dimensional cell cultures—examples of methods of preparation and the most important application. *International Journal of Molecular Sciences*, *21*(17), 6225.

Biju, T. S., Priya, V. V., & Francis, A. P. (2023). Role of three-dimensional cell culture in therapeutics and diagnostics: an updated review. *Drug Delivery and Translational Research*, *13*(9), 2239-2253.

Biju, T. S., Priya, V. V., & Francis, A. P. (2023). Role of three-dimensional cell culture in therapeutics and diagnostics: an updated review. *Drug Delivery and Translational Research*, *13*(9), 2239-2253.

Carvalho, M. P., Costa, E. C., & Correia, I. J. (2017). Assembly of breast cancer heterotypic spheroids on hyaluronic acid coated surfaces. *Biotechnology Progress*, *33*(5), 1346-1357.

Carvalho, M. P., Costa, E. C., Miguel, S. P., & Correia, I. J. (2016). Tumor spheroid assembly on hyaluronic acid-based structures: A review. *Carbohydrate Polymers*, *150*, 139-148.

Chaicharoenaudomrung, N., Kunhorm, P., & Noisa, P. (2019). Three-dimensional cell culture systems as an *in vitro* platform for cancer and stem cell modeling. *World Journal of Stem Cells*, *11*(12), 1065.

Chueh, B. H., Zheng, Y., Torisawa, Y. S., Hsiao, A. Y., Ge, C., Hsiong, S., ... & Takayama, S. (2010). Patterning alginate hydrogels using light-directed release of caged calcium in a microfluidic device. *Biomedical Microdevices*, *12*(1), 145-151.

Costa, E. C., de Melo-Diogo, D., Moreira, A. F., Carvalho, M. P., & Correia, I. J. (2018). Spheroids formation on non-adhesive surfaces by liquid overlay technique: Considerations and practical approaches. *Biotechnology Journal*, *13*(1), 1700417.

Cvetkovic, C., Raman, R., Chan, V., Williams, B. J., Tolish, M., Bajaj, P., ... & Bashir, R. (2014). Three-dimensionally printed biological machines powered by skeletal muscle. *Proceedings of the National Academy of Sciences*, *111*(28), 10125-10130.

De Leo, V., Marras, E., Maurelli, A. M., Catucci, L., Milano, F., & Gariboldi, M. B. (2024). Polydopamine-Coated Liposomes for Methylene Blue Delivery in Anticancer Photodynamic Therapy: Effects in 2D and 3D Cellular Models. *International Journal of Molecular Sciences*, *25*(6), 3392.

Demirel, G. (2021). Karaciğer hücreleri ve organoidlerin üç boyutlu kültürlerinde kullanılmak üzere geliştirilmiş biyomalzemeler. *Frontiers in Life Sciences and Related Technologies*, *2*(3), 111-119.

Demirel, G. (2023). Morphological investigation of liver cancer cells in gelatin/alginate/hyaluronic acid scaffolds, Master's Thesis, Istanbul University, Council of Higher Education Thesis Center. pp. 1-76.

Demirel, G., Cakıl, Y. D., Koltuk, G., Aktas, R. G., & Calıskan, M. (2024). The use of hyaluronic acid in a 3D biomimetic scaffold supports spheroid formation and the culture of cancer stem cells. *Scientific Reports*, *14*(1), 19560.

Di Cio, S., Marhuenda, E., Haddrick, M., & Gautrot, J. E. (2024). Vascularised cardiac spheroids-on-a-chip for testing the toxicity of therapeutics. *Scientific Reports*, *14*(1), 1-13.

Dobaño-López, C., Valero, J. G., Araujo-Ayala, F., Nadeu, F., Gava, F., Faria, C., ... & Pérez-Galán, P. (2024). Patient-derived follicular lymphoma spheroids recapitulate lymph node signaling and immune profile uncovering galectin-9 as a novel immunotherapeutic target. *Blood Cancer Journal*, *14*(1), 75.

Dutta, D., Heo, I., & Clevers, H. (2017). Disease modeling in stem cell-derived 3D organoid systems. *Trends in Molecular Medicine*, *23*(5), 393-410.

Emond, R., Griffiths, J. I., Grolmusz, V. K., Nath, A., Chen, J., Medina, E. F., ... & Bild, A. H. (2023). Cell facilitation promotes growth and survival under drug pressure in breast cancer. *Nature Communications*, *14*(1), 3851.

Fang, Y., & Eglen, R. M. (2017). Three-dimensional cell cultures in drug discovery and development. *Slas discovery: Advancing Life Sciences R&D*, *22*(5), 456-472.

Fennema, E., Rivron, N., Rouwkema, J., van Blitterswijk, C., & De Boer, J. (2013). Spheroid culture as a tool for creating 3D complex tissues. *Trends in Biotechnology*, *31*(2), 108-115.

Gu, B. K., Choi, D. J., Park, S. J., Kim, Y. J., & Kim, C. H. (2018). 3D bioprinting technologies for tissue engineering applications. *Cutting-Edge Enabling Technologies for Regenerative Medicine*, 15-28.

Handschel, J. G., Depprich, R. A., Kübler, N. R., Wiesmann, H. P., Ommerborn, M., & Meyer, U. (2007). Prospects of micromass culture technology in tissue engineering. *Head & face medicine*, *3*, 1-4.

Ingber, D. E., Mow, V. C., Butler, D., Niklason, L., Huard, J., Mao, J., ... & Vunjak-Novakovic, G. (2006). Tissue engineering and developmental biology: going biomimetic. *Tissue Engineering*, *12*(12), 3265-3283.

Jayme, C. C., Pires, A. F., Fernandes, D. S., Bi, H., & Tedesco, A. C. (2022). DNA polymer films used as drug delivery systems to early-stage diagnose and treatment of breast cancer using 3D tumor spheroids as a model. *Photodiagnosis and Photodynamic Therapy*, *37*, 102575.

Jordan, L. M. O., Vega, V. F., Shumate, J., Peles, A., Zeiger, J., Scampavia, L., & Spicer, T. P. (2024). Protocol for high throughput 3D drug screening of patient derived melanoma and renal cell carcinoma. *SLAS Discovery*, *29*(3), 100141.

Kahn-Krell, A., Pretorius, D., Ou, J., Fast, V. G., Litovsky, S., Berry, J., ... & Zhang, J. (2021). Bioreactor suspension culture: Differentiation and production of cardiomyocyte spheroids from human induced pluripotent stem cells. *Frontiers in Bioengineering and Biotechnology*, *9*, 674260.

Kim, J. H., An, G. H., Kim, J. Y., Rasaei, R., Kim, W. J., Jin, X., Woo, D. H., Han, C., Yang, S. R., Kim, J. H., & Hong, S. H. (2021). Human pluripotent stem-cell-derived alveolar organoids for modeling pulmonary fibrosis and drug testing. Cell Death Discovery, 7(1). https://doi.org/10.1038/s41420-021-00439-7.

Knowlton, S., Onal, S., Yu, C. H., Zhao, J. J., & Tasoglu, S. (2015). Bioprinting for cancer research. Trends in Biotechnology, 33(9), 504–513. https://doi.org/10.1016/J.TIBTECH.2015.06.007.

Kong, J., Lee, H., Kim, D., Han, S. K., Ha, D., Shin, K., & Kim, S. (2020). Network-based machine learning in colorectal and bladder organoid models predicts anti-cancer drug efficacy in patients. *Nature Communications*, *11*(1), 5485.

La-Beck, N. M., Liu, X., Shmeeda, H., Shudde, C., & Gabizon, A. A. (2021, January). Repurposing amino-bisphosphonates by liposome formulation for a new role in cancer treatment. In *Seminars in Cancer Biology* (Vol. 68, pp. 175-185). Academic Press.

Lancaster, M. A., & Knoblich, J. A. (2014). Organogenesis in a dish: modeling development and disease using organoid technologies. *Science*, *345*(6194), 1247125.

Langhans, S. A. (2018). Three-dimensional *in vitro* cell culture models in drug discovery and drug repositioning. *Frontiers in Pharmacology*, *9*(JAN), 334617.

Lee, S. Y., Koo, I. S., Hwang, H. J., & Lee, D. W. (2023). In Vitro three-dimensional (3D) cell culture tools for spheroid and organoid models. *SLAS Discovery*, *28*(4), 119-137.

Leedale, J. A., Kyffin, J. A., Harding, A. L., Colley, H. E., Murdoch, C., Sharma, P., ... & Bearon, R. N. (2020). Multiscale modelling of drug transport and metabolism in liver spheroids. *Interface Focus*, *10*(2), 20190041.

Li, Y., & Kumacheva, E. (2018). Hydrogel microenvironments for cancer spheroid growth and drug screening. *Science Advances*, *4*(4), eaas8998.

Lin, R. Z., & Chang, H. Y. (2008). Recent advances in three-dimensional multicellular spheroid culture for biomedical research. *Biotechnology Journal*, *3*(9-10), 1172-1184.

Liu, D., Chen, S., & Win Naing, M. (2021). A review of manufacturing capabilities of cell spheroid generation technologies and future development. *Biotechnology and Bioengineering*, *118*(2), 542-554.

Lu, P., Weaver, V. M., & Werb, Z. (2012). The extracellular matrix: A dynamic niche in cancer progression. *Journal of Cell Biology*, *196*(4), 395-406.

Maritan, S. M., Lian, E. Y., & Mulligan, L. M. (2017). An efficient and flexible cell aggregation method for 3D spheroid production. *Journal of Visualized Experiments: JoVE*, (121), 55544.

McCauley, H. A., & Wells, J. M. (2017). Pluripotent stem cell-derived organoids: using principles of developmental biology to grow human tissues in a dish. *Development*, *144*(6), 958-962.

Moroni, L., De Wijn, J. R., & Van Blitterswijk, C. A. (2008). Integrating novel technologies to fabricate smart scaffolds. *Journal of Biomaterials Science, Polymer Edition*, 19(5), 543-572.

Moshksayan, K., Kashaninejad, N., Warkiani, M. E., Lock, J. G., Moghadas, H., Firoozabadi, B., ... & Nguyen, N. T. (2018). Spheroids-on-a-chip: Recent advances and design considerations in microfluidic platforms for spheroid formation and culture. *Sensors and Actuators B: Chemical*, *263*, 151-176.

Murphy, S. V., & Atala, A. (2014). 3D bioprinting of tissues and organs. *Nature Biotechnology*, *32*(8), 773-785.

Nakazawa, Y., Miyano, M., Tsukamoto, S., Kogai, H., Yamamoto, A., Iso, K., ... & Machinaga, A. (2024). Delivery of a BET protein degrader via a CEACAM6-targeted antibody–drug conjugate inhibits tumour growth in pancreatic cancer models. *Nature Communications*, *15*(1), 2192.

Nanki, Y., Chiyoda, T., Hirasawa, A., Ookubo, A., Itoh, M., Ueno, M., ... & Aoki, D. (2020). Patient-derived ovarian cancer organoids capture the genomic profiles of primary tumours applicable for drug sensitivity and resistance testing. *Scientific Reports*, *10*(1), 12581.

Nath, S., & Devi, G. R. (2016). Three-dimensional culture systems in cancer research: Focus on tumor spheroid model. *Pharmacology & Therapeutics*, *163*, 94-108.

Olijnik, A. A., Rodriguez-Romera, A., Wong, Z. C., Shen, Y., Reyat, J. S., Jooss, N. J., ... & Khan, A. O. (2024). Generating human bone marrow organoids for disease modeling and drug discovery. *Nature Protocols*, 1-30.

Ozkan, S. N., & Ozturk, E. (2024). Growth and organotypic branching of lung-specific microvascular cells on 2D and in 3D lung-derived matrices. *Frontiers in Life Sciences and Related Technologies*, *5*(1), 6-14.

Pisheh, L., Matis, S., Taglieri, M., Di Gregorio, L., Benelli, R., & Poggi, A. (2024). EGFR-targeted antibody-drug conjugate to different aminobisphosphonates: Direct and indirect antitumor effects on colorectal carcinoma cells. *Cancers*, *16*(7), 1256.

Raghavan, S., Mehta, P., Horst, E. N., Ward, M. R., Rowley, K. R., & Mehta, G. (2016). Comparative analysis of tumor spheroid generation techniques for differential *in vitro* drug toxicity. *Oncotarget*, *7*(13), 16948-16961.

Ravi, M., Paramesh, V., Kaviya, S. R., Anuradha, E., & Paul Solomon, F. D. (2015). 3D cell culture systems: advantages and applications. *Journal of Cellular Physiology*, *230*(1), 16-26.

Rogmans, C., Dittrich, J., Hamm, E., Weimer, J. P., Holthaus, D., Arnold, N., ... & Hedemann, N. (2024). Inhibiting ADAM17 enhances the efficacy of olaparib in ovarian cancer spheroids. *Scientific Reports*, *14*(1), 26926.

Ruppen, J., Cortes-Dericks, L., Marconi, E., Karoubi, G., Schmid, R. A., Peng, R., ... & Guenat, O. T. (2014). A microfluidic platform for chemoresistive testing of multicellular pleural cancer spheroids. *Lab on a Chip*, *14*(6), 1198-1205.

Sato, T., Vries, R. G., Snippert, H. J., Van De Wetering, M., Barker, N., Stange, D. E., ... & Clevers, H. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*, *459*(7244), 262-265.

Schuster, B., Junkin, M., Kashaf, S. S., Romero-Calvo, I., Kirby, K., Matthews, J., ... & Tay, S. (2020). Automated microfluidic platform for dynamic and combinatorial drug screening of tumor organoids. *Nature Communications*, *11*(1), 5271.

Shah, V. M., Dorrell, C., Al-Fatease, A., Allen-Petersen, B. L., Woo, Y., Bortnyak, Y., ... & Alani, A. W. (2022). Microfluidics formulated liposomes of hypoxia activated prodrug for treatment of pancreatic cancer. *Pharmaceutics*, *14*(4), 713.

Simian, M., & Bissell, M. J. (2017). Organoids: a historical perspective of thinking in three dimensions. *Journal of Cell Biology*, *216*(1), 31-40.

Sittampalam, S., Eglen, R., Ferguson, S., Maynes, J. T., ... & Ferrer, M. (2015). Three-dimensional cell culture assays: are they more predictive of in vivo efficacy than 2D monolayer cell-based assays?. *Assay and Drug Development Technologies*, *13*(5), 254-261.

Spence, J. R., Mayhew, C. N., Rankin, S. A., Kuhar, M. F., Vallance, J. E., Tolle, K., ... & Wells, J. M. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro. *Nature*, *470*(7332), 105-109.

Sutherland, R. M., Inch, W. R., McCredie, J. A., & Kruuv, J. (1970). A multi-component radiation survival curve using an *in vitro* tumour model. *International Journal of Radiation Biology and Related Studies in Physics, Chemistry, and Medicine*, *18*(5), 491-495.

Takebe, T., Sekine, K., Enomura, M., Koike, H., Kimura, M., Ogaeri, T., ... & Taniguchi, H. (2013). Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature*, *499*(7459), 481-484.

Tanner, K., & Gottesman, M. M. (2015). Beyond 3D culture models of cancer. *Science Translational Medicine*, *7*(283), 283ps9.

Temple, J., Velliou, E., Shehata, M., Lévy, R., & Gupta, P. (2022). Current strategies with implementation of three-dimensional cell culture: the challenge of quantification. *Interface Focus*, *12*(5), 20220019.

Timmins, N. E., & Nielsen, L. K. (2007). Generation of multicellular tumor spheroids by the hanging-drop method. *Methods in Molecular Medicine*, *140*, 141-151.

Tostões, R. M., Leite, S. B., Serra, M., Jensen, J., Björquist, P., Carrondo, M. J., ... & Alves, P. M. (2012). Human liver cell spheroids in extended perfusion bioreactor culture for repeated-dose drug testing. *Hepatology*, *55*(4), 1227-1236.

Verma, A., Verma, M., & Singh, A. (2020). Animal tissue culture principles and applications. *Animal Biotechnology: Models in Discovery and Translation*, 269-293.

Wang, Y., & Jeon, H. (2022). 3D cell cultures toward quantitative high-throughput drug screening. *Trends in Pharmacological Sciences*, *43*(7), 569-581.

Whitesides, G. M. (2006). The origins and the future of microfluidics. *Nature*, *442*(7101), 368-373.

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Review article

Effect of metallic nanoparticles on cancer cell lines: A review on plant-based biosynthesis

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Abstract

The green synthesis method is an environmentally friendly, cost-efficient, and safe method for the production of metallic nanoparticles (MNPs). This method mainly relies on the use of plants and microorganisms as well. While plant-based MNPs are produced via the green synthesis method, the secondary metabolites of plants have the ability to enrich some functional properties of these MNPs. As a result of this, plant-based MNPs can be cytotoxic to some cancer cell lines. This review regarding the effect of plant-based MNPs anticancer activities on various cancer cell lines provides a summary of research articles in this area. Additionally, this review reports secondary metabolites of the plants used to synthesize MNPs. The present article offers a survey of plant species used with metallic nanoparticles, focusing on their anti-cancer properties in specific cancer cell lines. The objective is to provide researchers with a broad overview, facilitating exploration of plant–metal combinations.

Keywords: *Anti-cancer; anti-proliferation; cell line; green synthesis; nanoparticles; metal*

1. Introduction

Metallic nanoparticles (MNPs) are synthesized from salts of metals in a size range of 1-100 nm. Metal and metal oxide nanoparticles have unique physical and chemical functionalities. Given their small size, MNPs can cross biological membranes and biological barriers to influence cell metabolism. They can also be used in many biomedical applications as antimicrobial or therapeutic agents for diagnostic purpose and cancer treatments. (Khan et al., 2019; Franco et al., 2022; Khursheed et al., 2022). In the literature, zinc oxide (ZnO), iron oxide (Fe_3O_4NPs), and copper oxide nanoparticles (CuONPs) are commonly used for drug and gene delivery, biosensor design, cancer diagnosis, and treatment (Sharma et al., 2014). Although many types of metals are used in biomedical fields, silver nanoparticles (AgNP) are mostly preferred (Patra et al., 2018; Akhter et al., 2024). Since AgNPs have anti-angiogenic, antimicrobial, antiviral, antioxidant, and anti-cancer properties, their use in various nanoparticle applications in biomedicine is quite common (Iqbal et al., 2019). AgNPs have cytotoxic properties on cancer cells, arrest the cell cycle, and cause DNA damage and cell death by inducing oxidative stress (Liu et al., 2016). FeNPs can be used for many purposes, such as disease diagnosis and treatment, water decontamination, and cancer treatment (Montiel Schneider et al., 2022). Gold (Au) is a naturally inert material that is spontaneously resistant to bacteria, so it is widely used in biomedical applications (Ghobashy et al., 2024). ZnO nanoparticles have proven to be anti-fungal, anti-bacterial, and anti-cancer properties, and they have been used in various drug release systems (Ozcelik, 2023; Yalcin et al., 2023). Considering these properties, the use of nanoparticles in cancer treatment stands out due to their superior properties, such as high efficiency, selectivity, sensitivity, low toxicity, biosafety, and stability. Physical and chemical methods frequently used in

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nanoparticle production may require the use of high radiation, stabilizing agents, and toxic substances. Chemicals used to capture and reduce metal salts in particle formation increase free radical formation (Balmuri et al., 2017), thus increasing the toxicity of the nanoparticle. The green synthesis method has emerged as an eco-friendly alternative synthesis to existing chemical and physical methods. This method is easy to apply, cheap, fast, simple, eco-friendly, and safe since it does not contain stabilizing agents (Carrapico et al., 2023). Environmentally friendly synthesis is achieved by minimizing waste generation in green synthesis. It is also possible to produce particles with variable morphology and size by changing synthesis parameters (Gour et al., 2019). NPs can be produced by using various plants and microorganisms. However, the use of plants is more advantageous as they allow larger-scale production with faster NP synthesis. In addition, secondary metabolites of plants cover the particle during NP synthesis and provide various functions for the particle depending on the nature of the secondary metabolites. Given the fact that different plants contain different phytochemicals, the effect of the NP produced may vary, although the same metal salt is used. Concordantly, the cellular effects of synthesized MNPs can be modified and enriched.

In this review, nanoparticle production methods with a particular focus on plant-based green synthesis are explained, and the studies of the last decade examining the effects of Ag, Au, Fe, PbO, Pt, MgO, ZnO, and CeO nanoparticles, which are produced from plants by green synthesis, on cancer lines are compiled. In this context, the important contributions using plant-based synthesis are stated in detail, and their developmental aspects are presented as future perspectives in the field.

2. Production methods of metallic nanoparticles

There are top-down and bottom-up methods for MNP production. Bottom-up synthesis of NPs can be divided into three main groups: physical, chemical, and green synthesis (Fig. 1).

2.1. Physical methods

Evaporation-condensation, laser ablation, and mechanical milling are examples of physical techniques employed in synthesizing metallic nanoparticles. These physical methods typically aim to reduce bulk metals into nanoscale particles by applying high energy or mechanical forces. The evaporation-condensation process involves the application of a highly controlled vacuum to a metallic material, facilitating the evaporation of small particles that subsequently condense onto a target substrate. This method is preferred for the production of thin metallic layers (Pandey et al., 2011).

Another method is laser ablation, which is based on Cu or Ti vapor laser exposure on the metals (Al, Ag) in a liquid medium (Simakin et al., 2004). When these metal plates emit laser energy, small particles from metals break off and turn into nanoparticles (Sadrolhosseini et al., 2019). Depending on the laser parameters, the size of the nanoparticles can be changed. In addition, during long-term laser ablation, the rate of formation of new nanoparticles in the liquid medium may be reduced by saturation in the medium (Ghorbani et al., 2014).

The other method of metal nanoparticle production is the milling method. In this method, metal powders are made to be smaller particles through the use of rotating balls (Rajput et al., 2015). Although this method is cost-efficient for large-scale production, it has many disadvantages, such as high energy consumption and long processing time (Ullah et al., 2014).

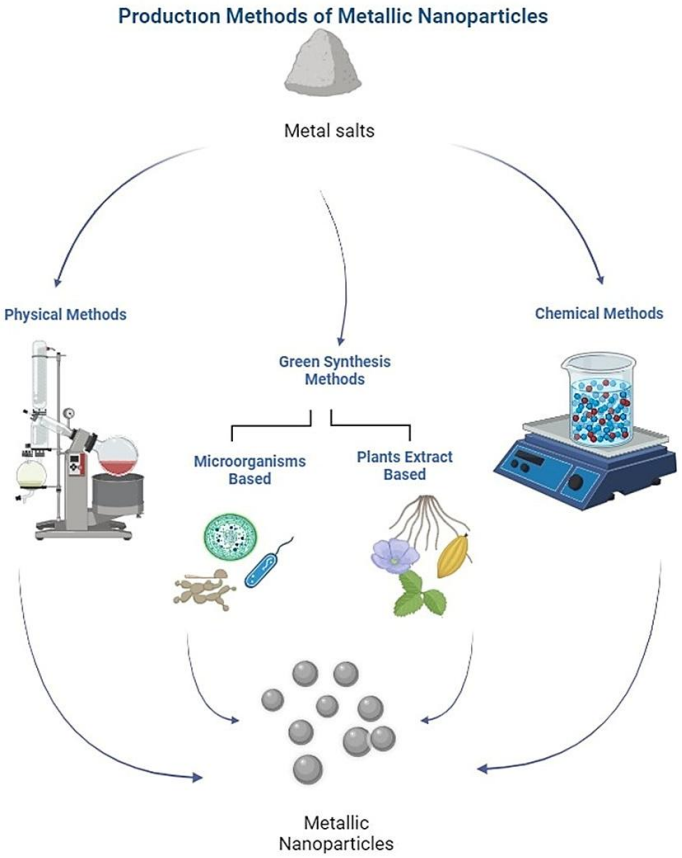

Fig. 1. Production methods of metallic nanoparticles (Created with Biorender).

In bottom-up nanoparticle production, the aforementioned methods are suitable, but these methods have some limitations such as the great deal of energy needed, high pressure, and temperature. In addition, these methods are rarely preferred for the large-scale formation of MNPs due to low production yields, high energy consumption, and cost (Dikshit et al., 2021).

2.2. Chemical methods

The most common methods to synthesize MNPs are chemical methods. In chemical methods, metal salts are reduced to metal nanoparticles by the use of some chemical agents for capping and stabilizing. In general, metal salts and chemical agents are mixed in a solvent that is toxic and corrosive during the generation of MNPs (Herlekar et al., 2014). Due to this, many chemical agents are used as reducing and capping agents, such as sodium borohydride ($NaBH_4$) (Banne et al., 2017), sodium citrate, Tollen's reagent, N, N-dimethylformamide (DMF), and formaldehyde (Norris et al., 2010). Compared to physical methods, chemical methods are mostly used for the industrial-scale production of MNPs due to their higher production yields. However chemical methods have some limitations such as toxicity problems, environmental hazards and unsustainability. Moreover, MNPs can lead to toxic nanoparticles depending on the use of some chemicals during their production. Thus, these methods are not safe MNP synthesis methods for clinical applications (Pal et al., 2007).

2.3. Green synthesis (biosynthesis)

Nanoparticles can be used in many applications in biomedical fields. There are also some concerns about toxicity. Despite the many advantages of physical and chemical methods, there is a need for safer and more environmentally friendly methods. Green synthesis methods have a huge potential to synthesize MNPs that are safe, environmentally friendly, sustainable, thermodynamically favorable, and cost-efficient (Razavi et al., 2015). Similar to other methods, many types of metal nanoparticles, such as Au, Ag, and Fe, can be produced via green synthesis methods (Singh et al., 2018). In this method, microorganisms or plant extracts provide some biomolecules. These molecules act as reducing, capping, and stabilizing agents. The green synthesis method has many advantages, such as the minimization of waste production throughout the process and the use of non-toxic solvents. Moreover, the preparation of metallic nanoparticles by green synthesis is cheap, fast, easy, and suitable for mass production. In addition, it is possible to produce metallic nanoparticles of different sizes by optimizing some parameters (temperature, pH, and pressure). Green synthesis metal nanoparticles can be used in many different applications, such as the use of antimicrobial agents, molecular detection, labeling, and optical imaging for diagnostic or therapeutic purposes (Gade et al., 2014). Although these methods do not include toxic chemicals for green synthesis, MNPs can have cytotoxic activity on various cancer cells. Thus, depending on the inclusion of biomolecules, some green synthesis-based MNPs can be used as anticancer agents on some cancer cell lines.

2.3.1. Microorganism-based synthesis

The microorganism-based green synthesis method includes the use of bacteria, fungi, or algae in metallic nanoparticle generation. In this method, metal salts are reduced to metal ions through electron transfer by enzymes or supernatants derived from microorganisms. When metal salts become metal ions, these small molecules begin to aggregate to form metallic nanoparticles. This method is a more environmentally friendly production method compared to chemical and physical methods. However, microorganism-based methods require the culturing of microorganisms, isolation of supernatant or enzyme, and filtration of extracts. Although the production of MNPs with microorganisms is a long-term process, microorganism culture is essential for this purpose. Similar to other methods, the microorganism-based method is suitable for the production of different types of metallic nanoparticles such as Ag, Au, Fe, Ti, PbO, Pt, MgO, ZnO, and CeO, (Gade et al., 2014; Molnár et al., 2018). In this method, metal salt can directly or indirectly interact with microorganisms. Microorganisms release a special biopolymer called extracellular polymeric substances (EPS). EPSs have some functional chemical groups, such as carboxylate (-COOH), amino (-NH_2), thiol (-SH), and alcohol (-OH). During the indirect interactions, biomolecules extracted from the microorganism are added to the medium for metal salts to be reduced to metallic nanoparticles (Escárcega-González et al., 2018). These chemical groups also make metallic nanoparticles biocompatible (Jeevanandam et al., 2022). However, the direct interaction method is based on real-time interaction with metal salts and microorganisms. During these interactions, some enzymes, such as nicotinamide adenine dinucleotide (NADH), play critical roles in the formation of MNPs. Despite the advantages of this method, the mechanism of generation of MNPs may vary because of the diversity of microorganisms. Thus, it is a great challenge to optimize the size, quantity, and morphology of MNPs. For these reasons, microorganism-based synthesis methods are not suitable for large-scale production or standardized synthesis of magnetic nanoparticles (Mittal et al., 2013).

In the literature, there are various studies of the microorganism-based MNPs. Haji et al. (2022) prepared AgNPs by using silver nitrate ($AgNO_3$) and extracts of *Acinetobacter baumannii* (Haji et al., 2022). In another study, AuNPs were produced by using ($HAuCl_4 \cdot 3H_2O$). ZnONPs were also prepared by using [$Zn(CH_3COO)_2 \cdot 2H_2O$] and the supernatant of *Leuconostoc* sp. The antibacterial activity of these MNPs was investigated against biofilms formed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Kabiri et al., 2023). Lahiri et al. (2021) prepared copper oxide nanoparticles (CuONPs) from $CuSO_4$ and *Streptomyces* sp. and evaluated their antimicrobial activity.

2.3.2. Plant extract-based synthesis

Plant-based green synthesis methods are simpler, easier, cheaper, and more useful than the other methods to produce MNPs on a large scale. Additionally, plants have various secondary metabolites for reducing metal salts to metal nanoparticles. These metabolites, such as steroids, saponins, carbohydrates, and flavonoids, which are called phytochemical molecules, act as reducing and capping agents during the formation of MNPs. Plant-based green synthesis methods also have more advantages than microorganism-based methods. The microorganism-based methods additionally require cell culture and cell extraction compared to the plant-based methods. As a result of this, whole extracts from plants (leaf, fruit, roots, etc.) can be used directly and are easier and cheaper to produce compared to the other methods. In the literature, many researches have been reported on the generation of MNPs using plant extracts. Similar to microorganism-based methods, particle size can be optimized by controlling parameters such as temperature and pH (Pal et al., 2019). Although both methods are environmentally friendly, plant-based methods require less energy and are faster to produce MNPs. In plant-based methods, the plant extracts and metal salts are mixed directly, and metal salts are reduced to MNPs during the process. MNPs gain functionality by phytochemicals from plants. Thus, it is possible to produce MNPs in one step. In the production of green synthesis of MNPs, plant tissues such as roots, leaves, fruits, and flowers are homogenized and mixed with metal precursor solutions (Mittal et al., 2013). The plant tissue to be used for nanoparticle synthesis is sequentially cleaned, mixed, boiled, and filtered to prepare the plant extract. This extract is then added to a solution containing metal salts (Nguyen et al., 2023). The most important feature of MNP production from plant extract is the reduction of metal salts of secondary metabolites (ketones, aldehydes, flavones, phenolic acids, amides, sugars, ketones, carboxylic acids, terpenoids, etc.) in plant extracts to MNPs while giving particles unique properties at the same time (Ovais et al., 2018). When plant extract is used in NP production, the secondary metabolites and plant-derived phytochemical agents surround the nanoparticle and give it various functions, such as anti-microbial and anti-cancer activities.

The percentage distribution of studies on AgNP, CuONP, PtNP, ZnONP, AuNP, PbO NP, and CeO_2 MNPs, based on 20

articles sourced from Scopus, PUBMED, Springer, and MDPI databases can be seen in Fig. 2. These studies were selected according to the inclusion and exclusion criteria considered during the preparation of this review. The graph aims to provide readers with a projection of the distribution of green-synthesized MNPs, produced from plant extracts and investigated for their anticancer activity over the last 10 years. Categorizing nanoparticle types offers a broad projection of the relevant literature (Vijayakumar et al., 2023; Batool et al., 2024; Muslim and Naji, 2024; Ullah et al., 2024).

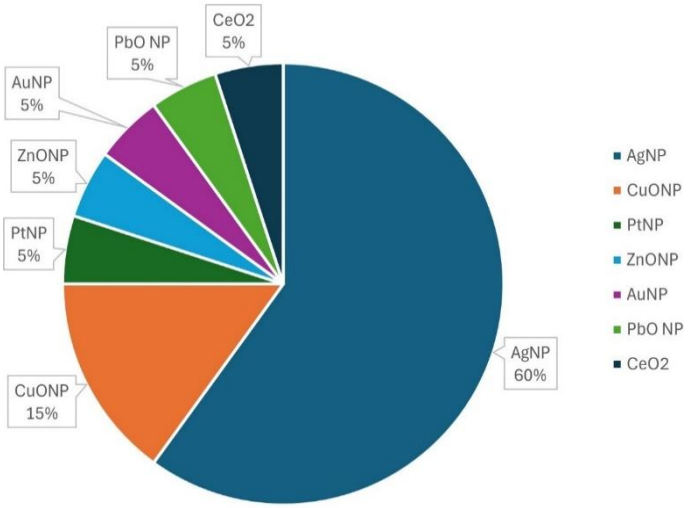

Fig. 2. The distribution of metal nanoparticles prepared by the green synthesis method in the literature. It was prepared on the basis of metal and metal oxide nanoparticles listed in Table 2. In accordance, the corresponding references are listed in Table 2.

The plants used for particle production, the secondary metabolites contained in these plants, and the studied biological effects are summarized in Table 1. Plant-based MNPs can acquire anticancer, antibacterial, antimicrobial, anti-arthritic, or antioxidant activities through the functional molecules derived from the plants they interact with during synthesis. These molecules enhance the properties and functionality of the nanoparticles, enabling them to exhibit various biological effects. The phytochemicals present in plant extracts are essential components that support the potential applications of these nanoparticles. Given this, plant-based MNPs can play a promising role in the treatment and prevention of various diseases (Demir et al., 2024; Grancharova et al., 2024; Revathi et ai., 2024).

According to Table 1, walnut fruit is especially rich in polyphenols, and it has been shown that polyphenol has a selective cytotoxic effect on cancer cells (Arulvasu et al., 2010; Dai and Mumper, 2010). In the literature, it has been reported that in research on *Punica granatum*, which contains flavonoids and tannins, these molecules' anti-cancer activities were shown on breast cancer cell lines (Moga et al., 2021). Another study reported the antimicrobial, anticancer, antidiabetic, and anti-inflammatory activities of *Saussurea costus*, which contains tannin and alkaloid derivatives (Singh et al., 2018). Salehi et al. (2016) demonstrated the induction of apoptotic pathways of cancer cell lines by using an extract that contains phenolic acid and flavonoids. In another research, the anti-inflammatory, anti-microbial, anti-fungal, anti-cancer, anti-arthritic, and anti-proliferative activities of *Curcuma wenyujin*, which contains terpenoid derivatives, were investigated. Its anti-cancer activities on many cancer cell lines, such as gastritis, prostate, ovarian, and uterine were also evaluated. *C. wenyujin* extract showed cytotoxic activity when applied to various cancer cell lines (Manzan et al., 2003; Zhou et al., 2014). Takemoto et al. (1983) demonstrated the anti-viral, anti-cancer, anti-diabetic, and anti-allergenic activities of *Glycyrrhiza glabra* (Liquorice), which is rich in flavonoid chalcone, isoflavone, and flavonols. In the literature, there is much research on ginseng extract. Ginseng's anti-proliferative activities have been shown on myeloma and HeLa cell lines (Davydov and Krikorian, 2000; Yesil-Celiktas et al., 2010). *Camellia sinensis* has anti-cancer, anxiolytic, anti-diabetic, anti-obesity, anti-inflammatory, analgesic, antipyretic, cytotoxic, and apoptogenic activities (Johnson 2011). It has many secondary metabolites, which are terpenoids, proanthocyanidins, and other phenolic compounds, flavones, and catechins. In another study, researchers evaluated the anti-proliferative and anti-cancer activities of rosemary extract on colon, pancreatic, and breast cancer cell lines. It is enriched in flavonoids, carnocytic acid, rosmarinic acid, and ursolic acid (Gutiérrez and Perez 2004; Movahedi et al., 2014).

In another study, *Raphanus sativus* (Longipinnatus), rich in flavonoids, and phenolics was reported to have anti-tumor activity (Gutiérrez and Perez 2004). The extract of *Teucrium polium*, which contains high levels of diterpenoids, flavonoids, iridoids, sterols, and terpenoids, was applied to cancer cell lines and displayed anti-cancer activity (Nematollahi-Mahani et al., 2007; Movahedi et al., 2014). Another plant, *Commiphora wightii* also has anti-microbial, anti-cancer, and anti-inflammatory activities due to the fact that it contains terpenoids and flavonoids. *C. wightii* has been confirmed to have anti-cancer activities. (Bhardwaj and Alia, 2019). *Mangifera indica* is rich in mangiferin and acts as an anti-oxidant, anti-bacterial, anti-fungal, anti-inflammatory, anti-allergic, and anti-cancer (Pattanayak 2013). *Rehmanniae radix* also has anti-inflammatory, anti-arthritic, anti-apoptotic, anti-cancer, and antioxidant activities. Its anti-cancer activities were shown on cervical cancer cells (Kim et al., 2006; Xia et al., 2019). Additionally, *Curcumae kwangsiensis* consists of steroids, saponin, tannin, terpenoids, alkaloids, glycoside, phenolic compounds, and flavonoids. *C. kwangsiensis* has anti-parasitic, anti-fungal, anti-viral, anti-bacterial, antioxidant, hypoglycemic, anti-diabetic, neuroprotective, and analgesic properties (Chen et al., 2021). In Chinese traditional medicine, *C. kwangsiensis* folium has been used in the treatment of ovarian cancer (Ebell et al., 2016). Anti-diabetic, anti-spasmodic, and anti-inflammatory properties of *Prosopis farcta* extracts, which mainly contain tannin, tryptamine, quercetin, and apigenin, have been described in studies (Asadollahi et al., 2010). Interestingly, *Scutellaria barbata* has been known as the magic herb that protects life in Asian societies. The cytotoxicity of *S. barbata* extract on various cancer cell lines has been studied so far (Lee et al., 2017; Suh et al., 2007; Marconett, et al., 2010; Chen et al., 2017; Kan et al., 2017; Yang et al., 2017; Zhang et al., 2017).

3. Effects of metallic nanoparticles on cancer cell lines

Cancer cell lines originate from cancerous tissue and are considered *in vitro* experimental models to test the cellular effects of natural and synthetic compounds as well as biomaterials. Anti-carcinogenic activity of any test agent depends on its ability to inhibit cancer cell proliferation, cancer cell migration, invasion, and angiogenesis, induce apoptosis and/or autophagy, and modulate the cell cycle and metabolism

Table 1
Plants used in the green synthesis of metallic nanoparticles and their bioactive compounds.

Plants	Plant Part	Bioactive compounds	Bioactivity	Reference
Adansonia digitata	fruit	terpenoids, flavonoids, sterol	*A. digitata* has analgesic and antipyretic, anti-inflammatory, antioxidant activity and antiviral activities.	(Kamatou et al., 2011)
Artemisia turcomanica	leaves	phenolic acid, flavonoid, alkaloid, terpenoid	*Artemisia turcomanica* has been shown to have a cytotoxic effect by inducing apoptosis in AGS cell line.	(Salehi et al., 2016)
Barleria buxifolia	leaves	alkaloids, terpenoids, triterpenoids, esters, aliphatic ketones, β-carotene	*B. buxifolia* extract demonstrates antioxidant, anti-bacterial, and anti-biofilm activities. These properties indicate its potential for protecting against oxidative stress, combating bacterial infections, and inhibiting the formation of biofilms.	(Sekar et al., 2022)
Camellia sinensis	leaves	terpenoid, phenolic compound, purine and alkaloid	*Camellia sinensis* has proven anti-cancer, anti-diabetic, anti-inflammatory and apoptogenic properties.	(Sharangi, 2009)
Centratherum anthelminticum	seed	phytochemicals, tetrahydroxy chalcone, tetrahydroxy flavone	*C. anthelminticum* has shown antioxidant and anti-proliferative effects in cancer cells.	(Lambertini et al., 2004; Qureshi et al., 2016)
Commiphora wightii	leaves	terpenoids, flavonoids, steroids, carbohydrates, sterols,	The anti-cancer and anti-inflammatory activities of *C. wightii* extract has been proven.	(Bhardwa and Alia, 2019)
Curcumae kwangsiensis	leaves	saponin, tannin, terpenoid, alkaloids, phenolic compound flavonoid	The successful effect of *C. kwangsiensis* extract in the treatment of ovarian cancer has been demonstrated.	(Sacchetti et al., 2005; Ebell et al., 2016)
Curcuma wenyujin	rhizome, stem	terpenoids and sesquiterpene derivatives	The anti-cancer properties of C. *wenyujin* have previously been demonstrated in gastritis, prostate, ovarian and uterine cancers.	(Zhou et al., 2014; Manzan et al., 2003)
Eclipta alba	leaves	coumestan, wedelolactone, desmethylwedelolactone, sitosterol	*E. alba* has anti-fungal, anti-bacterial, analgesic, anti-hepatotoxic, immunomodulatory, and antioxidant activities.	(Gupta et al., 2011)
Eleutherococcus senticosus (Siberian ginseng)	stem	Triterpenoidal saponins and phenylpropane derivatives	Ginseng extract has been shown to inhibit cancer cell growth when applied directly to myeloma (B-16) and Hela (S-3) cell lines.	(Takemoto et al., 1983; Davydov and Krikorian, 2000)
Glycyrrhzia glabra (Liquorice)	roots	flavonoid chalcon, isoflavone, flavonol,	Liquorice extract is antiviral, anti-cancer, anti-diabetic and has anti-allergenic activity.	(Pandian et al., 2017)
Jacobaea maritima	leaves	flavonoids, triterpenoids	*J. maritima* exhibits anti-cancer and anti-bacterial effects, highlighting its potential as a therapeutic agent in inhibiting cancer cell growth and combating bacterial infections.	(Grace and Khattab 1998; Althubiti et al.,2023)
Juglans regia (Walnut)	peeled walnut	polyphenol	Many studies have proven that polyphenol is safe against healthy cells and shows cytotoxicity against cancer cells.	(Dai and Mumper, 2010; Pandian et al., 2017)
Mangifera indica	leaves	mangiferin	*Mangifera indica* has been shown that high magniferin content of *M. indica* has anti-bacterial, anti-fungal, anti-viral, anti-inflammatory, and anti-cancer properties.	(Pattanayak and Nayak, 2013)
Moringa peregrina	leaves	flavonoids	*M. peregrina* has antioxidant, anti-microbial, anti-diabetic, anti-spasmodic, hypertension, hepatotoxicity, anti-inflammatory, anti-cancer properties	(Senthilkumar et al., 2018)
Ocimum sanctum	leaves	saponins, flavonoids, triterpenoids, and tannins	*O. sanctum* demonstrates anti-cancer activity by reducing tumor volume in fibrosarcoma tumors.	(Jaggi et al.,2003; Pattanayak et al.,2010)
Prosopis farcta	fruits	tannin, tryptamine, quercetin and apigenin	The anti-diabetic, anti-spasmodic and anti-inflammatory properties of *P. farcta* have been demonstrated.	(Asadollahi et al., 2010)
Punica granatum	crusts	flavonoid, tannin	*P. granatum* has been shown to have cytotoxic activity in the human breast cancer cell line.	(Arulvasu et al., 2010)
Raphanus sativus var. longipinnatus	leaves	flavanoid, phenol, alkaloid	Anti-tumor activity of *R. sativus var. longipinnatus* extract has been demonstrated.	(Gutiérrez and Perez, 2004)
Rehmanniae radix	roots	iridoid glycosides, phenylethanoid glycosides, monoterpenoidand triterpenes	In Hela cells, it was observed that *R. radix* extract increased apoptosis by increasing *Fas* expression.	(Xia et al., 2019; Kim et al., 2006)
Rhus coriaria	fruit	phenolic acids, favonoids, and tannins	*R. coriaria has* anti-cancer, antioxidant, anti-microbial, anti-infammatory, anti-fungal, hypoglycemic,	(Sakhr and El Khatib 2020; Elagbar et al.,

			hypolipidemic, neuroprotective, and anti-atherogenic properties	2020; Alsamri et al., 2021)
Rosmarinus officinalis (Rosemary)	leaves	flavonoid compounds carnosic acid, rosmarinic acid, and ursolic acid	Rosemary extract has been shown to have anti-proliferative properties on colon pancreatic and breast cancer cell lines. Carnasol causes the release of apoptosis-related proteins in cancer cells.	(Yesil-Celiktas et al., 2010; Gutiérrez and Perez, 2004)
Saussurea costus	roots	sesquiterpens, alkaloids, triterpenes, lignans, tannins	The anti-microbial, anti-cancer, anti-diabetic and anti-inflammatory properties of *S. costus* have been described in previous studies.	(Singh et al., 2018)
Scutellaria barbata	aqueous extract of the whole plant	flavonoid, alkaloid and saccharide	*S. barbata* extract has been shown to have anti-tumor activity on breast cancer, colorectal cancer, hepatocarcinoma, skin cancer, and ovarian and lung cancer.	(Marconett et al., 2010; Liu et al.,2015; Zhang et al., 2017)
Solanum lycopersicum	fruit	flavonoids	*S. lycopersicum* has anti-cancer, anti-microbial, anti-mutagenic, anti-inflammatory, anti-neurodegeneration, anti-platelet activities.	(Mousa et al., 2023).
Tectona grandis	leaves	terpenoids, apocarotenoids, phenolic compounds, flavonoids steroids/saponin	*T. grandis* exhibits significant antioxidant, anti-inflammatory, cytotoxic, analgesic, hypoglycemic, wound healing, and anti-plasmodial activities. These properties suggest its potential for therapeutic applications in managing oxidative stress, inflammation, cancer, pain, diabetes, wound healing, and malaria.	(Vyas et al., 2019)
Teucrium polium	leaves	diterpenoids, flavonoids, iridoids, sterols, and terpenoids	*T. polium* extract showed anti-tumor activity by inhibiting proliferation and colonization in BT20 breast cancer, DU145 prostate and A549 lung cancer cell line and MCF-7 cell line.	(Movahedi et al., 2014; Nematollahi-Mahani; 2007).
Zingiber officinale (Ginger)	roots	polyphenols, flavones, isoflavones, flavonoids, anthocyanins, coumarins, lignans, catechins, and isocatechins	Ginger has been confirmed to have anticancer activity against many types of cancer.	(Mukjerjee and Karati, 2022; Alkhathlan et al., 2021; Plengsuriyakarn et al., 2012; Khdary et al., 2023; Osman et al., 2020; Nachvak et al., 2023)

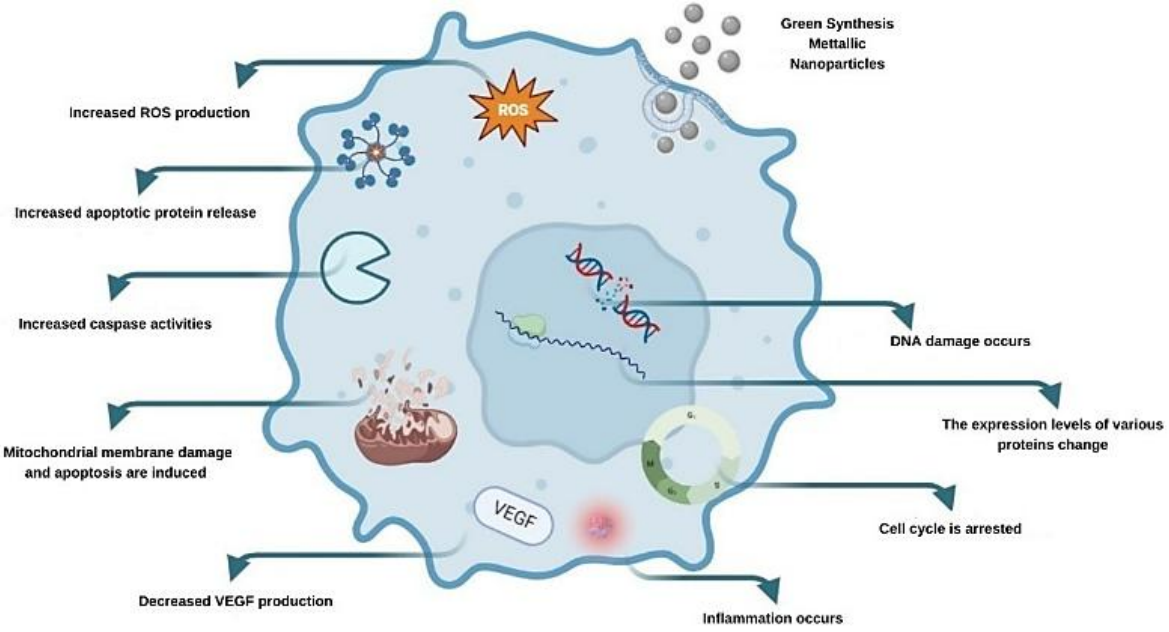

Fig. 3. The mechanism of actions of green-synthesized metal nanoparticles on cancer cells (Created with Biorender).

and ECM remodeling (Mehrotra et al., 2024; Perumal et al., 2024; Ramya et al., 2024). These effects are caused by direct interaction of the agent with cellular components and biomolecules, which inhibit biomolecule function or deregulate critical survival and apoptotic pathways, epigenetic gene regulatory effects, formation of free radicals inducing oxidative damage, or altered membrane fluidity. However, conclusions for therapeutic potentials should be drawn based on comparative studies on normal cells (Gharari et al., 2023; Ullah et al., 2024). In this context, if a nanoparticle is claimed to have anti-cancer activity, cancer cell lines serve as good, fast, and easy models to test cellular effects at molecular and biochemical levels upon direct application, which is also concordant with recent regulations on animal testing. 947 human cancer cell lines of 36

Table 2
Use of metallic nanoparticles produced by green synthesis method on various cancer cell lines.

MNP	Cell Line Tested	Plant	Bioactivity	Reference
AgNP	MCF-7 (breast cancer cells), L-929 (fibroblast cell line)	*Juglans regia*	AgNP showed cytotoxic effects in cancer cells compared to normal cells.	(Khorrami et al., 2018)
AgNP	MDA-MB-2 (Triple-negative breast cancer)	*Eclipta alba*	AgNPs exhibited higher anti-cancer activity on MDA-MB-2	(Mani et al., 2023).
AgNP	MCF-7, HeLa (cervical), HepG2 (liver), cell lines and L-929 (fibroblast cell line)	*Barleria buxifolia*	AgNPs exhibited anti-cancer activity on cancer cell lines but did not show cytotoxic effects against normal human cells	(Sekar et al., 2022)
AgNP	HTC116, HT29 cell line (human colon cancer cell lines)	*Zingiber officinale* (Ginger)	AgNPs demonstrated anti-cancer activity.	(Shanmugam et al., 2022; Alkhathlan et al., 2021;)
AgNP	HepG2 (human liver cancer cell lines)	*Tectona grandis*	AgNPs exhibited cytotoxic effects on cancer cell line.	(Younis et al., 2023)
AgNP	MCF-7, Caco-2 (colorectal cancer cell lines)	*Moringa peregrina*	AgNPs showed potential as a good anti-cancer agent.	(Al Baloushi et al., 2024)
AgNP	MCF-7, A-549) (lung cancer cell lines.	*Jacobaea maritima*	AgNPs exhibited cytotoxic effects on cancer cells.	(Althubiti et al., 2023)
AgNP	SW480 and HCT116 cell lines	*Adansonia digitata*	AgNPs exhibited cytotoxic effects on cancer cells.	(Almukaynizi et al., 2022)
AgNP	MDA-MB-231 (triple-negative breast cancer cell line)	*Centratherum anthelminticum*	AgNPs demonstrated anti-cancer activity by increasing cell death.	(Husain et al., 2020)
AgNP	AGS (gastric cancer cell line), L-929 (fibroblast cell line)	*Artemisia turcomanica*	AgNP plant-derived anti-proliferation property was concentration dependent. Apoptosis was induced rather than necrosis in AGS cells.	(Mousavi et al., 2018)
AgNP	MNK45 (human gastric cancer cell line)	*Teucrium polium*	AgNPs induced mitochondrial apoptosis in cancer cells by generating ROS.	(Hashemi et al., 2020)
AgNP	MCF-7, 3T3-L1 (embryonic cells)	*Commiphora wightii*	AgNPs induced apoptosis through DNA fragmentation by arresting the cell cycle in the G2M phase in the MCF-7 cell line.	(Malik et al., 2020)
AgNP AuNP	MCF-7, HaCaT (human keratinocyte cell line)	*Eleutherococcus senticosus* (*Siberian ginseng*)	AuNPs did not have any cytotoxic effect on cell lines. Therefore, AgNPs could be used therapeutically in breast cancer given their cytotoxic effect.	(Abbai et al., 2016)
AuNP	A498 (renal cancer cell lines), Sw-156	*Curcuma wenyujin*	AuNPs increased mitochondrial membrane permeability by generating ROS in A-498 cell line. AuNPs activated pro-apoptotic proteins and inhibited anti-apoptotic proteins.	(Liu et al., 2019)
AuNP	MCF-7, HEP-G2 (human liver cancer cell line)	*Glycyrrhzia glabra*	In the morphological analyzes, it was determined that AuNPs led cancer cells to apoptosis.	(Al-Radadi, 2021)
AuNP	HUVEC, Human HL60/vcr, 32D-FLT3-ITD, Murine C1498	*Camellia sinensis*	AuNPs did not show cytotoxic effect in HUVECs. AuNPs could be used in the treatment of acute myeloid leukemia.	(Ahmeda et al., 2020)
AuNP	PA-1, SW-626, SK-OV-3, HUVEC	*Curcumae kwangsiensis*	Dose-dependently, AuNPs inhibited growth in cancer cell lines.	(Chen et al., 2021)
AuNP	PANC-1 (pancreatic cancer cell)	*Scutellaria barbata*	Nps showed anti-cancer activity by increasing intracellular ROS production in PANC-1 cell line.	(Wang et al., 2019)
AgNP CuNP	human T-cell lymphoma (Jurkat) cell line.	*Ocimum sanctum*	AgNPs and CuNPs had anti-cancer activity.	(Ashokkumar et al., 2024)
CuONP	MCF-7, HCT-116, and HEK-293 cell lines	*Juglans regia* (*walnut*)	CuONPs demonstrated anti-cancer activity against breast and colon cancers.	(Abdollahzadeh et al., 2024)
CuONP	MCF-7 and normal NIH/3T3 cells.	*Moringa peregrina*	CuONPs exhibited cytotoxic activity against the MCF-7 cell line.	(Barani et al., 2024)
FeNP	4T1 (breast cancer cell line), C26 (colon carcinoma cell lines)	*Rosmarinus officinalis* (Rosemary)	The cytotoxicity of Rosemary-FeNP was higher than that of rosemary extract. Rosemary-FeNPs induced mitochondrial pathway apoptosis by down-regulating the anti-apoptotic protein BCL-2.	(Ahmeda et al., 2020)
MgO	MCF-7	*Saussurea costus*	MgONPs increased ROS generation and induced apoptosis by creating oxidative stress in MCF-7 cells.	(Malik et al., 2020)
CeO_2NP PbONP	HT-29 (colon cancer cell lines)	*Prosopis farcta*	PbO NPs were more cytotoxic than CeO_2 NPs. CeO_2 NPs could be used as a potential agent, especially for drug delivery systems.	(Nazaripour et al., 2021)
PtNP	MCF-7	*Punica granatum*	PtNPs induced apoptosis by arresting the cell cycle in the G0/G1 phase, increased oxidative stress and caused DNA damage.	(Sahin et al., 2018)

ZnONP	MCF-7, MDA-MB-231 cell lines	*Rhus coriaria*	ZnO NPs exhibited dose-dependent anti-cancer activity in both cancer cell lines.	(Mongy and Shalaby 2024)
ZnONP	A549 (lung cancer cells)	*Raphanus sativus var. longipinnatus*	Compounds in *R. sativus var. longipinnatus* extract increased the anti-cancer properties of ZnONP by targeting cancer-related proteins in the cell.	(Umamaheswari et al., 2021)
ZnONP	A549 (lung cancer cells)	*Mangifera indica*	NPs penetrated the membrane through ion channels and interacted with intracellular proteins.	(Rajeshkumar et al., 2018)
ZnONP	MG-63 (bone cancer cells)	*Rehmanniae radix*	Increased ROS caused apoptosis via the mitochondrial pathway.	(Cruz et al., 2020)

tumor types (Cancer Cell Line Encyclopedia (CCLE)) having the genomic diversity of their respective cancers and demonstrating the metabolic profiling and hormonal response status of the tumor microenvironment from which they were derived served as useful systems for medical research (Mirabelli et al., 2019). Additionally, HUVEC (human endothelial cell line), L-929 (normal fibroblast cell line), and 3T3-L1 (embryonic cell line) were used to evaluate the effect of particles on normal cells compared to cancer cells.

Various metal salts are selected due to their properties and reduced to MNPs with the determined plant extract. The mechanism of action of green-synthesized MNPs on cancer cells varies (Fig. 3). Nanoparticles tend to accumulate in cancer cells more than in healthy cells. Considering the side effects of traditional chemotherapy applications, the use of NPs with anti-cancer activity for therapeutic purposes has many advantages. MNPs can cross the cell membrane, induce free radical formation, conjugate with DNA, or cause cell death by affecting cell enzymes and transcription processes (Moghaddam et al., 2024; Shochah et al., 2024). In this context, both the anti-cancer activity of the metals selected for NP production and the plant-derived cytotoxic activity used in the production of green synthesis become critical. For example, AgNP are quite unique due to their diverse bioactivity spectrum with antioxidant, anti-fungal, anti-inflammatory, anti-viral, anti-angiogenic, and anti-microbial properties. Moreover, many studies have shown that MgONP have a cytotoxic effect on cancer cells. Studies on gold nanoparticles have demonstrated anti-cancer properties, and it has been stated that AuNPs can be used as a potential therapeutic agent in cancer treatment. Similarly, ZnONP have been shown to have anti-fungal, anti-bacterial, and anti-cancer activities and have been studied on various cancer cell lines due to their cytotoxic effects (Yalcin et al., 2020; Karahan et al., 2023; Genc and Celik, 2024).

Table 2 summarizes the MNPs produced, plants used for the biosynthesis process, and the test cell lines. Studies of the last decade are exemplified in this review. In a study conducted by Abdollahzadeh et al. (2024) copper(II) nitrate hexahydrate [$(Cu(NO_3)_2 \cdot 6H_2O)$] was mixed with walnut shell powder to synthesize CuONPs. These nanoparticles were then applied to different concentrations in MCF-7, HCT-116, and HEK-293 cell lines. According to the results, the anti-cancer activities of CuONPs are dependent on the particle size and have been reported anti-cancer activities on breast and colon cancer. Mongy and Shalaby prepared ZnONPs using *Rhus coriaria* fruit extract and zinc acetate dihydrate [$Zn(C_2H_3O_2)_2 \cdot 2H_2O$] and they investigated the anti-cancer activity of the particles on MCF-7 and MDA-MB-231 cell lines. They demonstrated that ZnONPs have dose-dependent anticancer activity through cell cycle arrest in the S phase (Mongy and Shalaby, 2024). In a similar study, AgNPs were prepared by mixing them with $AgNO_3$ and the plant *Eclipta alba*. When comparing between direct *plant* extract and AgNPs application, nanoparticles have been reported to have higher anti-cancer activities on triple-negative breast cancer (MDA-MB-2) cell lines (Mani et al., 2023). In another study, AgNPs were synthesized by using leaves of *Barleria buxifolia*, and nanoparticles were treated on human breast cancer (MCF-7), cervical (HeLa), and liver (HepG2) cell lines and fibroblast cells (L929). AgNPs showed selective anticancer activity on cancer cell lines (Sekar et al., 2022). Mousa et al. (2023) prepared ZnO nanoparticles using *Solanum lycopersicum* (tomato) extract and nanoparticles exhibited anti-cancer activity on ovarian cancer cell line (SKOV3). In another study, CuOMNPs were synthesized using *Moringa peregrina* leaf extracts and have been investigated for their anti-cancer activities on MCF-7 cell lines and normal NIH/3T3 cells. According to the results, nanoparticles have anti-cancer activities on cancer cells selectively (Barani et al., 2024). Ginger's anti-cancer activities have been demonstrated in previous studies so ginger-based green synthesis of AgNPs showed cytotoxicity on cancer cell lines (Alkhathlan et al., 2021; Shanmugam et al., 2022). Younis et al. (2023) prepared AgNPs from *Tectona grandis* leaf extract and reported cytotoxicity on the HepG2 cancer cell line via the induction of reactive oxygen species (ROS) generation. In another research, the anti-cancer activities of AgNPs produced from *Moringa peregrina* leaf extract were investigated on breast (MCF-7) and colon (Caco-2) cancer cell lines and demonstrated anti-cancer activities on cancer cells (Al Baloushi et al., 2024). Similarly, AgNPs synthesized from *Jacobaea maritima* leaves have been utilized in the treatment of breast cancer (MCF-7) and lung cancer (A-549) cell lines. According to the result, AgNPs exhibited cytotoxic activities on both cell lines with different inhibitory concentrations (IC_{50}) values, which were 1.37 μg mL^{-1} for the MCF-7 cell line and 1.98 μg mL^{-1} for the A-549 cell line (Althubiti et al., 2023). Almukaynizi et al. (2022) applied AgNPs produced from *Adansonia digitata* fruit via green synthesis on SW480 and HCT116 cell lines and demonstrated the effects of cell inhibition on cancer cells. AgNPs were also synthesized using *Centratherum anthelminticum* seed extract, and the resulting nanoparticles exhibited anti-cancer activity on the MDA-MB-231cell line by inducing apoptosis (Husain et al., 2020). Ashokkumar et al. (2024) prepared AgNPs and CuNPs using *Ocimum sanctum* leaves. AgNPs and CuNPs were applied at different concentrations on the human T-cell lymphoma (Jurkat) cell line, and synthesized nanoparticles showed anti-cancer activity at all concentrations.

In another study, Khorrami et al. (2018) prepared AgNPs using walnut extract and they applied AgNPs and direct walnut extract to MCF-7 breast cancer and L-929 fibroblast cell lines. When AgNPs and walnut extract at different concentrations (10-60 μg mL^{-1}) were treated on cancer cell lines, AgNPs were found to be more cytotoxic on MCF-7 cells than direct walnut extract. Additionally, green synthesized platinum nanoparticles (PtNPs) prepared from *Punica granutum* extract were applied to MCF-7 cells at different concentrations (2.5, 5, 10, 20, 40, and 50 μg

mL^{-1}), and cell death was induced by PtNPs (Sahin et al., 2018). Amina et al. synthesized MgONPs using *Saussurea costus* extract and they compared the cytotoxic activities of MgONPs and paclitaxel on MCF-7 cells. The results demonstrated that MgONP applied cell death rate was 84.3% compared to the 65.4% cell death rate of the paclitaxel-applied group at the same concentration (Amina et al., 2020). In another study, the anti-cancer activities of green synthesized AgNPs from *Artemisia turcomanica* and commercial AgNPs were compared on AGS (human gastritis adenocarcinoma cell line) and L-929 at different concentrations (3,5, 12.5, 25, 50, and 100 μg mL^{-1}). When comparing the cytotoxic effects of green synthesized AgNPs at different concentrations, the AGS cell line showed higher anti-cancer activity compared to L-929 cells (Mousavi et al., 2018).

AuNPs were produced from *Curcuma wenyujin* extract and tested on A498 renal cancer and SW-156 human kidney-derived cell lines. AuNPs were applied to cell lines at concentrations of 5, 10, 25, 30, 40, and 50 μg mL^{-1}. AuNPs activated pro-apoptotic proteins and inhibited anti-apoptotic proteins in the A498 cell line. Mitochondrial membrane damage increased 2-fold at 50 μg mL^{-1} AuNP application in comparison to 25 μg mL^{-1}. In addition, caspase9 and caspase3 activities also increased when AuNP application was increased to 50 μg mL^{-1} from 25 μg mL^{-1}. In another study, AuNPs were produced from *Glycyrrhiza glabra* extract (Al-Radadi, 2021). AuNPs' anti-cancer activity was evaluated on MCF-7 and HepG2 human liver cancer cell line at a concentration range of 0-500 μg mL^{-1}. AuNP application caused concentration-dependent inhibition of cell growth, and IC_{50} of the AuNPs were found to be 50 μg mL^{-1} and 23 μg mL^{-1} on MCF-7 and HepG2 cells, respectively.

In a study, both AgNP and AuNP were produced using *Siberian Ginseng*, and their bioactivities were tested on MCF-7 and HaCa-T human keratinocyte cell lines. Interestingly, AuNPs did not exert cytotoxic effects on both cell lines, even at 100 μg mL^{-1} concentration. On the other hand, the concentration-dependent cytotoxic effect of AgNPs on both cell lines was demonstrated. 100 μg mL^{-1} AgNP reduced the cell viability of MCF-7 cells by 50%, whereas it caused a 30% reduction in cell viability of HecaT cells. The possiblee application of AuNPs in the drug release of anti-cancer agents was further discussed (Abbai et al., 2016). In another study by which AuNP was produced from *Camellia sinensis* extract, the effectiveness of the particles was tested on C1498 murine acute myeloid leukemia cell line, incristine resistant acute promyleocytic leukemia (HL-60/VCR), 32D-FLT3-ITD (32D cells with FMS-like tyrosine kinase 3-internal tandem duplication mutation), and human normal endothelial (HUVEC) cell lines. Gold salt, daunorubicin, green synthesized AuNP, and *C. sinesis* extract were applied to cells separately in 1-1000 μg mL^{-1}. Each treatment decreased cell viability in a concentration-dependent manner. IC_{50} values of gold salt, plant extract, AuNP, and daunorubicin on the murine C1498 cell line were determined as 698, 389, 158, and 129 μg mL^{-1}, respectively. The cytotoxic effect of green synthesized AuNP was comparable to the chemotherapeutic drug daunorubicin. AuNPs also exerted concentration-dependent cytotoxicity on HL-60/VCR and 32D-FLT-ITD cell lines whereas they did not show any effect on the HUVEC cell line. The bioactivities of the FeNPs synthesized from rosemary plant extract, the rosemary extract, and the green synthesis FeNPs were investigated on 4T1 breast and C26 colon carcinoma cell lines in a concentration range of 3-200 μg mL^{-1}. 90% growth inhibition was achieved at 200 μg mL^{-1} of FeNPs. The IC_{50} of rosemary-FeNPs was 21 μg mL^{-1}, whereas it was 48 μg mL^{-1} for the extract, indicating a higher cytotoxic effect of green synthesized FeNPs in comparison to plant extract (Ahmeda et al., 2020). ZnONPs produced with *Raphanus sativus* extract were applied to the cells at a concentration of 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 μg mL^{-1} and incubated for 48 hours. It was observed that the cell viability was reduced by 50% when 40 μg mL^{-1} was applied (Umamaheswari et al., 2021).

In another study, AgNPs derived from *Teucrium polium* were applied to MNK45 gastric cancer cells within the concentration range of 12.5 μg mL^{-1} to 130 μg mL^{-1}. The ratio of cell death was elevated to 74% at the concentration of 130 μg mL^{-1}, which correlates with the fact that cell death increases with higher concentration (Hashemi et al., 2020). AgNPs were prepared from *Commiphora wightii* extract and treated for MCF-7 breast cancer and 3T3-L1 embryonic cells. It has also been reported that AgNPs induced more selective cell death in MCF-7 breast cancer cells compared to 3T3-L1 embryonic cells. For example, when AgNPs were applied at a concentration of 67 μg mL^{-1}, cell viability decreased by 50% in MCF-7 cells (Malik et al., 2020).

Similarly, ZnONPs prepared using *Magnifera indica* and ZnONPs and direct plant extract were applied to the A549 human lung cancer cell line. The increased ROS generation in cancer cells was dependent on the increasing concentration of ZnONPs. Compared with the direct application of plant extract, the anti-cancer activity of ZnONPs was found to be higher (Rajeshkumar et al., 2018). In another study, ZnONPs produced using *Rehmanniae radix* and treated on MG-63 human bone cancer cell line demonstrated a correlation between the concentration of ZnONPs and the induction of cytotoxic activity by the formation of ROS in the cells. It is also hypothesized that ZnONPs induce apoptosis in cancer cells (Chen et al., 2021).

AuNP produced using *Curcumae kwangsienis* extract were applied to 3 different human ovarian cancer lines which are PA-1 (human ovarian teratocarcinoma cell line), SW-626 (human ovarian metastatic cell line), and SK-OV-3 (human ovarian carcinoma cell line), and a normal human cell line (HUVEC) in the range of 0-1000 μg mL^{-1}. The particles did not exhibit any cytotoxic effects on HUVEC cells at any concentration. However, IC_{50} of AuNPs was found to be 153 μg mL^{-1} in PA-1 cells, 166 μg mL^{-1} in SW-626 cells, and 204 μg mL^{-1} in the SK-OV-3 cell line (Chen et al., 2021). A recent study showed that *Scutellaria barbata* extract was used in AuNP synthesis, and its anti-cancer activity on the PANC-1 human pancreatic cancer cell line was evaluated. NPs were applied at a concentration of 0-100 μg mL^{-1}, and an IC_{50} of 52 μg mL^{-1} has been determined (Wang et al., 2019).

Both lead oxide (PbONP) and selenium oxide (CeO_2NPs) nanoparticles were produced from *Prosopis farcta* extracts. HT-29 particles (human colon cancer cell line) were exposed to concentrations of PbONPs in the range of 1-500 μg mL^{-1}. The results showed that PbONPs did not exhibit any cytotoxic effects at concentrations below 30 μg mL^{-1}. The IC_{50} of PbONPs was determined to be 60 μg mL^{-1}. In contrast, CeO_2NPs exhibited a cell viability of 58% even at a concentration of 500 μg mL^{-1}. Therefore, PbO NPs have greater anti-cancer activity in cancer cells compared to CeO_2NPs (Nazaripour et al., 2021).

4. Conclusion

The examined articles here summarized the effects of metallic nanoparticles from plant sources, including Ag, Au, Fe,

PbO, Pt, MgO, ZnO, and CeO. It is worth noting that Ag and Au nanoparticles appear to be particularly common and effective in this context. This review suggests that metal nanoparticles produced from plants using green synthesis have cytotoxic properties on cancer cells through the functional phytochemicals derived from the plants they interact with during synthesis. Enhanced biological effects with reduced toxicity achieved through green biosynthesis increase the potential for healthcare applications. While physical and chemical production methods are also utilized for MNP synthesis, the green synthesis method is preferred due to its eco-friendly, practical, inexpensive, and reliable nature. Sources for green synthesis in the literature vary between plants and microorganisms, with plant-based sources being prominent due to their numerous advantages.

It is worth noting that the toxicity of nanoparticles on cell lines can vary depending on the concentration applied, the cell line used, and the combination of plant and metal. Additionally, compounds from plants used in MNP synthesis have been found to support the cytotoxic properties of MNPs. Optimization and standardization of cell line-based biological assays using different 2D and 3D *in vitro* experimental models are required in particular for the determination of the anti-carcinogenic potential of NPs.

Conflict of interest: The authors declare that they have no conflict of interests.

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References

Abbai, R., Mathiyalagan, R., Markus, J., Kim, Y. J., Wang, C., Singh, P., ... & Yang, D. C. (2016). Green synthesis of multifunctional silver and gold nanoparticles from the oriental herbal adaptogen: Siberian ginseng. *International Journal of Nanomedicine*, 3131-3143.

Abdollahzadeh, H., Pazhang, Y., Zamani, A., & Sharafi, Y. (2024). Green synthesis of copper oxide nanoparticles using walnut shell and their size dependent anticancer effects on breast and colorectal cancer cell lines. *Scientific Reports*, *14*(1), 20323.

Ahmeda, A., Zangeneh, A., & Zangeneh, M. M. (2020). Green synthesis and chemical characterization of gold nanoparticle synthesized using *Camellia sinensis* leaf aqueous extract for the treatment of acute myeloid leukemia in comparison to daunorubicin in a leukemic mouse model. *Applied Organometallic Chemistry*, *34*(3), e5290.

Akhter, M. S., Rahman, M. A., Ripon, R., Mubarak, M., Akter, M., Mahbub, S., ... & Sikder, M. T. (2024). A systematic review on green synthesis of silver nanoparticles using plants extract and their bio-medical applications. *Heliyon*, *10*(11), e29766.

Al Baloushi, K. S. Y., Senthilkumar, A., Kandhan, K., Subramanian, R., Kizhakkayil, J., Ramachandran, T., ... & Jaleel, A. (2024). Green synthesis and characterization of silver nanoparticles using *Moringa peregrina* and their toxicity on MCF-7 and Caco-2 Human Cancer Cells. *International Journal of Nanomedicine*, 3891-3905.

Alkhathlan, A. H., Al-Abdulkarim, H. A., Khan, M., Khan, M., Alkholief, M., Alshamsan, A., ... & Siddiqui, M. R. H. (2021). Evaluation of the anticancer activity of phytomolecules conjugated gold nanoparticles synthesized by aqueous extracts of *Zingiber officinale* (ginger) and *Nigella sativa* L. seeds (black cumin). *Materials*, *14*(12), 3368.

Almukaynizi, F. B., Daghestani, M. H., Awad, M. A., Althomali, A., Merghani, N. M., Bukhari, W. I., ... & Bhat, R. S. (2022). Cytotoxicity of green-synthesized silver nanoparticles by *Adansonia digitata* fruit extract against HTC116 and SW480 human colon cancer cell lines. *Green Processing and Synthesis*, *11*(1), 411-422.

Al-Radadi, N. S. (2021). Facile one-step green synthesis of gold nanoparticles (AuNp) using licorice root extract: Antimicrobial and anticancer study against HepG2 cell line. *Arabian Journal of Chemistry*, *14*(2), 102956.

Alsamri, H., Athamneh, K., Pintus, G., Eid, A. H., & Iratni, R. (2021). Pharmacological and antioxidant activities of *Rhus coriaria* L.(Sumac). *Antioxidants*, *10*(1), 73.

Althubiti, A. A., Alsudir, S. A., Alfahad, A. J., Alshehri, A. A., Bakr, A. A., Alamer, A. A., ... & Tawfik, E. A. (2023). Green synthesis of silver nanoparticles using *Jacobaea maritima* and the evaluation of their antibacterial and anticancer activities. *International Journal of Molecular Sciences*, *24*(22), 16512.

Amina, M., Al Musayeib, N. M., Alarfaj, N. A., El-Tohamy, M. F., Oraby, H. F., Al Hamoud, G. A., ... & Moubayed, N. M. (2020). Biogenic green synthesis of MgO nanoparticles using *Saussurea costus* biomasses for a comprehensive detection of their antimicrobial, cytotoxicity against MCF-7 breast cancer cells and photocatalysis potentials. *PLoS One*, *15*(8), e0237567.

Arulvasu, C., Prabhu, D., Manikandan, R., Srinivasan, P., Dinesh, D., Babu, G., & Sellamuthu, S. (2010). Induction of apoptosis by the aqueous and ethanolic leaf extract of *Vitex negundo* L. in MCF-7 human breast cancer cells. *International Journal of Drug Discovery*, *2*(1), 1-7.

Asadollahi, K., Abassi, N., Afshar, N., Alipour, M., & Asadollahi, P. (2010). Investigation of the effects of *Prosopis farcta* plant extract on rat's aorta. *Journal of Medicinal Plants Research*, *4*(2), 142-7.

Ashokkumar, M., Palanisamy, K., Ganesh Kumar, A., Muthusamy, C., & Senthil Kumar, K. J. (2024). Green synthesis of silver and copper nanoparticles and their composites using *Ocimum sanctum* leaf extract displayed enhanced antibacterial, antioxidant and anticancer potentials. *Artificial Cells, Nanomedicine, and Biotechnology*, *52*(1), 438-448.

Balmuri, S. R., Selvaraj, U., Kumar, V. V., Anthony, S. P., Tsatsakis, A. M., Golokhvast, K. S., & Raman, T. (2017). Effect of surfactant in mitigating cadmium oxide nanoparticle toxicity: implications for mitigating cadmium toxicity in environment. *Environmental Research*, *152*, 141-149.

Banne, S. V., Patil, M. S., Kulkarni, R. M., & Patil, S. J. (2017). Synthesis and characterization of silver nano particles for EDM applications. *Materials Today: Proceedings*, *4*(11), 12054-12060.

Barani, M., Mir, A., Roostaee, M., Sargazi, G., & Adeli-Sardou, M. (2024). Green synthesis of copper oxide nanoparticles via *Moringa peregrina* extract incorporated in graphene oxide: evaluation of antibacterial and anticancer efficacy. *Bioprocess and Biosystems Engineering*, *47*(11), 1915-1928.

Batool, A., Azizullah, A., Ullah, K., Shad, S., Khan, F. U., Seleiman, M. F., ... & Zeb, U. (2024). Green synthesis of Zn-doped TIO2 nanoparticles from *Zanthoxylum armatum*. *BMC Plant Biology*, *24*(1), 820.

Bhardwaj, M., & Alia, A. (2019). Commiphora wightii (Arn.) Bhandari. Review of its botany, medicinal uses, pharmacological activities and phytochemistry. *Journal of Drug Delivery and Therapeutics*, *9*(4-s), 613-621.

Carrapico, A., Martins, M. R., Caldeira, A. T., Mirão, J., & Dias, L. (2023). Biosynthesis of metal and metal oxide nanoparticles using microbial cultures: Mechanisms, antimicrobial activity and applications to cultural heritage. *Microorganisms*, *11*(2), 378.

Chen, C. C., Kao, C. P., Chiu, M. M., & Wang, S. H. (2017). The anti-cancer effects and mechanisms of *Scutellaria barbata* D. Don on CL1-5 lung cancer cells. *Oncotarget*, *8*(65), 109340.

Chen, J., Li, Y., Fang, G., Cao, Z., Shang, Y., Alfarraj, S., ... & Duan, X. (2021). Green synthesis, characterization, cytotoxicity, antioxidant, and anti-human ovarian cancer activities of *Curcumae kwangsiensis* leaf aqueous extract green-synthesized gold nanoparticles. *Arabian Journal of Chemistry*, *14*(3), 103000.

Cruz, D. M., Mostafavi, E., Vernet-Crua, A., Barabadi, H., Shah, V., Cholula-Díaz, J. L., ... & Webster, T. J. (2020). Green nanotechnology-based zinc oxide (ZnO) nanomaterials for biomedical applications: a review. *Journal of Physics: Materials*, *3*(3), 034005.

Dai, J., & Mumper, R. J. (2010). Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules*, *15*(10), 7313-7352.

Davydov, M., & Krikorian, A. D. (2000). *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. (Araliaceae) as an adaptogen: a closer look.

Journal of Ethnopharmacology, *72*(3), 345-393.
Demir, C., Aygun, A., Gunduz, M. K., Altınok, B. Y., Karahan, T., Meydan, I., ... & Sen, F. (2024). Production of plant-based ZnO NPs by green synthesis; anticancer activities and photodegradation of methylene red dye under sunlight. *Biomass Conversion and Biorefinery*, 1-16.
Dikshit, P. K., Kumar, J., Das, A. K., Sadhu, S., Sharma, S., Singh, S., ... & Kim, B. S. (2021). Green synthesis of metallic nanoparticles: Applications and limitations. *Catalysts*, *11*(8), 902.
Ebell, M. H., Culp, M. B., & Radke, T. J. (2016). A systematic review of symptoms for the diagnosis of ovarian cancer. *American Journal of Preventive Medicine*, *50*(3), 384-394
Elagbar, Z. A., Shakya, A. K., Barhoumi, L. M., & Al-Jaber, H. I. (2020). Phytochemical diversity and pharmacological properties of *Rhus coriaria*. *Chemistry & Biodiversity*, *17*(4), e1900561.
Escárcega-González, C. E., Garza-Cervantes, J. A., Vázquez-Rodríguez, A., & Morones-Ramírez, J. R. (2018). Bacterial exopolysaccharides as reducing and/or stabilizing agents during synthesis of metal nanoparticles with biomedical applications. *International Journal of Polymer Science*, *2018*(1), 7045852.
Franco, D., Calabrese, G., Guglielmino, S. P. P., & Conoci, S. (2022). Metal-based nanoparticles: Antibacterial mechanisms and biomedical application. *Microorganisms*, *10*(9), 1778.
Gade, A., Gaikwad, S., Duran, N., & Rai, M. (2014). Green synthesis of silver nanoparticles by *Phoma glomerata*. *Micron*, *59*, 52-59.
Genc, D., & Celik, I. (2024). Investigation of the effects of *Eremurus spectabilis* Bieb. lyophilized and nanoparticle extracts on the cellular and enzymatic immune system in experimentally-induced hepatocellular carcinogenesis in rats. *Frontiers in Life Sciences and Related Technologies*, *5*(2), 95-100.
Gharari, Z., Hanachi, P., Sadeghinia, H., & Walker, T. R. (2023). Eco-friendly green synthesis and characterization of silver nanoparticles by *Scutellaria multicaulis* leaf extract and its biological activities. *Pharmaceuticals*, *16*(7), 992.
Ghobashy, M. M., Alkhursani, S. A., Alqahtani, H. A., El-damhougy, T. K., & Madani, M. (2024). Gold nanoparticles in microelectronics advancements and biomedical applications. *Materials Science and Engineering: B*, *301*, 117191.
Ghorbani, H. R. (2014). A review of methods for synthesis of Al nanoparticles. *Oriental Journal of Chemistry*, *30*(4), 1941-1949.
Gour, A., & Jain, N. K. (2019). Advances in green synthesis of nanoparticles. *Artificial Cells, Nanomedicine, and Biotechnology*, *47*(1), 844-851.
Grace, M. H., & Khattab, A. M. (1998). Chemical constituents and molluscicidal activity of *Senecio cineraria* DC.
Grancharova, T., Simeonova, S., Pilicheva, B., & Zagorchev, P. (2024). Gold nanoparticles in Parkinson's disease therapy: A focus on plant-based green synthesis. *Cureus*, *16*(2).
Gupta, R., Sharma, A. K., Dobhal, M. P., Sharma, M. C., & Gupta, R. S. (2011). Antidiabetic and antioxidant potential of β-sitosterol in streptozotocin-induced experimental hyperglycemia. *Journal of Diabetes*, *3*(1), 29-37.
Gutiérrez, R. M. P., & Perez, R. L. (2004). *Raphanus sativus* (Radish): Their chemistry and biology. *The Scientific World Journal*, *4*(1), 811-837.
Haji, S. H., Ali, F. A., & Aka, S. T. H. (2022). Synergistic antibacterial activity of silver nanoparticles biosynthesized by carbapenem-resistant Gram-negative bacilli. *Scientific Reports*, *12*(1), 15254.
Hashemi, S. F., Tasharrofi, N., & Saber, M. M. (2020). Green synthesis of silver nanoparticles using *Teucrium polium* leaf extract and assessment of their antitumor effects against MNK45 human gastric cancer cell line. *Journal of Molecular Structure*, *1208*, 127889.
Herlekar, M., Barve, S., & Kumar, R. (2014). Plant-mediated green synthesis of iron nanoparticles. *Journal of Nanoparticles*, *2014*(1), 140614.
Husain, J. H., Arumugam, D., Nawabjohn, M. S., Kumaran, S., & Pandurangan, A. K. (2024). Green synthesis of silver nanoparticles using *Centratherum anthelminticum* extract against breast cancer cells. *Asian Pacific Journal of Cancer Prevention: APJCP*, *25*(8), 2711.
Iqbal, S., Fakhar-e-Alam, M., Akbar, F., Shafiq, M., Atif, M., Amin, N., ... & Farooq, W. A. (2019). Application of silver oxide nanoparticles for the treatment of cancer. *Journal of Molecular Structure*, *1189*, 203-209.
Jaggi, R. K., Madaan, R., & Singh, B. (2003). Anticonvulsant potential of holy basil, *Ocimum sanctum* Linn., and its cultures. *Indian Journal of Experimental Biology*, *41*, 1329-1333.
Jeevanandam, J., Kiew, S. F., Boakye-Ansah, S., Lau, S. Y., Barhoum, A., Danquah, M. K., & Rodrigues, J. (2022). Green approaches for the synthesis of metal and metal oxide nanoparticles using microbial and plant extracts. *Nanoscale*, *14*(7), 2534-2571.
Johnson, J. J. (2011). Carnosol: a promising anti-cancer and anti-inflammatory agent. *Cancer Letters*, *305*(1), 1-7.
Kabiri, F., Aghaei, S. S., Pourbabaee, A. A., Soleimani, M., & Komeili Movahhed, T. (2023). Antibiofilm and cytotoxic potential of extracellular biosynthesized gold nanoparticles using actinobacteria *Amycolatopsis* sp. KMN. *Preparative Biochemistry & Biotechnology*, *53*(3), 265-278.
Kamatou, G. P. P., Vermaak, I., & Viljoen, A. M. (2011). An updated review of Adansonia digitata: A commercially important African tree. *South African Journal of Botany*, *77*(4), 908-919.
Kan, X., Zhang, W., You, R., Niu, Y., Guo, J., & Xue, J. (2017). *Scutellaria barbata* D. Don extract inhibits the tumor growth through down-regulating of Treg cells and manipulating Th1/Th17 immune response in hepatoma H22-bearing mice. *BMC Complementary and Alternative Medicine*, *17*, 1-10.
Karahan, H., Tetik, N., & Colgecen, H. (2023). Phytofabrication of silver nanoparticles using callus extracts of natural tetraploid *Trifolium pratense* L. and its bioactivities. *Frontiers in Life Sciences and Related Technologies*, 18-28.
Khan, I., Saeed, K., & Khan, I. (2019). Nanoparticles: Properties, applications and toxicities. *Arabian journal of chemistry*, *12*(7), 908-931.
Khdary, N. H., Alangari, A. A., Katubi, K. M., Alanazi, M., Alhassan, A., Alzahrani, S. D., ... & Alanazi, I. O. (2023). Synthesis of Gingerol-Metals Complex and *in-vitro* Cytotoxic Activity on Human Colon Cancer Cell Line. *Cancer Management and Research*, 87-98.
Khorrami, S., Zarrabi, A., Khaleghi, M., Danaei, M., & Mozafari, M. R. (2018). Selective cytotoxicity of green synthesized silver nanoparticles against the MCF-7 tumor cell line and their enhanced antioxidant and antimicrobial properties. *International Journal of Nanomedicine*, 8013-8024.
Khursheed, R., Dua, K., Vishwas, S., Gulati, M., Jha, N. K., Aldhafeeri, G. M., ... & Singh, S. K. (2022). Biomedical applications of metallic nanoparticles in cancer: Current status and future perspectives. *Biomedicine & Pharmacotherapy*, *150*, 112951.
Kim, J. Y., Jo, O. H., Choe, C. M., & Cho, H. B. (2006). Rhizoma *Rehmanniae* induced apoptosis in human cervical carcinoma HeLa cells. *The Journal of Korean Obstetrics and Gynecology*, *19*(1), 69-80.
Lahiri, D., Nag, M., Sheikh, H. I., Sarkar, T., Edinur, H. A., Pati, S., & Ray, R. R. (2021). Microbiologically-synthesized nanoparticles and their role in silencing the biofilm signaling cascade. *Frontiers in Microbiology*, *12*, 636588.
Lambertini, E., Piva, R., Khan, M. T. H., Lampronti, I., Bianchi, N., Borgatti, M., & Gambari, R. (2004). Effects of extracts from Bangladeshi medicinal plants on *in vitro* proliferation of human breast cancer cell lines and expression of estrogen receptor α gene. *International Journal of Oncology*, *24*(2), 419-423.
Lee, S. R., Kim, M. S., Kim, S., Hwang, K. W., & Park, S. Y. (2017). Constituents from *Scutellaria barbata* inhibiting nitric oxide production in LPS-stimulated microglial cells. *Chemistry & Biodiversity*, *14*(11), e1700231.
Liu, P., Jin, H., Guo, Z., Ma, J., Zhao, J., Li, D., ... & Gu, N. (2016). Silver nanoparticles outperform gold nanoparticles in radiosensitizing U251 cells *in vitro* and in an intracranial mouse model of glioma. *International Journal of Nanomedicine*, 5003-5014.
Liu, R., Pei, Q., Shou, T., Zhang, W., Hu, J., & Li, W. (2019). Apoptotic effect of green synthesized gold nanoparticles from *Curcuma wenyujin* extract against human renal cell carcinoma A498 cells. *International Journal of Nanomedicine*, 4091-4103.
Liu, Y., Deng, A. J., Ma, L., Zhang, H. J., Zhang, Z. H., Wu, L. Q., ... & Qin, H. L. (2015). Chemical constituents of the roots of *Macleaya microcarpa* and activation efficacy of benzophenanthridine alkaloids for the transcription of xbp1 gene. *Yao xue xue bao=Acta Pharmaceutica Sinica*, *50*(2), 207-210.
Malik, U. U., Siddiqui, I. A., Ilyas, A., Hashim, Z., Staunton, L., Kwasnik, A., ... & Zarina, S. (2020). Identification of differentially expressed proteins from smokeless tobacco addicted patients suffering from Oral squamous cell carcinoma. *Pathology & Oncology Research*, *26*, 1489-1497.

Mani, S. T., Jayakumar, P., Pavithra, M. E., Saranya, K., Rathinavel, T., & Ammashi, S. (2023). Green synthesis and characterization of silver nanoparticles from *Eclipta alba* and its activity against triple-negative breast cancer cell line (MDA-MB-231). *Molecular Biotechnology*, 1-11.

Manzan, A. C. C., Toniolo, F. S., Bredow, E., & Povh, N. P. (2003). Extraction of essential oil and pigments from *Curcuma longa* [L.] by steam distillation and extraction with volatile solvents. *Journal of Agricultural and Food Chemistry*, *51*(23), 6802-6807.

Marconett, C. N., Morgenstern, T. J., San Roman, A. K., Sundar, S. N., Singhal, A. K., & Firestone, G. L. (2010). BZL101, a phytochemical extract from the *Scutellaria barbata* plant, disrupts proliferation of human breast and prostate cancer cells through distinct mechanisms dependent on the cancer cell phenotype. *Cancer Biology & Therapy*, *10*(4), 397-405.

Mehrotra, S., Goyal, V., Dimkpa, C. O., & Chhokar, V. (2024). Green synthesis and characterization of ginger-derived silver nanoparticles and evaluation of their antioxidant, antibacterial, and anticancer activities. *Plants*, *13*(9), 1255.

Mirabelli, P., Coppola, L., & Salvatore, M. (2019). Cancer cell lines are useful model systems for medical research. *Cancers*, *11*(8), 1098.

Mittal, A. K., Chisti, Y., & Banerjee, U. C. (2013). Synthesis of metallic nanoparticles using plant extracts. *Biotechnology Advances*, *31*(2), 346-356.

Moga, M. A., Dimienescu, O. G., Bălan, A., Dima, L., Toma, S. I., Bîgiu, N. F., & Blidaru, A. (2021). Pharmacological and therapeutic properties of *Punica granatum* phytochemicals: possible roles in breast cancer. *Molecules*, *26*(4), 1054.

Moghaddam, N. A., Eskandari, A., Khodadadi, B., Hafezi, Y., Paduvilan, J. K., & Yaraki, M. T. (2024). Green synthesis of bimetallic AgZnO Nanoparticles: Synergistic anticancer effects through regulation of gene expression for lung cancer treatment. *Results in Engineering*, 102329.

Molnár, Z., Bódai, V., Szakacs, G., Erdélyi, B., Fogarassy, Z., Sáfrán, G., ... & Lagzi, I. (2018). Green synthesis of gold nanoparticles by thermophilic filamentous fungi. *Scientific Reports*, *8*(1), 3943.

Mongy, Y., & Shalaby, T. (2024). Green synthesis of zinc oxide nanoparticles using *Rhus coriaria* extract and their anticancer activity against triple-negative breast cancer cells. *Scientific Reports*, *14*(1), 13470.

Montiel Schneider, M. G., Martín, M. J., Otarola, J., Vakarelska, E., Simeonov, V., Lassalle, V., & Nedyalkova, M. (2022). Biomedical applications of iron oxide nanoparticles: Current insights progress and perspectives. *Pharmaceutics*, *14*(1), 204.

Mousa, A. B., Moawad, R., Abdallah, Y., Abdel-Rasheed, M., & Zaher, A. M. A. (2023). Zinc oxide nanoparticles promise anticancer and antibacterial activity in ovarian cancer. *Pharmaceutical Research*, *40*(10), 2281-2290.

Mousavi, B., Tafvizi, F., & Zaker Bostanabad, S. (2018). Green synthesis of silver nanoparticles using *Artemisia turcomanica* leaf extract and the study of anti-cancer effect and apoptosis induction on gastric cancer cell line (AGS). *Artificial Cells, Nanomedicine, and Biotechnology*, *46*(sup1), 499-510.

Movahedi, A., Basir, R., Rahmat, A., Charaffedine, M., & Othman, F. (2014). Remarkable anticancer activity of *Teucrium polium* on hepatocellular carcinogenic rats. *Evidence-Based Complementary and Alternative Medicine*, *2014*(1), 726724.

Mukjerjee, S., & Karati, D. (2022). A mechanistic view on phytochemistry, pharmacognostic properties, and pharmacological activities of phytocompounds present in *Zingiber officinale*: A comprehensive review. *Pharmacological Research-Modern Chinese Medicine*, *5*, 100173.

Muslim, A. M., & Naji, I. S. (2024). Green synthesis of CuO nanoparticles mediated *Rhazya stricta* plant leaves extract characterization and evaluation of their antibacterial and anticancer activity (*in vitro* study). *Iraqi Journal of Physics*, *22*(3), 93-105.

Nachvak, S. M., Soleimani, D., Rahimi, M., Azizi, A., Moradinazar, M., Rouhani, M. H., ... & Miryan, M. (2023). Ginger as an anticolorectal cancer spice: A systematic review of *in vitro* to clinical evidence. *Food Science & Nutrition*, *11*(2), 651-660.

Nazaripour, E., Mousazadeh, F., Moghadam, M. D., Najafi, K., Borhani, F., Sarani, M., ... & Khatami, M. (2021). Biosynthesis of lead oxide and cerium oxide nanoparticles and their cytotoxic activities against colon cancer cell line. *Inorganic Chemistry Communications*, *131*, 108800.

Nematollahi-Mahani, S. N., Rezazadeh-Kermani, M., Mehrabani, M., & Nakhaee, N. (2007). Cytotoxic effects of *Teucrium polium*. On some established cell lines. *Pharmaceutical Biology*, *45*(4), 295-298.

Nguyen, N. T. T., Nguyen, T. T. T., Nguyen, D. T. C., & Van Tran, T. (2023). Green synthesis of $ZnFe_2O_4$ nanoparticles using plant extracts and their applications: A review. *Science of The Total Environment*, *872*, 162212.

Norris, C. B., Joseph, P. R., Mackiewicz, M. R., & Reed, S. M. (2010). Minimizing formaldehyde use in the synthesis of gold−silver core−shell nanoparticles. *Chemistry of Materials*, *22*(12), 3637-3645.

Osman, A. M. E., Taj Eldin, I. M., Elhag, A. M., Elhassan, M. M. A., & Ahmed, E. M. M. (2020). *In-vitro* anticancer and cytotoxic activity of ginger extract on human breast cell lines. *Khartoum Journal of Pharmaceutical Sciences*, *1*(1), 26-29.

Ovais, M., Khalil, A. T., Islam, N. U., Ahmad, I., Ayaz, M., Saravanan, M., ... & Mukherjee, S. (2018). Role of plant phytochemicals and microbial enzymes in biosynthesis of metallic nanoparticles. *Applied Microbiology and Biotechnology*, *102*, 6799-6814.

Ozcelik, S. (2023). Investigation of antimicrobial effects of zinc-based nanoparticles on food-borne pathogens. *Frontiers in Life Sciences and Related Technologies*, *4*(3), 132-137.

Pal, G., Rai, P., & Pandey, A. (2019). Green synthesis of nanoparticles: A greener approach for a cleaner future. In *Green Synthesis, Characterization and Applications of Nanoparticles* (pp. 1-26). Elsevier.

Pal, S., Tak, Y. K., & Song, J. M. (2007). Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram-negative bacterium *Escherichia coli*. *Applied and Environmental Microbiology*, *73*(6), 1712-1720.

Pandey, P. A., Bell, G. R., Rourke, J. P., Sanchez, A. M., Elkin, M. D., Hickey, B. J., & Wilson, N. R. (2011). Physical vapor deposition of metal nanoparticles on chemically modified graphene: observations on metal-graphene interactions. *Small*, *7*(22), 3202-3210.

Pandian, N., & Chidambaram, S. (2017). Antimicrobial, cytotoxicty and anti cancer activity of silver nanoparticles from *Glycyrrhiza glabra*. *International Journal of Pharmaceutical Sciences and Research*, *8*(4), 1633.

Patra, J. K., Das, G., Fraceto, L. F., Campos, E. V. R., Rodriguez-Torres, M. D. P., Acosta-Torres, L. S., ... & Shin, H. S. (2018). Nano based drug delivery systems: recent developments and future prospects. *Journal of Nanobiotechnology*, *16*, 1-33.

Pattanayak, M., & Nayak, P. L. (2013). Ecofriendly green synthesis of iron nanoparticles from various plants and spices extract. *International Journal of Plant, Animal and Environmental Sciences*, *3*(1), 68-78.

Pattanayak, P., Behera, P., Das, D., & Panda, S. K. (2010). *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. *Pharmacognosy reviews*, *4*(7), 95.

Perumal, P., Sathakkathulla, N. A., Kumaran, K., Ravikumar, R., Selvaraj, J. J., Nagendran, V., ... & Rathinasamy, S. (2024). Green synthesis of zinc oxide nanoparticles using aqueous extract of shilajit and their anticancer activity against HeLa cells. *Scientific Reports*, *14*(1), 2204.

Plengsuriyakarn, T., Viyanant, V., Eursitthichai, V., Tesana, S., Chaijaroenkul, W., Itharat, A., & Na-Bangchang, K. (2012). Cytotoxicity, toxicity, and anticancer activity of *Zingiber officinale* Roscoe against cholangiocarcinoma. *Asian Pacific Journal of Cancer Prevention*, *13*(9), 4597-4606.

Qureshi, S. A., Rais, S., Usmani, R., Zaidi, S. S. M., Jehan, M., Lateef, T., & Azmi, M. B. (2016). *Centratherum anthelminticum* seeds reverse the carbon tetrachloride-induced hepatotoxicity in rats. *African journal of Pharmacy and Pharmacology*, *10*(26), 533-539.

Rajeshkumar, S., Kumar, S. V., Ramaiah, A., Agarwal, H., Lakshmi, T., & Roopan, S. M. (2018). Biosynthesis of zinc oxide nanoparticles using *Mangifera indica* leaves and evaluation of their antioxidant and cytotoxic properties in lung cancer (A549) cells. *Enzyme and microbial technology*, *117*, 91-95.

Rajput, N. (2015). Methods of preparation of nanoparticles-a review. *International Journal of Advances in Engineering & Technology*, *7*(6), 1806.

Ramya, B., Khusro, A., Indra, V., Agastian, P., Almutairi, M. H., & Almutairi, B. O. (2024). Green synthesis of silver nanoparticles using *Justicia adhatoda* leaves extract and its anticancer effect on human lung carcinoma via induced apoptosis mechanism. *Results in Chemistry*, *7*, 101472.

Razavi, M., Salahinejad, E., Fahmy, M., Yazdimamaghani, M., Vashaee, D., & Tayebi, L. (2015). Green chemical and biological synthesis of nanoparticles and their biomedical applications. *Green processes for nanotechnology: From Inorganic to Bioinspired Nanomaterials*, 207-235.

Revathi, S., Sutikno, S., Hasan, A. F., Altemimi, A. B., ALKaisy, Q. H., Phillips, A. J., ... & Abedelmaksoud, T. G. (2024). Green synthesis and characterization of silver nanoparticles (AgNP) using *Acacia nilotica* plant extract and their anti-bacterial activity. *Food Chemistry Advances*, *4*, 100680.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chemistry*, *91*(4), 621-632.

Sadrolhosseini, A. R., Mahdi, M. A., Alizadeh, F., & Rashid, S. A. (2019). Laser ablation technique for synthesis of metal nanoparticle in liquid. *Laser Technology and its Applications*, 63-83.

Sahin, B., Aygun, A., Gunduz, H., Sahin, K., Demir, E., Akocak, S., & Sen, F. (2018). Cytotoxic effects of platinum nanoparticles obtained from pomegranate extract by the green synthesis method on the MCF-7 cell line. *Colloids and Surfaces B: Biointerfaces*, *163*, 119-124.

Sakhr, K., & El Khatib, S. (2020). Physiochemical properties and medicinal, nutritional and industrial applications of Lebanese Sumac (Syrian Sumac-*Rhus coriaria*): A review. *Heliyon*, *6*(1).

Salehi, S., Shandiz, S. A. S., Ghanbar, F., Darvish, M. R., Ardestani, M. S., Mirzaie, A., & Jafari, M. (2016). Phytosynthesis of silver nanoparticles using *Artemisia marschalliana* Sprengel aerial part extract and assessment of their antioxidant, anticancer, and antibacterial properties. *International Journal of Nanomedicine*, 1835-1846.

Sekar, V., Balakrishnan, C., Kathirvel, P., Swamiappan, S., Alshehri, M. A., Sayed, S., & Panneerselvam, C. (2022). Ultra-sonication-enhanced green synthesis of silver nanoparticles using *Barleria buxifolia* leaf extract and their possible application. *Artificial Cells, Nanomedicine, and Biotechnology*, *50*(1), 177-187.

Senthilkumar, A., Karuvantevida, N., Rastrelli, L., Kurup, S. S., & Cheruth, A. J. (2018). Traditional uses, pharmacological efficacy, and phytochemistry of *Moringa peregrina* (Forssk.) Fiori. -a review. *Frontiers in Pharmacology*, *9*, 465.

Shanmugam, K. R., Shanmugam, B., Venkatasubbaiah, G., Ravi, S., & Reddy, K. S. (2022). Recent Updates on the Bioactive Compounds of Ginger (Zingiber officinale) on Cancer: A Study with Special Emphasis of Gingerol and Its Anticancer Potential: Effect of Ginger and Its Compounds in Cancer Subjects. In *Handbook of Oxidative Stress in Cancer: Therapeutic Aspects* (pp. 1-18). Singapore: Springer Nature Singapore.

Sharangi, A. B. (2009). Medicinal and therapeutic potentialities of tea (*Camellia sinensis* L.)–A review. *Food Research International*, *42*(5-6), 529-535.

Sharma, B., Purkayastha, D. D., Hazra, S., Gogoi, L., Bhattacharjee, C. R., Ghosh, N. N., & Rout, J. (2014). Biosynthesis of gold nanoparticles using a freshwater green alga, *Prasiola crispa*. *Materials Letters*, *116*, 94-97.

Shochah, Q. R., & Jabir, F. A. (2024). Green synthesis of Au/ZnO nanoparticles for anticancer activity and oxidative stress against MCF-7 cell lines. *Biomass Conversion and Biorefinery*, *14*(14), 15283-15296.

Simakin, A. V., Voronov, V. V., Kirichenko, N. A., & Shafeev, G. A. (2004). Nanoparticles produced by laser ablation of solids in liquid environment. *Applied Physics A*, *79*, 1127-1132.

Singh, J., Dutta, T., Kim, K. H., Rawat, M., Samddar, P., & Kumar, P. (2018). 'Green' synthesis of metals and their oxide nanoparticles: applications for environmental remediation. *Journal of Nanobiotechnology*, *16*, 1-24.

Takemoto, T., Nishimoto, N., Nakai, S., Takagi, N., Hayashi, S., Odashima, S., & Wada, Y. (1983). Pfaffic acid, a novel nortriterpene from *Pfaffia paniculata* Kuntze. *Tetrahedron Letters*, *24*(10), 1057-1060.

Ullah, I., Khalil, A. T., Zia, A., Hassan, I., & Shinwari, Z. K. (2024). Insight into the molecular mechanism, cytotoxic, and anticancer activities of phyto-reduced silver nanoparticles in MCF-7 breast cancer cell lines. *Microscopy Research and Technique*, *87*(7), 1627-1639.

Ullah, I., Rauf, A., Khalil, A. A., Luqman, M., Islam, M. R., Hemeg, H. A., ... & Quradha, M. M. (2024). Peganum harmala L. extract-based Gold (Au) and Silver (Ag) nanoparticles (NPs): Green synthesis, characterization, and assessment of antibacterial and antifungal properties. *Food Science & Nutrition*.

Ullah, M., Ali, M. E., & Abd Hamid, S. B. (2014). Surfactant-assisted ball milling: a novel route to novel materials with controlled nanostructure-a review. *Reviews on Advanced Materials Science*, *37*.

Umamaheswari, A., Prabu, S. L., John, S. A., & Puratchikody, A. (2021). Green synthesis of zinc oxide nanoparticles using leaf extracts of *Raphanus sativus* var. Longipinnatus and evaluation of their anticancer property in A549 cell lines. *Biotechnology Reports*, *29*, e00595.

Vijayakumar, S., Chen, J., Sánchez, Z. I. G., Tungare, K., Bhori, M., Durán-Lara, E. F., & Anbu, P. (2023). *Moringa oleifera* gum capped MgO nanoparticles: Synthesis, characterization, cyto-and ecotoxicity assessment. *Int J Biol Macromol*, *233*, 123514.

Vyas, P., Yadav, D. K., & Khandelwal, P. (2019). *Tectona grandis* (teak)–A review on its phytochemical and therapeutic potential. *Natural Product Research*, *33*(16), 2338-2354.

Wang, L., Xu, J., Yan, Y., Liu, H., Karunakaran, T., & Li, F. (2019). Green synthesis of gold nanoparticles from *Scutellaria barbata* and its anticancer activity in pancreatic cancer cell (PANC-1). *Artificial Cells, Nanomedicine, and Biotechnology*, *47*(1), 1617-1627.

Xia, T., Dong, X., Jiang, Y., Lin, L., Dong, Z., Shen, Y., ... & Qin, L. (2019). Metabolomics profiling reveals rehmanniae radix preparata extract protects against glucocorticoid-induced osteoporosis mainly via intervening steroid hormone biosynthesis. *Molecules*, *24*(2), 253.

Yalcin, B., Akcan, D., Yalcin, I. E., Alphan, M. C., Senturk, K., Ozyigit, I. I., & Arda, L. (2020). Effect of Mg doping on morphology, photocatalytic activity and related biological properties of $Zn_{1-x}Mg_xO$ nanoparticles. *Turkish Journal of Chemistry*, *44*(4), 1177-1199.

Yalcin, B., Arda, L., Yalcin, I. E., Senturk, K., Alphan, M. C., Akcan, D., & Ozyigit, I. I. (2023). Exploration of the improving effect of Cd-doping on structural, photocatalytic, and biological properties of ZnO nanoparticles. *Journal of Nanoparticle Research*, *25*(7), 146.

Yang, N., Zhao, Y., Wang, Z., Liu, Y., & Zhang, Y. (2017). Scutellarin suppresses growth and causes apoptosis of human colorectal cancer cells by regulating the p53 pathway. *Molecular Medicine Reports*, *15*(2), 929-935.

Yesil-Celiktas, O., Sevimli, C., Bedir, E., & Vardar-Sukan, F. (2010). Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines. *Plant Foods for Human Nutrition*, *65*, 158-163.

Younis, H. M., Hussein, H. A., Khaphi, F. L., & Saeed, Z. K. (2023). Green biosynthesis of silver and gold nanoparticles using Teak (*Tectona grandis*) leaf extract and its anticancer and antimicrobial activity. *Heliyon*, *9*(11).

Zhang, L., Ren, B., Zhang, J., Liu, L., Liu, J., Jiang, G., ... & Li, W. (2017). Anti-tumor effect of *Scutellaria barbata* D. Don extracts on ovarian cancer and its phytochemicals characterisation. *Journal of Ethnopharmacology*, *206*, 184-192.

Zhou, R., Xu, L., Ye, M., Liao, M., Du, H., & Chen, H. (2014). Formononetin inhibits migration and invasion of MDA-MB-231 and 4T1 breast cancer cells by suppressing MMP-2 and MMP-9 through PI3K/AKT signaling pathways. *Hormone and Metabolic Research*, *46*(11), 753-760.

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