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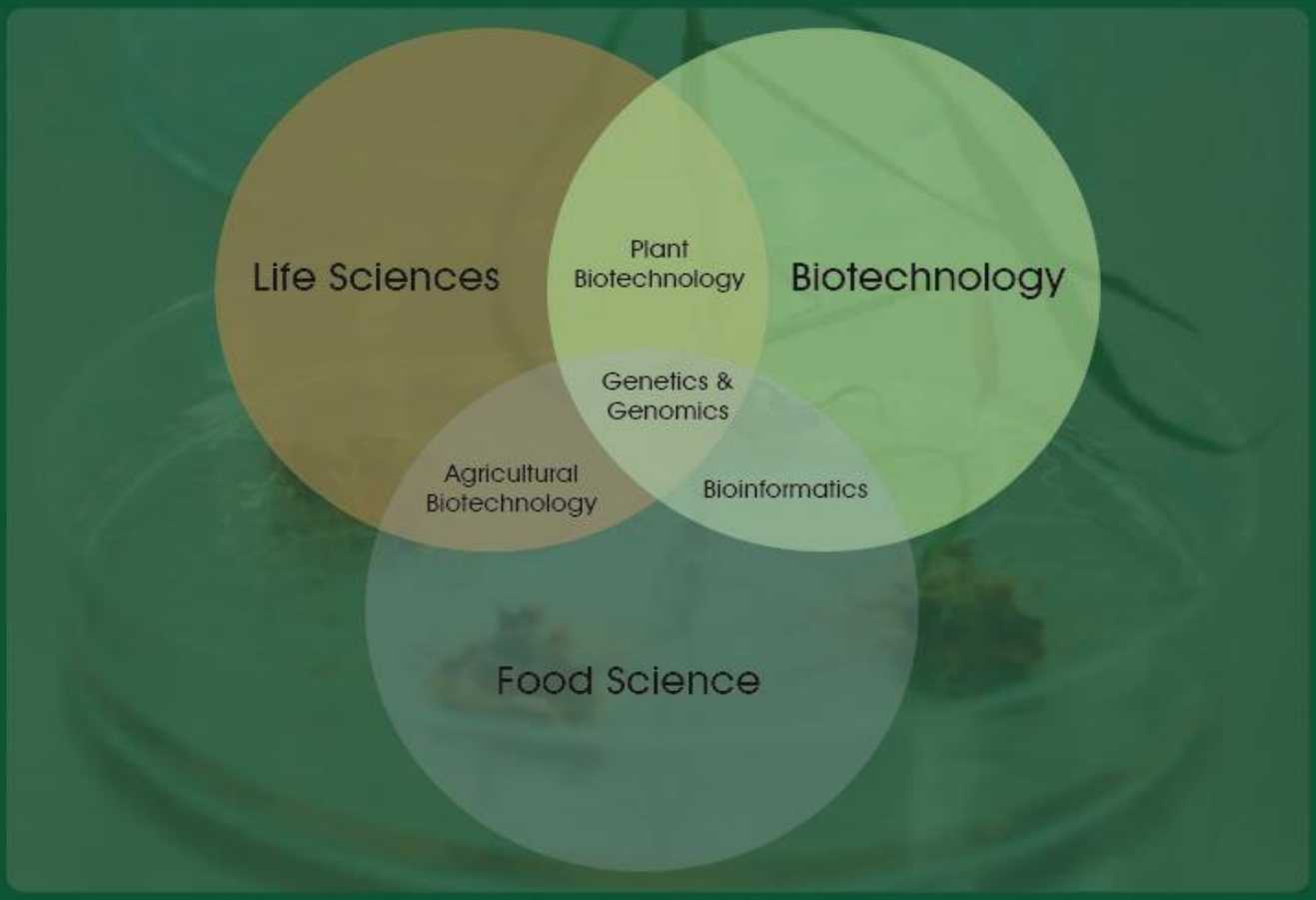

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Editörden;

Değerli okurlar ve yazarlar,

"International Journal of Life Sciences and Biotechnology" olarak dergimizin on beşinci sayısını yayın hayatına sunmaktan mutluluk ve onur duyuyoruz. "International Journal of Life Sciences and Biotechnology" dergisi araştırma- geliştirme ve uygulama ilkeleri baz alınarak yayınlanan uluslararası hakemli açık erişimli akademik bir elektronik dergidir.

"International Journal of Life Sciences and Biotechnology" dergisi Yaşam Bilimleri, Biyoloji, Biyoteknoloji, Biyomühendislik, Ziraat Bilimleri, Gıda Biyoteknolojisi ve Genetik alanlarındaki ilgili araştırmacılara, kurum ve kuruluşlara teorik ve pratik uygulamalarda katkı sağlamayı, tarafsızlık ve bilim etiği ilkelerine bağlı kalarak çözüm temelli, yenilikçi ve katma değeri olan çalışmalara odaklanan, günceli ve geleceği tartışan çalışmaların yayınlanmasını hedeflemektedir.

Bu düşüncelerle 2023 yılı birinci sayısını yayınladığımız "International Journal of Life Sciences and Biotechnology" dergisini, makaleleri ile onurlandıran akademisyenlere, Fikir / Görüş / Öneri / Katkı ve Eleştirileri ile değerlendirme süreçlerine katkılarından dolayı hakem ve yayın kurullarında yer alan kıymetli bilim insanlarına yürekten teşekkür ediyoruz. Bir sonraki sayıda görüşmek ümidiyle…

15.04. 2023
Editör
Dr. Öğrt. Üyesi Yilmaz KAYA

From The Editor;

Dear Readers and Authors,

As “International Journal of Life Sciences and Biotechnology”, we are pleased and honored to present the 15th issue of the journal. "International Journal of Life Sciences and Biotechnology" is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, We are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in the first issue of 2023 "International Journal of Life Sciences and Biotechnology". Hope to see you in the next issue…

15. 04. 2023

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Assist. Prof. Dr. Yilmaz KAYA

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Research Article

Yigit U., et al., Antifungal activity and optimization procedure of silver nanoparticles green synthesized with *Prunus laurocerasus* L. (cherry laurel) leaf extract. International Journal of Life Sciences and Biotechnology, 2023. 6(1): p. 1-20. DOI: 10.38001/ijlsb.1168628

Antifungal activity and optimization procedure of silver nanoparticles green synthesized with *Prunus laurocerasus* L. (cherry laurel) leaf extract

Ugur Yigit[1], Yaren Gurel[1], Hasan Ilhan[2], Muharrem Turkkan[1,*]

ABSTRACT

In this study, green synthesis conditions of silver nanoparticles (AgNPs) synthesized with *Prunus laurocerasus* (cherry laurel) leaf extract as reducing and coating agent were optimized using Box-Behnken design (BBD). Three important synthesis factors such as the concentration (M) of silver nitrate ($AgNO_3$), pH of cherry laurel leaf extract, and reaction temperature (°C) were used as independent variables of the model, and the absorbance intensity originating from AgNPs was employed as a dependent variable. Statistical analyzes showed that the optimized conditions for the predicted absorbance at 405 nm (2.35 A.U) were determined at a concentration of 0.01 M $AgNO_3$, a pH of 9.0, and a temperature of 50°C. The validity of the developed model was verified, and the average absorbance from six experimental runs was recorded as 2.26 (A.U) with an error of 14.86%. The synthesized AgNPs were characterized using Ultraviolet–Visible (UV–Vis) Spectroscopy, Fourier Transform Infrared (FT–IR) Spectroscopy, and Scanning Electron Microscopy–Energy Dispersive X-ray Spectroscopy (SEM–EDS). In addition, *in vitro* trials revealed that the synthesized AgNPs exhibited antifungal activity against all five fungal kiwifruit pathogens tested. The EC_{50} values of synthesized AgNPs were 10.88, 9.30, 7.15, 25.16, and 53.77 µg/ml for *Phytopythium vexans*, *Globisoprangium sylvaticum*, *G. intermedium*, *Phytophthora citrophthora*, and *Rhizoctonia solani*, respectively. With the exception of the MIC values of two *Globisporangium* species (120 µg/ml), both MIC and MFC values of the remaining three species were found to be above 150 µg/ml. The results of this study indicate that AgNPs synthesized with cherry laurel leaf extract should be further investigated for use in the control of fungal root and stem rot diseases in kiwifruit.

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Prunus laurocerasus, silver nanoparticle, Box-Behnken design, antifungal activity

Introduction

Today, silver (Ag) nanoparticles (NPs) are of great interest due to their optical, catalytic, mechanical, electrical, and biosensing properties [1, 2, 3]. AgNPs also have strong antimicrobial activity and are widely used in the pharmaceutical industry as an ingredient in the preparation of human health drugs [4]. In many studies, it has been shown that AgNPs can be synthesized by chemical and physical methods, but it has become

[1] Ordu University, Faculty of Agriculture, Department of Plant Protection, 52200 Ordu, Turkey
[2] Ordu University, Faculty of Science, Department of Chemistry, 52200 Ordu, Turkey
*Corresponding Author: Muharrem Türkkan, e-mail: muharremturkkan@gmail.com; muharremturkkan@odu.edu.tr

necessary to find an alternative method due to the use of large amounts of toxic chemicals in the synthesis process [5, 6, 7, 8, 9, 10, 11]. On the other hand, the biological approach based on living organisms (bacteria, fungi, lichens, plants, etc.) offers a reliable, simple, rapid, non-toxic, and environmentally friendly solution [12, 13, 14, 15]. Among these bio-resources, plants are more popular as they are readily accessible, non-toxic, and easily processed. Plants also have many biologically active compounds such as polyphenols, organic acids, and proteins that serve not only as reducing agents but also as capping agents making synthesis an easy process [16, 17]. The green synthesis of nano-sized silver nanoparticles using alfalfa sprouts (variety Mesa) was first reported by Gardea-Torresdey [18]. Recently, extracts of various plant parts such as leaves, flowers, fruits, rhizomes, seeds, etc. have been successfully used in the synthesis of AgNPs. *Azadirachta indica* (leaf extract) [1], *Aloe vera* (leaf extract) [1], *Camellia sinensis* (plant extract) [21], *Cinnamon zeylanicum* (bark extract) [22], *Jatropha curcas* (seed extract) [23], *Musa paradisiaca* (peel extract) [24], *Acalypha indica* (leaf extract) [25], *Macrotyloma uniflorum* (seed extract) [26], *Prosopis juliflora* (leaf extract) [27], *Pimenta dioica* (leaf extract) [28], *Alstonia scholaris* (bark extract) [29], *Eucalyptus oleosa* (plant extract) [30], *Artemisia absinthium* (leaf extract) [31], *Atrocarpus altilis* (leaf extract) [32], *Camellia japonica* (leaf extract) [33], *Rubus glaucus* (fruit extract) [34], *Nigella arvensis* (leaf extract) [35], *Prosopis juliflora* (bark extract) [36], *Pueraria tuberosa* (tuber extract) [37], *Berberis vulgaris* (leaf and root extracts) [38], *Erodium cicutarium* (plant extract) [39], *Teucrium polium* (stem and flower extracts) [40], *Zingiber officinale* (rhizome extract) [41], *Clitoria ternatea* (flower extract) [42], *Diospyros malabarica* (fruit extract) [43], *Malva parviflora* (leaf extract) [44], and *Psidium guajava* (leaf extract) [45] are examples of plants used in AgNPs synthesis. Of these, the AgNPs synthesized with extracts of *A. absinthium* and *M. parviflora* have been shown to be a potent inhibitor against *Alternaria alternata*, *Helminthosporium rostratum*, *Fusarium solani*, *F. oxysporum*, *Phytophthora capsici*, and *P. parasitica* [31, 44]. In addition, several others have been reported to exhibit antibacterial activity [22, 25, 38].

Prunus laurocerasus L. (cherry laurel), which belongs to the Rosaceae family, is an evergreen shrub or small tree native to Europe, the Caucasus, Iran and Türkiye [46]. Cherry laurel is cultivated in the Black Sea region of Türkiye for its fruits and is known as Karayemiş or Taflan. In Turkish traditional medicine, cherry laurel leaves are used in

the treatment of asthma, coughs, and dyspepsia [47, 46]. Şahan also determined that aqueous and ethanolic extracts of cherry laurel leaves have antifungal activity against various bread molds (*Aspergillus*, *Mucor*, *Penicillium*, and *Rhizopus*) [48]. Previous studies have shown that cherry laurel is a rich source of various biochemicals such as protein, sugar, ascorbic acid, minerals, and antioxidants [46, 48]. Furthermore, Karabegovic et al. determined that both leaf and fruit extracts contain high amounts of phenolic and flavonoid compounds [49]. Thus, these compounds may serve to produce AgNPs by mediating the reduction of silver ions.

Response surface methodology (RSM), a statistical and graphical technique, is a widely used methodology for designing models and analyzing various manufacturing problems [50]. RSM helps to identify factors, examine interactions and optimal conditions, compute the optimum level of variables, and ensure maximum production in a constant number of experiments.

In this study, silver nanoparticles synthesized using cherry laurel leaf extract were optimized using Box-Behnken design (BBD). AgNPs synthesized under optimized conditions were characterized by Ultraviolet–Visible (UV–Vis) Spectroscopy, Fourier Transform Infrared (FT–IR) Spectroscopy, and Scanning Electron Microscopy–Energy Dispersive X-ray Spectroscopy (SEM–EDS). In addition, their antifungal activities on some important fungal disease agents that cause root and stem rot in kiwifruits were evaluated under *in vitro* conditions in the laboratory.

Material and Methods

Plant material, chemicals, and fungal cultures

The leaves of *Prunus laurocerasus* (cherry laurel) were collected from Gülyalı district of Ordu, Türkiye (40°57'58.8"N 38°00'00.4"E).

Silver nitrate ($AgNO_3$), sodium hydroxide (NaOH) and hydrochloric acid (HCl) from Merck (Darmstadt, Germany), and potato dextrose agar (PDA) medium were purchased from BD Difco (Sparks, MD, USA).

Phytopythium vexans, *Globisoprangium sylvaticum*, *G. intermedium*, *Phytophthora citrophthora*, and *Rhizoctonia solani* AG 4 HG-I isolates causing root and stem rot in kiwifruit were obtained from fungal culture collection of the Faculty of Agriculture, Department of Plant Protection, Mycology Laboratory (Ordu University).

Preparation of plant extract

Cherry laurel leaves were surface cleaned with running tap water to remove the debris, then washed with distilled water and dried with a paper towel. Afterward, they were dried in an oven at 60°C for 4 days and ground into a fine powder using a blender. Twenty grams of the leaf powder were mixed with 100 ml of distilled water in a 500 ml flask and stirred continuously for 30 min at 80°C using a heater stirrer. After cooling, the extracts were centrifuged and filtered through Whatman No. 1 filter paper and then kept in a refrigerator at 4°C.

Green synthesis of AgNPs

In this experiment, solutions of $AgNO_3$ in distilled water at 0.001, 0.0055, and 0.01 M concentrations were prepared and used as the metal source (Ag) for AgNPs biosynthesis. To synthesize AgNPs, 10 ml of cherry laurel leaf extracts at different pHs (5, 7, and 9) was added to 90 ml of the above aqueous solutions and vigorously stirred at various temperatures (25, 50, and 75°C) for 30 min. The reduction of Ag+ ions to Ag° ions was confirmed by the color change from colorless to dark brown. The precipitates of each sample were obtained by centrifuging at 10000 rpm for 15 min. The collected NPs were washed three times with double distilled water and the washed samples were suspended in 5 ml of distilled water.

Statistical optimization of AgNPs synthesis

Box-Behnken design (BBD) from RSM was used to evaluate the effect of independent variables (silver nitrate concentration, pH of plant leaf extract, and reaction temperature) on the synthesis of AgNPs and also to find an optimum condition. Each variable was coded at three levels of +1 (high), 0 (middle) and -1 (low) (**Table 1**). A Box-Behnken was designed, consisting of a total of 17 runs with five repetitions at the central point (**Table 2**). The following quadratic polynomial equation was used to compute the relationship between the three independent variables and the response.

$$Y=\beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_{11}A^2+\beta_{22}B^2+\beta_{33}C^2+\beta_{12}AB+\beta_{13}AC+\beta_{23}BC$$

where Y is the predicted response (predicted absorbance of AgNPs), A, B, and C are the independent variables, β_0 is the regression coefficient at center point, β_1, β_2, and β_3 are the linear coefficient; β_{11}, β_{22}, and β_{33} are the quadratic coefficients, and β_{12}, β_{13}, and β_{23} are the second order interaction coefficients.

All analyzes were performed with version 13 of the Design Expert program (Stat-Ease, Inc., USA). The program was used for regression analysis of the data obtained and to estimate the regression equation coefficient. The fitted model was then plotted in the form of perturbation, 3D response surface, and 2D contour plots to illustrate the relationship between responses. Finally, experimental sets were made using the suggested optimum combination to validate the developed model.

Table 1 Level of variables chosen for Box-Behnken design

Coded factors	Variables	Units	Minimum	Maximum	Coded Low	Coded High	Mean	Std. Dev.
A	$AgNO_3$ concentration	M	0.001	0.01	-1 ↔ 0.001	+1 ↔ 0.01	0.0055	0.0032
B	pH of cherry laurel leaf extract	-	5.00	9.00	-1 ↔ 5.00	+1 ↔ 9.00	7.00	1.41
C	Reaction temperature	^oC	25.00	75.00	-1 ↔ 25.00	+1 ↔ 75.00	50.00	17.68

Table 2 Experimental design using Box-Behnken showing coded and actual values along with the experimental and predicted responses

Run order	$AgNO_3$ concentration (M)	pH of cherry laurel leaf extract	Reaction temperature (^oC)	Absorbance (405 nm)	
				Actual	Predicted
1	-1	0	-1	0.5857	0.7601
2	1	0	1	1.7246	1.5500
3	0	0	0	1.5988	1.6600
4	0	1	1	1.8395	1.9600
5	0	0	0	1.7943	1.6600
6	1	0	-1	1.7112	1.7100
7	1	-1	0	0.3532	0.4772
8	0	-1	-1	1.1539	1.0300
9	0	0	0	1.5968	1.6600
10	-1	1	0	0.7398	0.6158
11	1	1	0	2.2945	2.3500
12	-1	0	1	0.6021	0.6046
13	0	1	-1	2.1188	2.0700
14	-1	-1	0	0.3674	0.3145
15	0	0	0	1.5703	1.6600
16	0	-1	1	0.7753	0.8257
17	0	0	0	1.7173	1.6600

Characterization of AgNPs

UV–Vis analysis of the synthesized AgNPs was performed with a UV–Vis Spectrometer (Lambda 35, Perkin Elmer, Inc., MA, USA). A 200 µl solution containing AgNPs was

diluted with 2 ml of distilled water. The spectra of AgNPs were obtained in the wavelength range of 200-700 nm at 1 nm resolution using a quartz cuvette. Baseline correction was done using distilled water as the blank.

Infrared spectra of the AgNPs were recorded on Fourier Transform Infrared (FT–IR) Spectroscopy (Spectrum 65, Perkin Elmer, Inc., MA, USA). One mg of the synthesized NPs was mixed with 200 mg KBr, then pressed into a pellet. FT–IR spectra from the nanoparticles were collected at the wave number range of 4000-400 cm^{-1} in the transmittance mode.

Chemical composition and morphology analysis of the AgNPs were carried out using a Hitachi Scanning Electron Microscope (SEM) (SU-1510, Hitachi High-Technologies Corp., Tokyo, Japan) coupled with Energy Dispersive X-ray Spectroscopy (EDS).

Antifungal effect of the synthesized AgNPs on fungi

Antifungal activities of the synthesized AgNPs against the fungi were tested according to Türkkan with a minor modification [51].

Different concentrations of AgNPs (15, 30, 60, 90, 120, and 150 μg/ml) were added to autoclaved and then cooled PDA medium at 55°C. A 15 ml aliquot of modified PDA medium was aseptically dispensed into a Petri plate (8-cm-dia.), with an unmodified PDA plate used as a control. A 5 mm diameter mycelial disc from 7-day-old fungal cultures was placed in the center of each plate and incubated at 25°C in the dark after sealing the plates with Parafilm. When control fungal colonies covered plates (2-7 days), all colony diameters were measured at two perpendicular points. The growth values were converted into percent inhibition of the mycelial growth inhibition (MGI) in relative to controls using the formula MGI (%) = [(dc − dt)/dc]× 100, where dc represents the fungal growth diameter of the control and dt represents the fungal growth diameter of the modified plates. The experiment was performed twice with 3 replications for each fungus.

Probit analysis (IBM SPSS Statistics, Version 22; IBM Company, Chicago, USA) was used to compute the concentrations of the AgNPs that caused a 50% reduction (EC_{50}) in mycelial growth of the fungi. The minimum inhibitory concentration (MIC) value, which totally inhibited mycelial growth, was also found by parallel experiments.

Toxicity (fungistatic/fungicidal) of the AgNPs was evaluated according to Thompson and Tripathi et al. [52, 53]. PDA discs taken from modified plates that exhibited no fungal growth were re-inoculated with unmodified PDA plates and then monitored for 9 days at

25°C revivals of growth. At the end of this period, the minimum AgNPs concentration required to completely and irreversibly inhibit fungal growth was recorded as the minimum fungicidal concentration (MFC) value.

Results and Discussion

Optimization of process parameters by response surface methodology (RSM)

In this study, the Box-Behnken design was used to determine the optimum experimental condition for the synthesis of AgNPs. Considering the peak intensity, three independent synthesis factors including the concentration of $AgNO_3$ solution, pH of the plant leaf extract, and reaction temperature were investigated to obtain the optimal surface plasmon resonance (SPR) band of the synthesized AgNPs.

In preliminary studies, pH was shown to be one of the most effective variables in the synthesis of AgNPs. Therefore, a series of syntheses were performed to determine the pH range in the research, with a one-at-a-time approach based on varying the pH (4-10) and constant amounts of other two factors ($AgNO_3$ = 0.001 M and temperature = 50°C). Silver nanoparticle synthesis occurred in the pH range of 5-10 but did not produce SPR as AgNP was suppressed at pH 4 (**Fig 1**). Furthermore, a fairly broad SPR peak (470 nm) was observed at pH 5.0; this may be related to the agglomeration of NPs at acidic pH, which results in an increase in the size of the NPs [54]. In contrast, at pH 9.0, a relatively narrow SPR peak was observed with λmax at 405 nm which can be due to an increase in the small diameters of nanoparticles. Veerasamy et al. reported that high pH values facilitate the binding of more AgNPs with many functional groups available for silver bonding [55]. Vanaja et al. also stated that nanoparticle formation was ascended with increasing pH [54]. Therefore, the pH values (5, 7, and 9) of acidic, neutral, and alkaline solutions were used in the Box-Behnken design.

The Box-Behnken design for three factors was constituted with a total of 17 runs. The absorbance intensity of the SPR was used as the response of the Box-Behnken design and modeling resulting from the absorbance of 17 AgNP synthesized at 405 nm (**Table 2**).

In order to determine the optimal values of AgNP synthesis, the Design Expert program proposed a quadratic model to correlate the relationship between experimental factors and response. The developed model is expressed in terms of coded (1) and actual (2) factors as follows:

$$Y \quad (\text{Absorbance}) = 1.66+0.4736A+0.5429B-0.0785C-0.5164A^2-0.2004B^2+0.0168C^2+$$

$0.3922AB-0.0008AC+0.0248BC$ (1)

Y (Absorbance)$= -1.97563+81.00556A+0.708307B-0.009263C-25500A^2 -0.050100B^2+0.000027C^2+43.58056AB-0.006667AC+0.000496BC$ (2)

Where Y is the obtained absorbance (405 nm) as an indication of the SPR intensity; A is silver nitrate concentration; B is pH value of plant leaf extract; and C is the reaction temperature.

The significance of this quadratic model was checked with an analysis of variance using the F-test (**Table 3**). The model F-value of 28.39 implies that the model is statistically significant. There is only a 0.01% chance that an F-value this large could occur due to noise while p-values are 0.0001. P-values less than 0.05 indicate that the developed quadratic model and model terms are significant. In this case, A, B, AB, A^2 and B^2 are significant model terms. The Lack of Fit F-value of 4.75 means that the Lack of Fit is negligible compared to pure error and is a good indicator for the model.

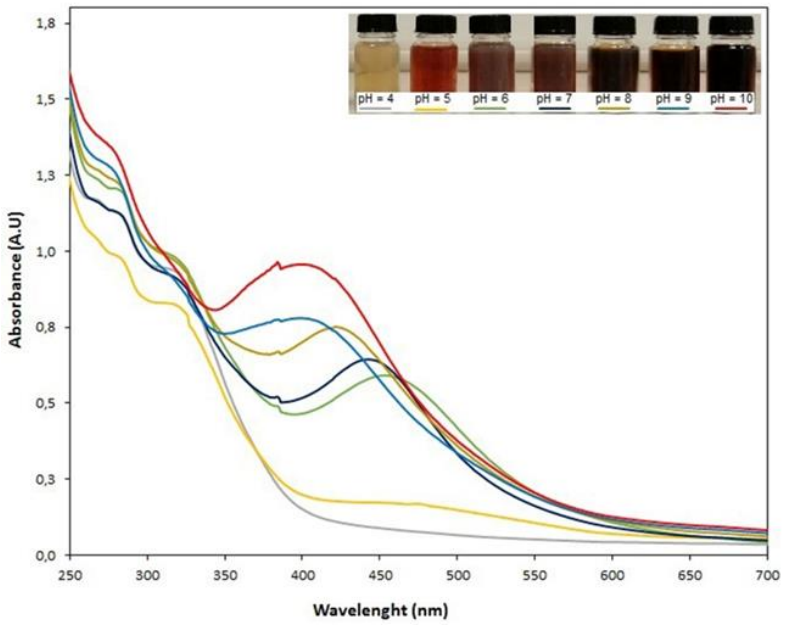

Fig 1 Visual observations and UV–Vis spectra of reaction mixtures with different pHs at fixed values of $AgNO_3$ concentration (0.001 M) and reaction temperature (50°C)

In our study, the regression coefficient ($R^2 = 0.9733$) means that 97.33% of both observed and predicted data can be explained by using this model (**Fig 2a**, **Table 4**). It is reported that a suitable statistical model should result in $R^2 \sim 1$ [56]. Adequate precision measures the signal-to-noise ratio, and a ratio greater than 4 is desirable. The ratio of 17.0674

indicated an adequate signal; hence, the model can be used to continue the design process. In the developed model, the residuals show a random distribution between the predicted and actual (observed) values, which shows that all the residual values lie along a straight line without large deviations, confirming the normality of the error distribution (**Fig 2b**). It is also shown in **Fig 2(c)** that not only is the residue randomly distributed on both sides of the zero line but is also within the acceptable range. Mondal and Purkait stated that the residuals between predicted and actual values should be in the range of ±3%, demonstrating that the constructed model is sufficient [57]. The perturbation plot shows that $AgNO_3$ concentration (A) and pH of the plant leaf extract (B) have a large positive effect on increasing the amount of AgNP synthesis, but the effect of the reaction temperature (C) is quite limited (**Fig 2d**).

In the study, 3D response surface and 2D contour plots were used to show the interactions among variables and their mutual effects on the response (absorbance) (**Fig 3**). **Fig 3(a)** shows the relationship between $AgNO_3$ concentration (A) and pH of the plant leaf extract (B), which has a similar effect on the AgNPs synthesis. As can be seen, AgNPs synthesis was at its highest when both were increased. On the other hand, the increase in the reaction temperature (°C) limited the AgNPs synthesis to some extent.

Table 3 Analysis of variance (ANOVA) for quadratic model

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	6.16	9	0.6847	28.39	0.0001	significant
A-$AgNO_3$ concentration (M)	1.79	1	1.79	74.39	< 0.0001	
B-pH of cherry laurel leaf extract	2.36	1	2.36	97.75	< 0.0001	
C-Reaction temperature (°C)	0.0493	1	0.0493	2.04	0.1958	
AB	0.6154	1	0.6154	25.52	0.0015	
AC	2.25E-06	1	2.25E-06	0.0001	0.9926	
BC	0.0025	1	0.0025	0.1022	0.7585	
A^2	1.12	1	1.12	46.55	0.0002	
B^2	0.1691	1	0.1691	7.01	0.033	
C^2	0.0012	1	0.0012	0.0491	0.8309	
Residual	0.1688	7	0.0241			
Lack of Fit	0.1318	3	0.0439	4.75	0.0832	not significant
Pure Error	0.037	4	0.0093			
Cor Total	6.33	16				

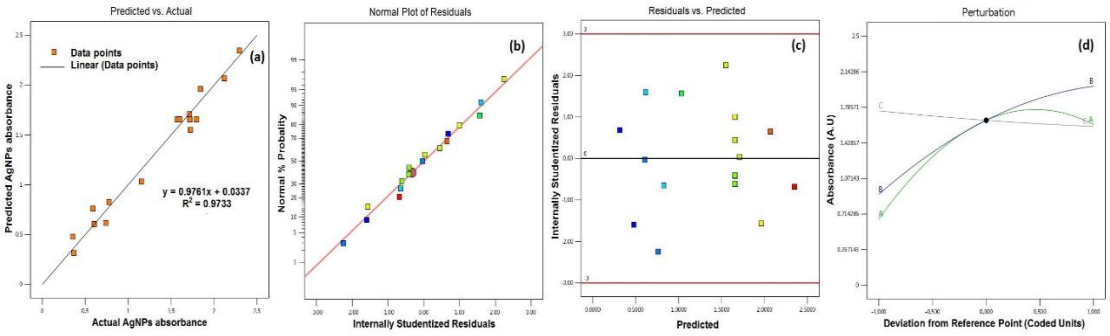

Fig 2 Diagnostic plots of AgNPs optimized using Box-Behnken design: (a) predicted versus actual, (b) normality of residuals, (c) residuals versus predicted, and (d) perturbation

Table 4. Fit and model summary statistics

Std. Dev.	0.1553	R^2	0.9733
Mean	1.33	Adjusted R^2	0.9391
C.V. %	11.71	Predicted R^2	0.6578
		Adeq Precision	17.0674
PRESS	2.17	-2 Log Likelihood	-30.16
BIC	-1.83	AICc	26.5

Depending on the other two variables, it is seen that they may lead to higher AgNP formation in the range of approximately 25-50°C (**Fig 3b**; **3c**). Similarly, Sun et al. found that the increase in temperature did not have a significant effect on the production efficiencies of AgNPs [58]. Sanghi and Verma stated that silver ion reduction at higher pH was favorable and proceeded at a higher rate [59]. Furthermore, Nikaeen et al. reported that the interaction of pH and silver concentration has a greater effect on the synthesis of AgNPs than other factors such as reaction temperature and time [60].

In the present study, the optimum values of $AgNO_3$ concentration, cherry laurel leaf extract pH, and reaction temperature for the synthesis of AgNPs at 405 nm are 0.01 M, 9.0, and 50°C, respectively, and the absorbance value of the corresponding AgNPs is 2.35. We repeated the experimental synthesis of AgNPs using the parameters mentioned above and obtained an absorbance value of 2.26 with an error value of 14.86% (**Fig 4**), which is less than 20% (0.2) error value. Since the error value is lower than the standard value (0.2) of Design Expert software, it has been verified that it is sufficient for the optimization of the model parameters.

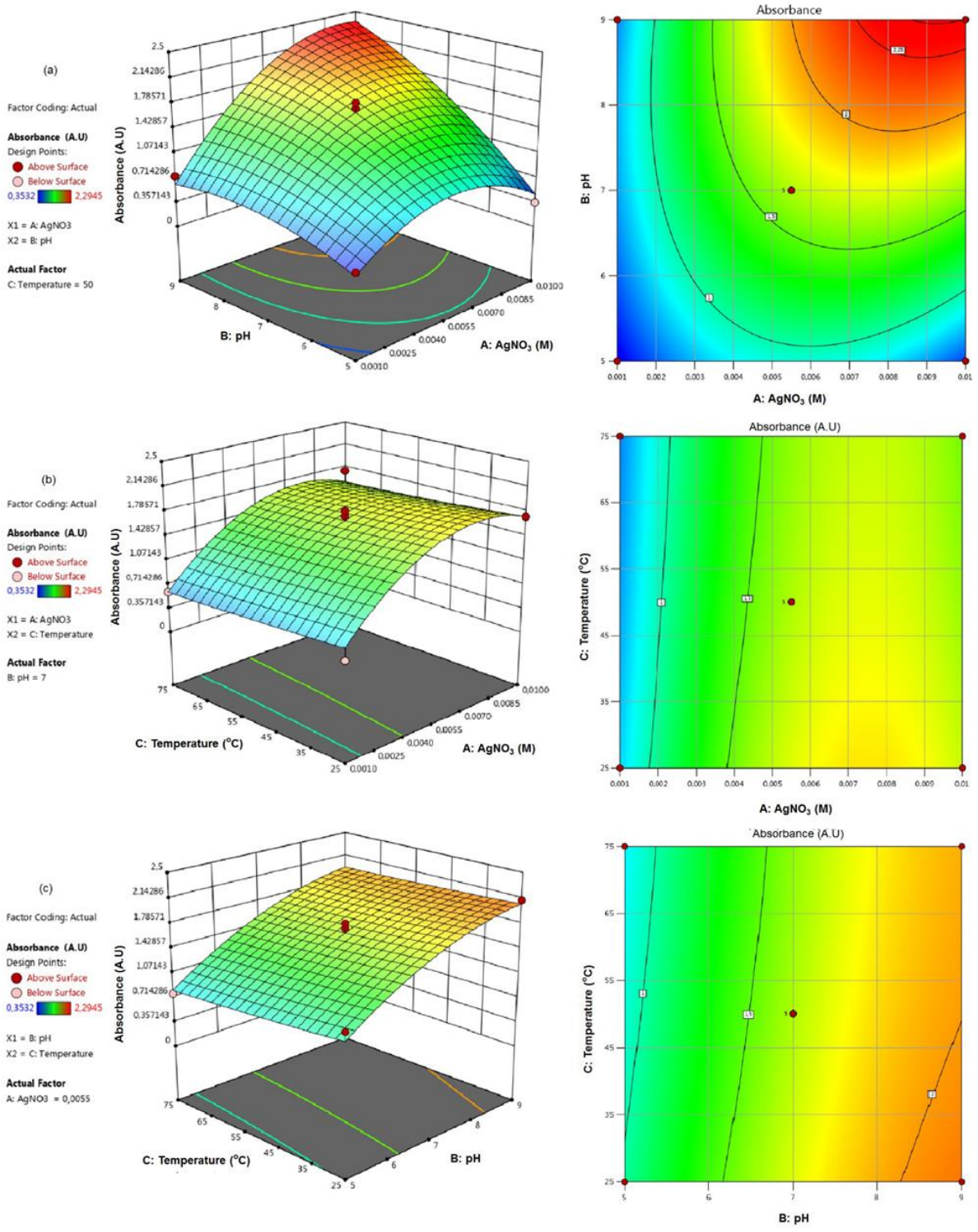

Fig 3 3D response surface and 2D contour plot for interaction effect of two-parameter on absorption response at fixed values of other parameters: (a) influence of $AgNO_3$ concentration and cherry laurel extract pH on absorbance, (b) influence of $AgNO_3$ concentration and reaction temperature on absorbance, and (c) influence of cherry laurel extract pH and reaction temperature on absorbance

Characterization of AgNPs

UV–Vis spectral analysis

The solutions containing different runs in the Box-Behnken design caused a wide color change, ranging from yellowish brown to dark brown, at the end of the reaction time (**Fig 5**). This color change indicates that the silver ions in the solution are reduced from Ag^{+1} to Ag^{0}. It may be due to the excitation of the SPR [61], which is the typical feature of AgNPs. **Fig 5** shows the UV–Vis spectra of silver colloid as a function of the reactions of Ag ions with the plant leaf extract. The characteristic SPR bands of AgNPs in the study were observed between 405 and 472 nm, which was within the range previously reported for AgNPs [60, 62]. The different absorption spectra in the formation of AgNPs indicate the existence of different morphology and size variations [26].

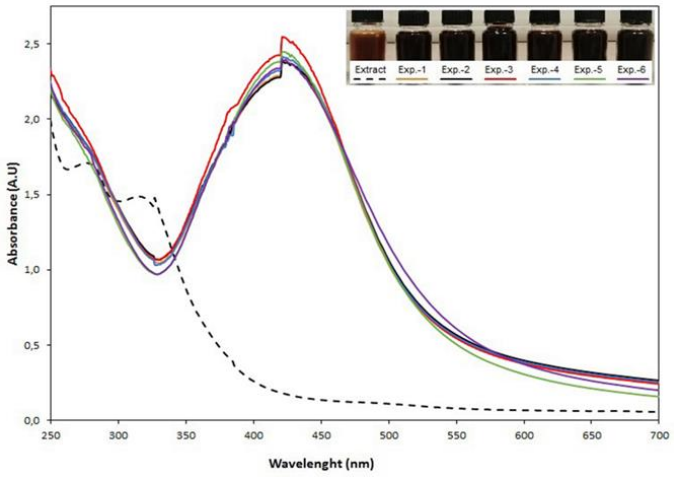

Fig 4 Visual observations and UV–Vis spectra of AgNPs synthesized by six experimental runs under optimum conditions

Fig 5 Changes in solution colors confirming the formation of AgNPs in different experimental runs

FT–IR studies

To get information about molecules and functional groups in newly synthesized AgNPs, an FT–IR spectrum was used. **Fig 6** illustrates the FT–IR spectrum of stabilized AgNPs. The spectra indicate nanoparticle absorption bands at about 3370, 2920, 1604, 1357, 1242, 1026, 832 and 601 cm^{-1}. Phenolic metabolites of cherry laurel leaf extract caused a

strong peak at 3370 cm^{-1}. The band at 2920 cm^{-1} was related to the stretching vibration of the alkane C–H bond. Perhaps the presence of the carbonyl group causes the C=O stretching. The C=C stretching vibration of aromatic rings was responsible for the peak at 1604 cm^{-1}. The C-N stretching of the aromatic amine group was shown by the peak at 1357,1319 cm^{-1} in the spectrum. Furthermore, the peaks at 1242 and 1026 cm^{-1} were corresponded to C–N stretching, which occurred as a result of the presence of the amine groups. Another band at 832 cm^{-1} was typical of the aromatic ring. According to the results, cherry laurel leaf extract contained proteins, phenolic compounds, flavonoids, and aromatic groups, which were reported to be responsible for the reduction of Ag ions to nanoparticles [19, 63, 64, 65].

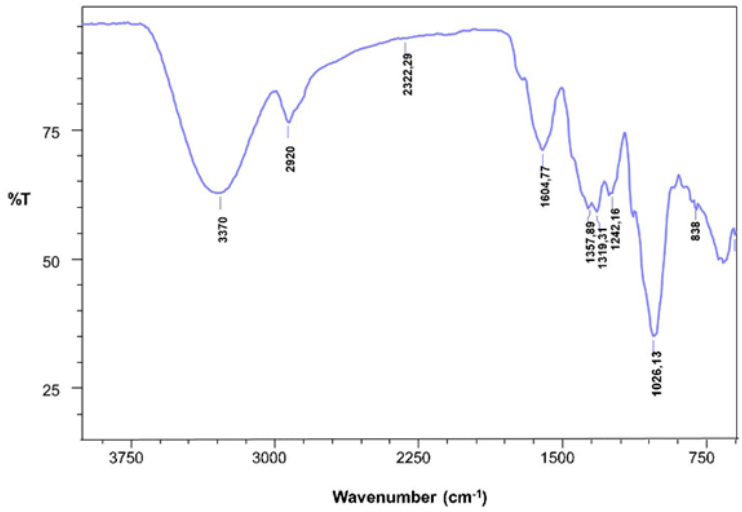

Fig 6 FT–IR spectra representing functional groups associated with cherry laurel leaf extract-mediated reduction and stabilization of silver ions

SEM–EDS analysis

The size, shape, and morphologies of the synthesized AgNPs were characterized by SEM. According to the SEM images of the silver nanoparticles (**Fig 7a**), the AgNPs had an almost spherical morphology, with an average particle size of 80.39 nm. It is possible to determine the composition and metal distribution of a sample using SEM and Energy Dispersive X-ray Spectroscopy (EDS). Because of the SPR of AgNPs, Magudapatty and groups demonstrated that optical absorption peaks of silver nanoparticles emerged at almost 3 keV [66]. AgNPs with a crystalline character were found to provide a peak in the 3 keV area in our EDS profiles (**Fig 7b**).

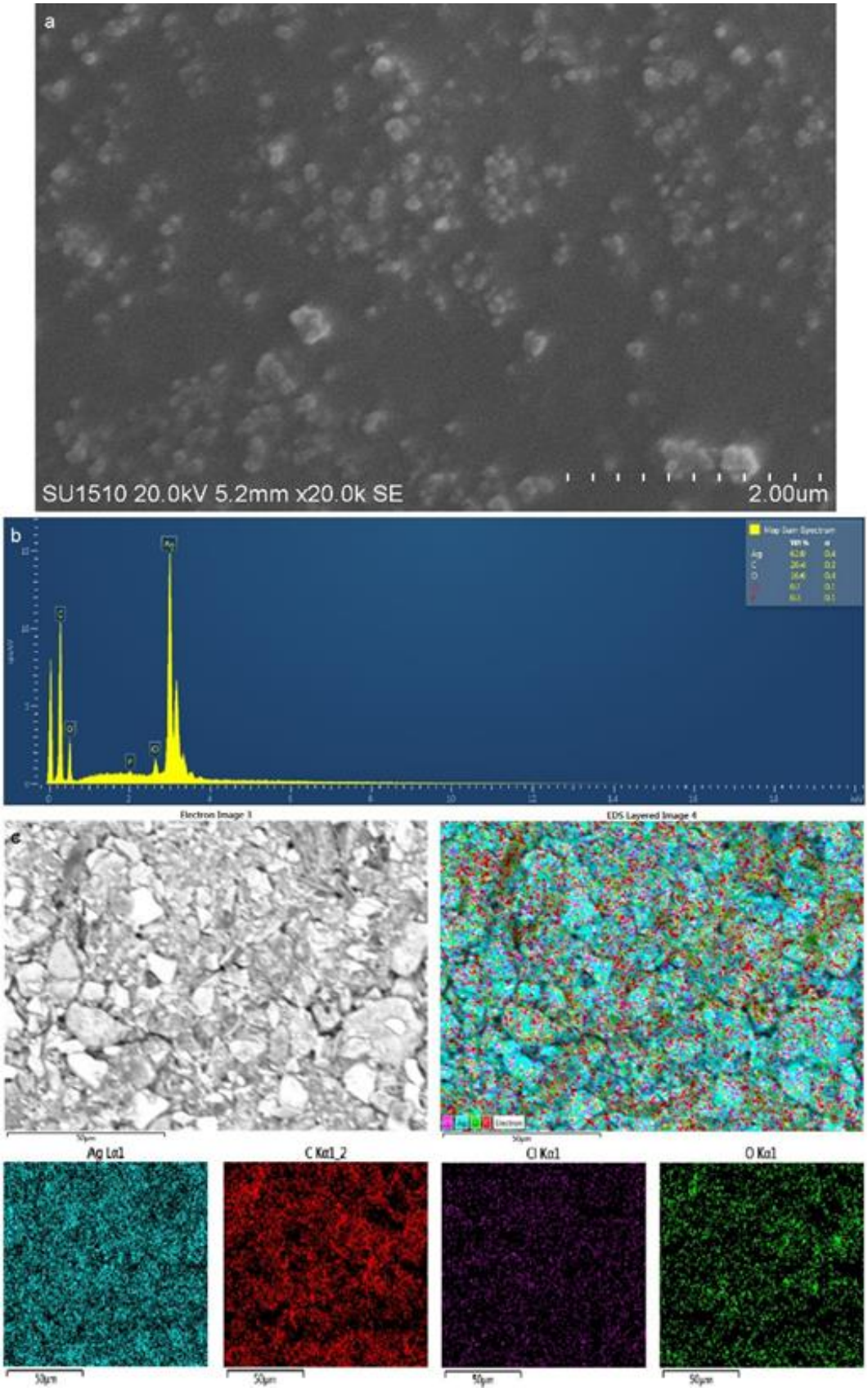

Fig 7 Element analysis and purity assessment of the synthesized AgNPs are based on (a) SEM images, (b) EDS spectrum, (c) SEM–EDS mapping approach

EDS spectra showed that oxygen (O), carbon (C), phosphorus (P), and chlorine (CI) were found in the residual materials that surround NPs and the SEM grid that was used to prepare them. The synthesized AgNPs had a strong Ag atom signal compared to the signals from C, O, Cl, and P. **Fig 7(c)** displayed the EDS mapping of green synthesized AgNPs. The EDS mapping result for Ag, C, O, and CI was shown in the image of respective blue, red, green, and purple colors. AgNPs were confirmed by the EDS elemental mapping analysis, which revealed a signal of Ag (blue). In addition, the SEM results showed that the particles are well dispersed and uniformly distributed.

Antifungal activity of the synthesized AgNPs

The results of the antifungal activity of AgNPs presented in **Table 5** show that the synthesized AgNPs reduced the mycelial growth of all five fungi tested. Among the fungi, *Globisporangium intermedium* was found to have the most sensitive against the synthesized AgNPs, followed by *G. sylvaticum* and *Phytopythium vexans*. The AgNPs at 120 µg/ml concentration completely inhibited *G. intermedium* and *G. sylvaticum*. However, this effect was observed to be fungistatic and re-developed when inoculums of both fungi were transferred to a fresh PDA medium. On the other hand, for the remaining three fungi (*P. vexans*, *Phytophthora citrophthora*, and *Rhizoctonia solani*), both MIC and MFC values of the synthesized AgNPs were greater than 150 µg/ml. In previous studies, it was found that AgNPs synthesized by biological method exhibited strong antifungal activity against various oomycetes (*Phytophthora capsici*, *P. cinnamomi*, *P. infestans*, *P. katsurae*, *P. palmivora*, *P. parasitica*, *P. tropicalis*) and fungi (*Alternaria alternata*, *Botrytis cinerea*, *Curvularia lunata*, *Helminthosporium rostratum*, *Fusarium solani*, *F. oxysporum*, *Macrophomina phaseolina*, *R. solani*, and *Sclerotinia sclerotiorum*) [31, 67, 44]. It is assumed that the antifungal activity of AgNPs is related to their very small structure, shape, and form and that AgNPs act by disrupting the normal functions of cell organelles after penetrating the microbial cell [68].

Table 5 EC_{50}, MIC and MFC values of the synthesized AgNPs inhibiting mycelial growth of kiwifruit fungal root and stem rot agents

Fungi	EC_{50}[a] (µg/ml)	MIC[b] (µg/ml)	MFC[c] (µg/ml)
Phytopythium vexans	10.88	> 150.00	> 150.00
Globisoprangium sylvaticum	9.30	120.00	> 150.00
G. intermedium	7.15	120.00	> 150.00
Phytophthora citrophthora	25.16	> 150.00	> 150.00
Rhizoctonia solani	53.77	> 150.00	> 150.00

[a]The concentration that caused 50% reduction.[b]Minimum inhibitory concentration.[c]Minimum fungicidal concentration.

Conclusion

In this study, AgNPs were synthesized by a green method using cherry laurel (*Prunus laurocerasus*) leaf extract. Based on the RSM, the Box-Behnken design was used to optimize three important experimental parameters in the biosynthesis process of AgNPs, such as $AgNO_3$ concentration, pH of the plant leaf extract, and reaction temperature. The optimum conditions of the experimental parameters are computed as 0.01 M, pH 9.0, and 50°C through a three-factor Box-Behnken design with 17 runs and this reaction condition was experimentally verified. The synthesized AgNPs were characterized by UV–Vis, SEM–EDS, and FT–IR analyzes. In addition, the synthesized AgNPs exhibited antifungal activity against some kiwifruit fungal pathogens, such as *P. vexans*, *G. sylvaticum*, *G. intermedium*, *P. citrophthora*, and *R. solani*.

Abbreviations

AgNPs; silver nanoparticles; $AgNO_3$; silver nitrate; NaOH; sodium hydroxide; HCl; hydrochloric acid; PDA; potato dextrose agar; BBD; Box-Behnken design; RSM; response surface methodology; UV–Vis spectroscopy; ultraviolet–visible spectroscopy; FT–IR; fourier transform infrared spectroscopy; SEM; scanning electron microscopy; EDS; energy dispersive X-ray spectroscopy; SPR; surface plasmon resonance.

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Data Availability statement

The authors confirm that data supporting the findings of this study are available in the article and Supplementary material. All data are included in Uğur Yiğit doctoral (Ph.D.) thesis. Raw data supporting the findings of this study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

Conflict of interest

The authors declare no conflict of interest.

Ethical standards

The study is proper with ethical standards

Authors' contributions

All authors contributed to the study's conception and design. Material preparation, data collection and analysis were performed by Uğur Yiğit, Yaren Gürel, and Muharrem Türkkan. Muharrem Türkkan wrote the original draft of the manuscript. Uğur Yiğit, and Hasan İlhan reviewed and edited the manuscript. All authors read and approved the final manuscript.

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The study does not include human and animal experiments.

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Research article

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An Improved Method for Efficient DNA Extraction from Grapevine

Tulay Oncu Oner[1], Melih Temel[1], Seda Pamay[1], Altin Kardelen Abaci[1] Hilal Betul Kaya[1*]

ABSTRACT

Grapevine (*Vitis vinifera* L.) is one of the oldest and most important perennial crops worldwide which has been the subject of extensive genetic studies including gene mapping, genetic transformation, and DNA fingerprinting. Grapevines are rich in polysaccharides, polyphenolic compounds, and various secondary metabolites, many of which have significant importance in food, agrochemical, and pharmaceutical industries. While metabolites are one of the indicators of quality of grapevines, the presence of them makes grapevine one of the most difficult plants to extract DNA from. These metabolites not only affect DNA extraction procedures but also downstream reactions such as restriction digestion and PCR. Development of new genotyping techniques based on sequencing such as genotyping by sequencing (GBS) requires high-quality DNA for digestion and sequencing. To date, several protocols have been developed for DNA extraction from grapevine. In this study, three different protocols with modifications were compared for DNA extraction performance from grapevine leaves from four different cultivars. Efficiencies of these methods were determined by extracted DNA's quantity and quality. To confirm the suitability for GBS, extracted DNA was digested with restriction enzymes. Although all protocols were based on the traditional CTAB method, they resulted in different DNA yield and restriction digestion efficiency. The modified protocol including PVP-40 and ß-mercaptoethanol was found to be the most efficient method to obtain high quality and quantity grapevine DNA that is amenable to restriction digestion.

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Introduction

Grapevine (*Vitis vinifera* L.), which belongs to Vitaceae family, is one of the most important fruit crops worldwide since it is used in the production of wine, table grapes, dried grapes, and grape juice [1-3]. Besides grape berries, grapevine leaves also have been traditionally used in Mediterranean cuisine and have cultural importance [4]. Grapevine leaves are generally large and petiolate, and appear to be shaped like a hand [5]. They are rich in carotenoids, vitamins, minerals, volatile compounds, and phenolic compounds [6, 7]. In particular, flavonoids, tannins, anthocyanins and procyanidins are the phenolic compounds found in grapevine leaves [8]. Grapes and their byproducts have been used

[1] Department of Bioengineering, Engineering Faculty, Manisa Celal Bayar University, Manisa, Turkey
*Corresponding Author: Hilal Betul Kaya, e-mail: hilalbetul.kaya@cbu.edu.tr

in traditional medicine, including the treatment of skin diseases, bleeding, nausea, inflammation, pain, diarrhea, and gastroenteritis, for thousands of years [9].

Grapevine has been the subject of extensive genetic studies including genetic transformation, DNA fingerprinting, QTL and association mapping [10]. Although DNA extraction from grape berries, stems, and seeds have been shown in different studies [11-13], leaves are the most widely used plant organ for DNA extraction. Genomic DNA extraction from grapevine leaves has always been problematic because of large amounts of secondary metabolites [14, 15]. High polyphenol, polysaccharide and other secondary metabolite content can interfere with DNA extraction and purification [16, 17]. Especially difficult are the oxidized form of polyphenols, as they can covalently bind to DNA interfering with the purification steps in DNA extraction [18, 19]. Also, polysaccharides can affect downstream digestion and amplification processes by inhibiting restriction enzyme and *Taq* polymerase activity, respectively. Polysaccharides in the extracted DNA increase the viscosity of the solution which makes it difficult to work with and affects the quality of DNA [20-23].

Molecular markers are generally used in DNA fingerprinting, population structure and genetic mapping studies [24, 25]. Various molecular markers such as amplified fragment length polymorphism (AFLP) [26], sequence characterized amplified region (SCAR) [27], random amplified polymorphic DNA (RAPD) [28], sequence-related amplified polymorphism (SRAP) [29] and simple sequence repeat (SSR) [30] have been utilized to identify genetic diversities and population structures of grapevine. With the advancement of next-generation sequencing (NGS) techniques, the cost of marker identification decreased considerably. Results were obtained faster and more reliably while novel applications such as genotyping-by-sequencing (GBS) have been developed [31]. GBS offers a reliable and fast approach that can be applied to a large number of samples simultaneously by reducing genome complexity and providing high SNP coverage [32]. The low cost and flexibility of the method make GBS a preferred tool for plant genetic studies [33]. Yet, GBS requires high quality DNA suitable for enzymatic reactions such as digestion and amplification [34].

DNA extraction process efficiency depends on a number of factors including plant tissue, material storage conditions, and extraction buffer components such as detergents,

chelating and reducing agents [16, 35]. To extract DNA from plants, various types of plant tissues such as seeds, leaves, roots, callus, and endosperm can be used, with optimization required for each tissue type. Collection method of tissue and storage conditions are also important parameters for successful DNA extraction [36]. To avoid degradation of DNA, liquid nitrogen or silica gels can be used and plant tissue should be stored at proper conditions [35].

DNA extraction method should be efficient, rapid, simple and cost-effective especially if working with a large number of samples [37]. In the past, various DNA extraction methods were developed for grapevine [10, 38-40]. In addition to manual extraction methods, commercial kits have become available. However, these kits are expensive and usually yield a small amount of DNA. Therefore, developing a standardized high yield and cost-efficient DNA extraction protocol amenable to modern applications is crucial for future grapevine studies [41, 42].

The aim of the present study is to evaluate three different DNA extraction protocols with modifications and select the best one in terms of yield, quality, and suitability for restriction digestion. The result is an optimized DNA extraction protocol from grapevine using leaf samples. This protocol does not only provide extracted DNA suitable for GBS and other sequencing methods but can also be used as a useful guideline that may be applied to other plants.

Materials and Methods

Plant material

Fresh young grapevine leaves from four different cultivars, Alphonse, Hamburg misketi, Royal, and Cardinal, were collected from the vineyard in Manisa Viticulture Research Institute, Turkey (38ºN, 27ºE). Grapevine leaves were immediately frozen in the liquid nitrogen and then stored at -80˚C until DNA extraction.

Comparison of DNA extraction protocols

Fresh grapevine leaves (0.2 g) were ground using liquid nitrogen and a mortar and pestle to a fine powder, and then transferred into 2 ml eppendorf tubes. After this common step, the following protocols were used. A schematic overview of workflow for the protocols is shown in Fig 1.

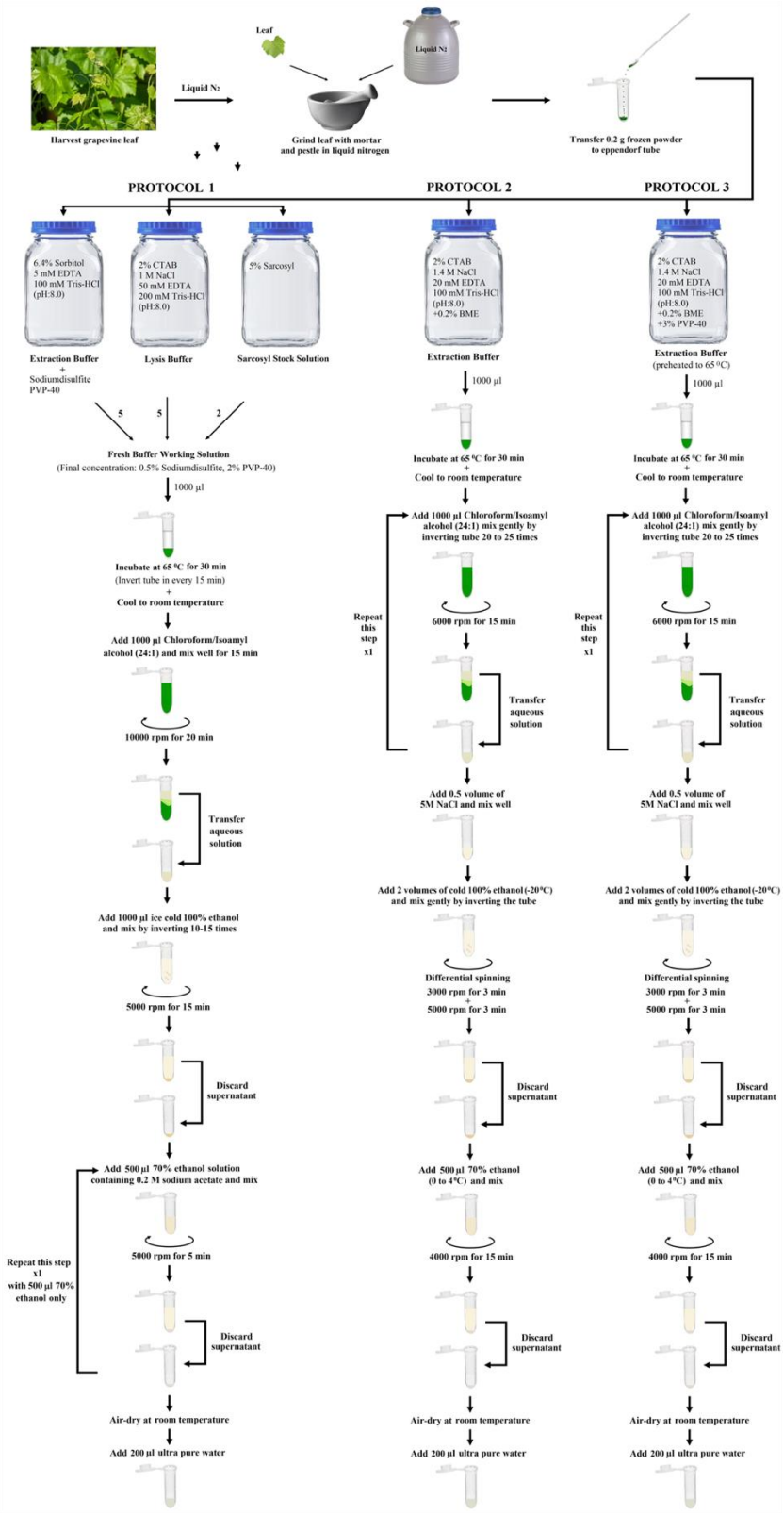

Fig 1 Workflow for implemented DNA extraction protocols. BME: ß-mercaptoethanol, CTAB: cetyl-trimethyl ammonium bromide, EDTA: ethylene diamine tetra acetic acid, N_2: nitrogen, PVP-40: polyvinylpyrrolidone

DNA extraction protocol 1

The extraction of DNA was performed using the in-house DNA extraction protocol of the Diversity Arrays Technology (DArT) with some modifications. *(https://www.diversityarrays.com/orderinstructions/plant-dna-extraction-protocol-for-dart/).* The CTAB-based DArT DNA extraction protocol includes extraction buffer, lysis buffer and sarcosyl stock solutions as follows: Extraction buffer was prepared with 6.4% (w/v) sorbitol, 5 mM EDTA (pH: 8.0), 100 mM TrisHCl (pH: 8.0) in ddH_2O. Lysis buffer contained 2% (w/v) CTAB, 1 M NaCl, 50 mM EDTA, 200mM TrisHCl (pH: 8.0). Sarcosyl stock was 5% (w/v). Final concentrations of sodium disulfite and PVP-40 in the working solution were 0.5% (w/v) and 2% (w/v), respectively. Fresh working solution was prepared by dissolving sodium disulfite and PVP-40 in extraction buffer and mixing this solution with lysis buffer and sarcosyl stock at a ratio of 5:5:2. Leaf tissues were ground in mortar and pestle under liquid nitrogen to fine powder and suspended in 1 ml fresh buffer solution kept at 65 ºC. The sample was incubated at 65 ºC for 1 h with gentle shaking and inverting the tubes every 15 minutes. After incubation, the mixture was cooled down for 5 min and 1 ml of chloroform: isoamyl alcohol (24:1) was added and mixed well for 15 min. The mixture was centrifuged at 10000 rpm for 20 min. The water phase was transferred to new eppendorf tube and 1 ml ice cold absolute ethanol (isopropanol in the original protocol by DArT) was added and mixed by inverting 10-15 times. The mixture was centrifuged at 5000 rpm for 15 min. Supernatant was discarded carefully and, diverging from the DArT protocol, the pellet was washed with 500 µl of 70% ethanol solution containing 0.2 M sodium acetate and again with 500 µl of 70% ethanol solution only. The mixture was centrifuged at 5000 rpm for 5 min. The supernatant was decanted carefully, and the pellet was dried at room temperature. Finally, the DNA pellet was dissolved in 200 µl of ultra-pure water (TE buffer was used in the original protocol by DArT) at 65 ºC for 30 min. Genomic DNA was treated with 1 µl RNAse A (10 mg/ml) and incubated at 37 ºC for 15 min to remove RNA contamination. DNA was stored at -20 ºC for future use.

DNA extraction protocol 2

The extraction of DNA was performed with the protocol described by Lodhi *et al.* (1994) with the following modifications: preheated extraction buffer, 0.2 g of leaf tissue powder was used instead of 0.5 g. Extraction buffer was prepared using 2% CTAB (w/v), 1.4 M

NaCl, 100 mM Tris-HCl (pH: 8.0) and 20 mM EDTA and added 0.2% of ß-mercaptoethanol (w/v) just before use. Differently from Lodhi *et al.* (1994), extraction buffer was preheated in a 65 ºC water bath and 1 ml extraction buffer was added to the eppendorf tube containing grapevine leaf powder and mixed well by vortexing. Addition of PVP-40 was omitted before incubation. The sample was incubated at 65 ºC for 30 min and then cooled to room temperature (instead of incubation at 60 ºC for 25 min). Then 1 ml of chloroform:isoamyl alcohol (24:1) was added (Lodhi et al. used chloroform:octanol) and mixed gently by inverting the tubes 20 to 25 times to form an emulsion. This mixture was centrifuged at 6000 rpm for 15 min. The aqueous phase was transferred to a new eppendorf tube. If the supernatant was not clear, this chloroform:isoamyl alcohol step was repeated. 5 M NaCl equal to half the volume of supernatant was added and mixed well. Thereafter two volumes of cold 100% ethanol (-20 ºC) were added and mixed gently by inverting the tubes until DNA strands began to appear. The mixture was centrifuged at 3000 rpm for 3 min and immediately after at 5000 rpm for 3 min at room temperature. This differential spinning step kept the DNA at the bottom of the eppendorf tube. Supernatant was discarded and the pellet was washed with cold 70% ethanol (0 to 4 ºC) and then air-dried at room temperature. Finally, instead of TE buffer (Lodhi *et al*. 2014), the pellet was dissolved in 200 µl of ultra-pure water. This 200 µl DNA solution was treated with 1 µl RNase A (10 mg/ml) and incubated at 37 ºC for 15 minutes. DNA was stored at -20 ºC for future use.

DNA extraction protocol 3

DNA extraction Protocol 3 mirrored Protocol 2 but included the addition of 3% PVP-40 (w/v) (polyvinylpyrrolidone) in the extraction buffer. While PVP-40 was added separately to the leaf slurry after addition of the extraction buffer (Lodhi et al., 2014) in the original protocol, PVP-40 was added directly to the extraction buffer in this study. Preheated extraction buffer (preheated to 65 ºC) was also used in this protocol which was different from Lodhi *et al.* (1994). The same incubation and centrifuge conditions were implemented in this protocol as in Protocol 2, as well as resuspension, RNAse treatment, and storage.

DNA quantification and quality assessment

DNA quantification was performed with Qubit dsDNA BR Assay Kit (Invitrogen, cat no. Q32850) on a Qubit 3.0 Fluorometer (Invitrogen) using an aliquot of 1 µl genomic DNA.

The instrument was calibrated with the Quant-iT dsDNA BR Assay (stated assay range between 2–1000 ng; sample starting concentration between 100 pg/µl and µg/µl) according to the manufacturer's instructions.

Based on DNA concentration derived from the Qubit measurements, total yield was obtained by multiplying the DNA concentration by the volume of the total DNA sample.

$$\text{Total DNA yield } (\mu g) = \text{DNA concentration} \times \text{total DNA sample volume (ml)}$$

In addition, the presence and quality of genomic DNA was evaluated by electrophoresis. DNA was stained with Sybr Safe (Invitrogen), separated by 1% agarose gel for 1 h at 200 V using 1 x TAE (40 mM Tris-acetate, 1 mM EDTA) running buffer in Thermo Scientific™ Owl™ A2 Large Gel Systems, and then visualized by UV gel documentation system E-box VX2/20LM (Vilber Lourmat).

Restriction digestion with *EcoR*I enzyme

DNA extracted from the grapevine leaves was subjected to restriction digestion with *EcoR*I enzyme (R0101S, NEB), which cuts DNA at the sequence GTTAAC. A mixture was prepared using 1 µg genomic DNA, 1 µl *EcoR*I restriction enzyme and 5 µl 10X NEBuffer and the reaction volume brought up to 50 µl with nuclease free water. This mixture was incubated at 37 ºC for 30 min. Then the enzyme was inactivated by incubation at 65 ºC for 20 min. Digested DNA were run on a 1% agarose gel to test the efficiency of the digestion.

Application of the best protocol to twenty-two different cultivars

Based on the results of the experiments from different protocols described above, the best DNA extraction method, Protocol 3, was implemented with some modifications as follows. To test the method's efficiency in various cultivars, twenty-two different grapevine cultivars was used for DNA extraction. Instead of grinding sample in a mortar and pestle, grinding was performed on a Precellys Evolution tissue homogenizer (Bertin instruments, Montigny-le-Bretonneux, France). DNA pellet was dissolved in 200 µl of TE buffer. All the other steps were as described in Protocol 3 and DNA quantification and quality assessment were performed as mentioned above.

Results and Discussion

In molecular genetic studies of plants, extraction of DNA of good quality and quantity is critically important. Quality of the extracted DNA can change depending on the tissue

type of the plants as well as collection and storage conditions of the sample [20]. DNA extraction can be problematic especially in plants which consist of high amounts of secondary metabolites, phenolic compounds and polysaccharides such as grapevine [43]. In this study, CTAB-based protocols reported by Diversity Arrays Technology (DArT) and Lodhi *et al.* (1994) were modified and implemented, showing different DNA yields and restriction digestion efficiencies. The CTAB-based DArT DNA extraction protocol (Protocol 1) has been successfully used in a wide variety of plants including maize [44], safflower [45] and cowpea [46]. This protocol contains PVP-40, sorbitol and sarcosyl reagents that can help remove polyphenols and polysaccharides that are attached to the DNA [47, 48]. Although clear DNA bands without protein contamination were achieved when Protocol 1 was used, a huge amount of RNA contamination was also obtained (Fig 2a) indicating the consistently applied RNAse A treatment was less efficient for this DNA extraction. The yield of DNA obtained was low ranging from 6.4 µg to 12 µg from 0.2 g fresh leave samples with Protocol 1 (Table 1).

Table 1 DNA yield and concentration values obtained from four samples using three different extractions protocols

		Protocol 1	**Protocol 2**	**Protocol 3**
Sample 1	DNA concentration (ng/µl)	32	272	112
	Total DNA yield (µg)	6.4	54.4	22.4
Sample 2	DNA concentration (ng/µl)	40	164	126
	Total DNA yield (µg)	8	32.8	25.2
Sample 3	DNA concentration (ng/µl)	60	113	91
	Total DNA yield (µg)	12	22.6	18.2
Sample 4	DNA concentration (ng/µl)	45	158	85
	Total DNA yield (µg)	9	31.6	17

The protocol reported by Lodhi *et al.* (1994) was a modified version of the protocol reported by Doyle and Doyle (1987). Lodhi *et al.* (1994) added NaCl and PVP-40 to the extraction buffer to remove polysaccharides and polyphenols, respectively. In this study, we tried extraction buffers with PVP-40 (Protocol 3) and without PVP-40 (Protocol 2) to understand the effect of PVP-40 in the extraction buffer. The protocol 2 extraction method provided the highest amount of DNA ranging from 22.6 µg to 54.4 µg while the DNA yields from Protocol 3 ranged from 17 µg to 25.2 µg from 0.2 g leaf tissue. According to agarose gel results, extracted DNA by Protocol 2 showed higher amount of protein and

RNA contaminations than DNA obtained from Protocol 3 (Fig 2a). Although RNAse treatment was applied to all DNA samples, various amount of RNA contamination was also obtained in all protocols.

The addition of PVP-40 in the extraction buffer increases the DNA quality by removing RNA and phenolics from plants high in polyphenolic compounds, such as, betula and grape leaves [49]. However, it is also reported that the presence of PVP-40 increased the DNA yield [49, 50]. In the present study, addition of PVP-40 decreased the RNA and protein contamination, but it did not increase the DNA yield. Lodhi *et al.* (1994) reported that grinding of leaf samples to the very fine powder resulted in sheared DNA. However, in this study, grinding to the very fine powder under liquid nitrogen did not result in degradation as shown in Fig 2a.

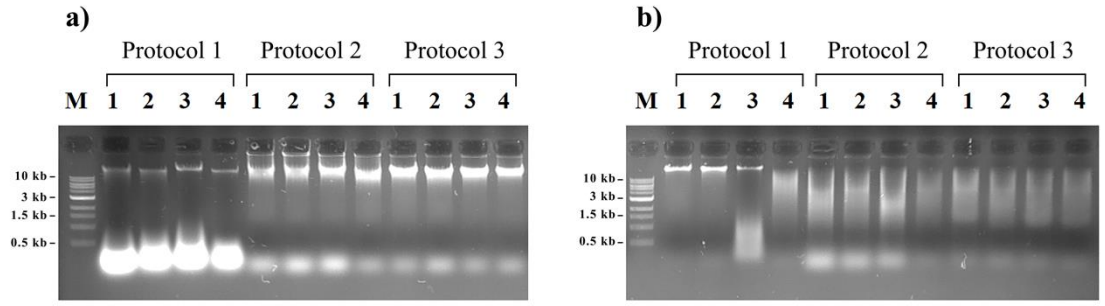

Fig 2 Result from agarose gel electrophoresis analysis of **a)** DNA extracted using three protocols from four different fresh young grapevine leaves (1: Alphonse, 2: Hamburg misketi, 3: Royal, 4: Cardinal). 1 kb DNA ladder (N3232S, New England BioLabs) was used to estimate the molecular size of the fragments. Lane M: 1 kb DNA ladder. Sample lanes 1 to 4 show DNA extracted using Protocol 1, Protocol 2 and Protocol 3, respectively. **b)** restriction enzyme digestion for three protocols. 1 kb DNA ladder was used to estimate the molecular size of the fragments. Lane M: 1 kb DNA ladder. Sample lanes 1 to 4 show restriction digestion results of DNA extracted Protocol 1, Protocol 2, and Protocol 3, respectively

UV spectrophotometry, dsDNA-specific fluorimetry and quantitative PCR are the most common techniques for DNA quantification [51]. The most frequently used instrument for spectrophotometric analysis, Nanodrop, is based on similar principles as a conventional spectrophotometer but has additional features. The Qubit system uses fluorochromes that specifically bind dsDNA to measure the concentration of DNA. Although Nanodrop has advantages over Qubit such as ease-of use, well-established, and no reagent requirement, Qubit is the more preferred instrument, especially for sequencing, due to its highly reproducible and consistent results [52]. It has been reported

that NanoDrop overestimates DNA concentration [53]. In this study, Qubit 3.0 Fluorometer (Invitrogen) was used for DNA quantification and consistent results were obtained with agarose gel electrophoresis.

DNA quality and quantity requirements change depending on the platform and genotyping techniques by NGS. In the GBS protocol, the first step is an initial digest of sample DNA by restriction enzyme to reduce genome representation [32]. Various compounds including proteins, polysaccharides, phenolic compounds, and other plant secondary metabolites in the DNA sample may inhibit restriction digestion and PCR [10, 54]. For this reason, DNA quality is a critical parameter for the succes of GBS. Genomic DNA should have a clear band greater than 10 kb while digested samples should exhibit a smear. In the present study, effectiveness of digestion was assessed by *EcoR*I restriction enzyme. Analysis of digestion of DNAs by *EcoR*I showed that inconsistent results were obtained when the Protocol 1 used (Fig 2b). However, all DNAs from Protocol 2 and Protocol 3 were fully digested as shown in Fig 2b. In the present study, addition of PVP-40 did not affect the restriction digestion as shown in Fig 2b.

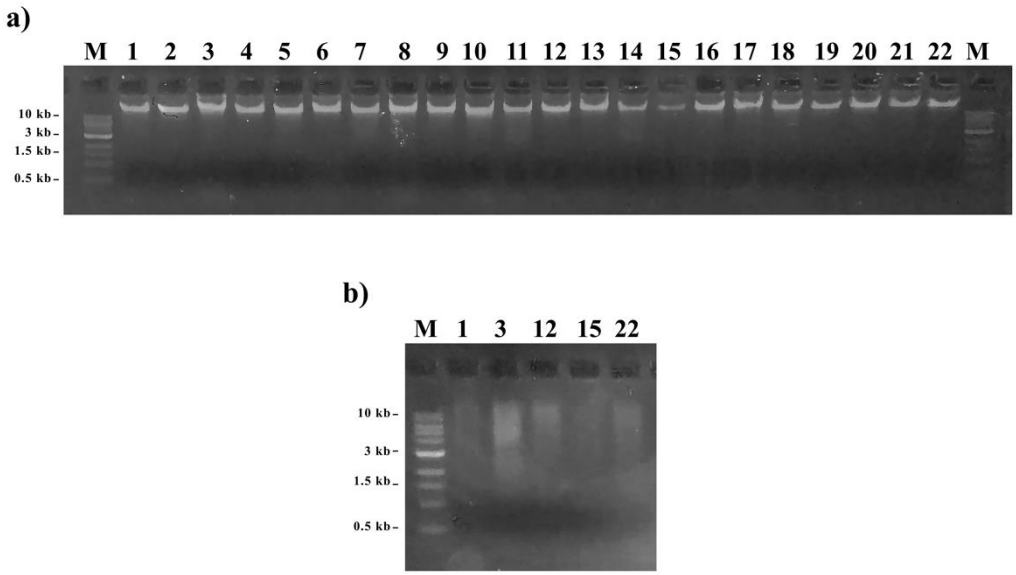

Fig 3 Agarose gel electrophoresis of undigested and digested genomic DNA samples of grapevine. a) Genomic DNA from twenty-two different grapevines, b) Genomic DNA of five random selected grapevine samples digested with *Eco*RI restriction enzyme, Lane M: 1 kb DNA ladder

The SNPs detected by GBS are widely used for genetic diversity analysis [55-58], characterization of population structure [59, 60], QTL mapping [61, 62] and genome-

wide association studies [63-65] (GWAS), and genomic selection [66, 67] in many horticultural crops including grapevine [68-72]. As DNA extraction and purification methods affect GBS results, they also affect the genetic analysis which use GBS data. To date, various DNA extraction protocols have been applied to woody plants including grapevine. Marsal *et al.* (2013) extracted DNA from young leaves, mature leaves, seeds and stems of grapevine for SSR analysis by using ten different DNA extraction methods including three common commercial kits. They reported that the use of the cationic detergents such as CTAB and DTAB gave better results than SDS in grapevine. The authors also showed that using the combination of CTAB and DTAB provided good DNA yields. However, in our study using only CTAB was enough to meet the requirements of DNA quality and quantity for GBS. They also reported that higher DNA yield was obtained from young leaves than from mature leaves, seeds, and stems. Akkurt (2012) also investigated the effects of sample collection time and samples grown in both vineyard and greenhouse in addition to different DNA extraction protocols [73]. He reported that higher DNA yield was obtained from vineyard sample when they implemented the DNA extraction protocol by Lodhi *et al.* (1994). Yet, none of these studies evaluated the suitability of the DNAs for NGS.

Table 2 DNA yield and concentration values of 22 grapevine cultivars using Protocol 3

Sample ID	**DNA concentration (ng/µl)**	**Total yield (µg)**	**Sample ID**	**DNA concentration (ng/µl)**	**Total yield (ng)**
1	92	18.4	**12**	92	18.4
2	97	19.4	**13**	97	19.4
3	104	20.8	**14**	87	17.4
4	96	19.2	**15**	99	19.8
5	98	19.6	**16**	95	19.0
6	89	17.8	**17**	92	18.4
7	87	17.4	**18**	89	17.8
8	94	18.8	**19**	95	19.0
9	102	20.4	**20**	112	22.4
10	107	21.4	**21**	97	19.4
11	93	18.6	**22**	91	18.2

For confirmation, the best performing protocol, Protocol 3, was implemented for genomic DNA extraction of different cultivars of grapevine. Similar band intensities were observed for the extracted DNA from twenty-two grapevines cultivars (Fig 3a) of which fresh leaves were collected and stored at -80 ºC upon flash freezing in liquid N_2. None of

DNA samples exhibited significant smearing which indicates degradation of DNA sample. DNA concentrations ranged from 87 ng/µl to 112 ng/µl (Table 2). In the case of restriction digestion, five random genomic DNAs were digested with *EcoR*I which produced excellent digestion smears as shown in Fig 3b.

Conclusion

In conclusion, we described a simple and rapid protocol that can be reliably used for routine DNA extraction from grapevine leaf tissue and meet the requirements of DNA quality and quantity for GBS. The modified Lodhi extraction method may be used for other tree species that include high levels of polysaccharides, polyphenolic compounds, and various secondary metabolites.

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Data Availability statement
The authors confirm that data supporting the findings of this study are available in the article.

Compliance with ethical standards
Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards

Authors' contributions
Hilal Betul Kaya conceived and designed the study. All authors performed the experiments and contributed to the preparation of the manuscript.

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Research article

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Production of Tetracycline Hydrochloride-Collagen-Doped Chitosan Nanofiber Scaffolds And Investigation of Their Antibacterial Properties

Ibrahim Altun[1]*, Ismail Tiyek[2], Eda Altun[1], Idiris Altun[3]

ABSTRACT

The study aims to develop a noble biomaterial that can accelerate the healing process without the risk of infection by loading tetracycline hydrochloride and collagen hemostatic agent into the chitosan tissue scaffold. After the trials, a good tissue scaffold was obtained from chitosan and PVA by electro-spinning. To increase the hemostatic features of this biomaterial, a 10% (by weight) collagen hemostatic agent was added to the PVA chitosan blend. After the amount of collagen hemostatic agent in the chitosan tissue scaffold was set, various amounts of tetracycline were added and 5 different biomaterials were developed to augment the antibacterial and wound healing properties. Antibiotic concentration in the biomaterial was IV 10% in the first, 15% in the second, 20% in the third, 25% in the fourth, and 30% in the fifth sample. Finally, the effects of the obtained biomaterials on the nosocomial bacteria (gram-positive: Staphylococcus Aureus, gram-negative: Pseudomonas Aeruginosa) were analyzed with in-vitro tests at Kahramanmaras Sutcu Imam University, School of Medicine Department of Microbiology laboratories. As a result of the examination, it was examined how much the biomaterial should be and how effective it was against bacterial growth on the first, third, and fifth days. It is thought that the biomaterial material will be very effective in emergencies and surgical procedures.

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Introduction

Piterresi et al. posted in 2012 the characterization and manufacturing of electrospun fibers of the proposed --poly(N-2-hydroxyethyl)-DL-aspartame-graft-polylactic acid (PHEA-g-PLA) copolymer for the cap potential to close by the release of ibuprofen. Before the electrospinning technique, a physically certain medicated solution with PHEA-g-PLA and/or a chemically certain medicated solution with PHEA-g-PLA was modified into organized. The synthesis of PHEA-g-PLA copolymer become done with the usage of an answer of 1. eighty g of PHEA in 36 ml of anhydrous DMSO and 1.7 ml of DEA as a catalyst. The reaction becomes mounted

[1] Ministry of National Education, Teacher, Kahramanmaras, 46050, Turkey
[2] Textile Engineering, Faculty of Engineering and Architecture, Kahramanmaras Sutcu Imam University, Kahramanmaras, 46050, Turkey
[3] Neurosurgery, Faculty of Medicine, Kahramanmaras Sutcu Imam University, Kahramanmaras, 46050, Turkey
*Corresponding Author: İbrahim ALTUN e-mail: ibobey46@hotmail.com

below the Argon fuel line for twenty-4 hours at 40 0C.PHEA-gPLA-IBU medicated copolymer answer; 117,15 mg of IBU become dissolved in 1. five ml of anhydrous DMC and saved for a half-hour at -14 0C withinside the presence of 7 mg of DMAP and 117,15 mg of DCC. Then, the IBU solution becomes modified and added dropwise to a 500 mg PHEA-g-PLA solution in 4. five ml of DCM. This chemical synthesis reaction took place at -14 0C for 1 hour, and then it become at room temperature for 3 hours. The nanofibers of these samples, on the alternative hand, have been created with the useful resource of electrospinning after obtaining the medicated copolymer solution with the physical combination of five% IBU and PHEA-g-PLA polymer at the most useful ratios in the solvent combination of acetone and N, N-dimethylformamide. Dulbekko phosphate buffer (DBSO) answer become used for launch testing. A piece of decreased nanofiber withinside the buffer answer modified and dissolved, and a sample is modified and become taken at sure time durations. These samples were examined with the useful resource of the UV-vis technique. Images of drug-loaded nanofiber structures were inquisitive about the useful resource of SEM [1].

Nanofiber ground was modified into received for the primary time with the usage of an electrospinning approach from a combination of the aqueous answer of poly (vinyl alcohol) polymer, which has extremely good nanofiber formation ability and mechanical houses of propolis extract, which can not be drawn alone. They received linear nanofibers that could provide the drug release mechanism. In their experiments, they truly located that the nanofiber ground modified form in the SEM image of the nanofiber surfaces they received at a 5 percent PVA solution and 10 cm walking distance. They claimed that as PVA attention improved, so did the linear fiber form, and the connection between walking distance and fiber form has become at the least proportional. The maximum helix shape became located within the nanofiber ground image of the solution prepared with 3 percent propolis extract and 11 percent PVA. As a result of scanning electron microscope pix, the pleasant fiber structures were received within the pix of nanofiber surfaces produced at a fifteen cm walking distance in an electrospinning tool with the resource of the use of getting prepared propolis extract solutions at 7% and 9% PVA concentrations at 60 0C. It has been determined that the factors decided within the natural wound flora are formed in vitro and are effective in competition with the S. Aureus microorganism, one of the gram-first-rate microorganisms that reason the most wound formation on human skin. They concluded that due to the fact the bacterial boom surrounding the affected character's wound flora may be removed or reduced with the usage of the composite

plaster, the affected character will do away with the thing results of the drug due to systematic or oral antibiotic intake and will avoid the price of antibiotics withinside the route of the treatment [11].

In taking a study with the resource of Taepabioon et al. In 2006, they efficiently prepared the fibers with the resource of electrospinning the usage of the drug-loaded biodegradable polymer PVA (polyvinyl alcohol) and examined the usability of these fibers as a drug transport system. It was modified right into a 10% PVA (polyvinylalcohol) solution with the resource of dissolving sufficient PVA (polyvinylalcohol) powder in distilled water at eighty C for 3 hours. As a drug model, 4 tremendous non-steroidal anti-inflammatory tablets with water solubility residences: sodium salicylate (SS) (water-soluble), diclofenac sodium (DS) (slightly water-soluble), Naproxen (NAP), and Indomethacin (IND) (every water-soluble and insoluble) had been used. These tablets have been combined one after the alternative with 10% PVA solutions at 20% and 10% ratios. Drug-loaded nanofibers of prepared answers had been acquired through the use of the electrospinning technique, and nuclear magnetic resonance (NMR) became used to make clear the chemical form of drug-loaded PVA fibers [12].

Yu et al., in their, examination posted in 2012, investigated the release houses of nonsteroidal anti-inflammatory drug active substance KET (ketoprofen), which they used as a drug model, from CA (cellulose acetate) polymer fibers. Eleven mg of CA and more than one g of KET, one hundred ml of DMAc (dimethylacetamide), acetone, and ethanol (4:1:1 ratio) were dissolved within the solvent. Then, KET-loaded CA fibers had been prepared with the useful resource of an electrospinning approach. The houses of nanofibers in conjunction with morphology, crystalline shape, form, length, and composition were evaluated using FESEM (problem emission scanning electron microscopy). a hundred mg of fiber samples derived from nanofibers changed into taken and dissolved in 600 ml of ninety-percentage physiological saline (PS) at 321 C. The Fickian diffusion mechanism of KET, which is amorphous and dispersed in six CA-based drug-loaded fibers, changed into used to research a non-stop drug release profile for one hundred forty-four hours. The oscillation 38 changed into theoretically examined with the useful resource of the use of the UV-vis approach at the samples taken over time, and on the give up of this appearance, they decided that the oscillation kinetics modified into 0th order [13].

In their have a look at in 2005, Zeng et al. investigated the usability of drug-loaded PLLA (Poly (L-lactide)) nanofibers in controlled drug delivery systems. Paclitaxel, doxorubicin, and

doxorubicin hydrochloride were used as drug models. PLLA (Poly (L-lactide)) polymer solution changed into prepared in a 2:1 chloroform-acetone solvent combination at the attention of 3.9 wt%. 15.5% paclitaxel, 1.6% doxorubicin hydrochloride, and 1.6% doxorubicin drugs were added to the prepared PLLA (Poly (L-lactide)) polymer answers. The morphology of the prepared drug-loaded nanofibers and the images of the drug-loaded cloth were examined with the resource of SEM (scanning electron microscopy). In the in vitro release of the paclitaxel drug model, it changed into studied in 25 ml of 0.05 mol/L Tris-HCl buffer solution (pH = 8.6). In addition, the release of drug-loaded PLLA (Poly (L-lactide)) polymer form was modified whilst studied with proteinase K (0.01 mg/ml). For the in vitro release of doxorubicin hydrochloride, it is 3.0 x 10-3 mg/ml prepared in a proteinase K enzyme buffer solution. The drug-loaded fibers have been dissolved withinside the enzyme solution, samples have been taken at sure time durations, and UVvis analyses have been carried out. Similarly, an in vitro test of Doxorubicin changed into performed. The studies executed are, briefly, The person releases of these three first-rate drugs from drug-loaded PLLA nanofibers were investigated, every with the presence of proteinase K enzyme and without the usage of proteinase K enzyme. As a result, they discovered that Paclitaxel changed into not released withinside the absence of the proteinase K enzyme, however, Doxorubicin and Doxorubicin Hydrochloride have been released. The Proteinase K enzyme degraded PLLA nanofibers and released drugs. In addition, they proved from kinetic studies that the release of Paclitaxel and Doxorubicin drugs is of the 0th order [14].

Material and Methods

It is of incredible significance to save blood loss in wounds due to accidents, emergencies, diverse diseases, and surgical interventions and to heal wounds speedy without the threat of infection [1]. Although bleeding might also additionally appear easy in such cases, prevention of blood loss is of essential significance. Also, one of the largest risks for open or closed wounds is infection. Antibiotics have to end up crucial safety in opposition to the threat of infection [1]. In this study, Tetracycline Hydrochloride antibiotic and Collagen anti-bleeding agent became loaded onto the tissue scaffold received with the aid of using blending Polyvinyl alcohol (PVA) and Chitosan. Thus, it's far aimed to achieve a biomaterial that hastens the recovery technique of wounds without the threat of infection. For this, first of all, a tissue scaffold became received from Polyvinyl alcohol (PVA) and Chitosan with the aid of using the electrospinning method. Collagen astringent and Tetracycline Hydrochloride had been delivered to the tissue scaffold

on the charges stated withinside the content. Codes had been given to the samples received. SEM pictures of the samples are given in Figure 1(a) above [1].

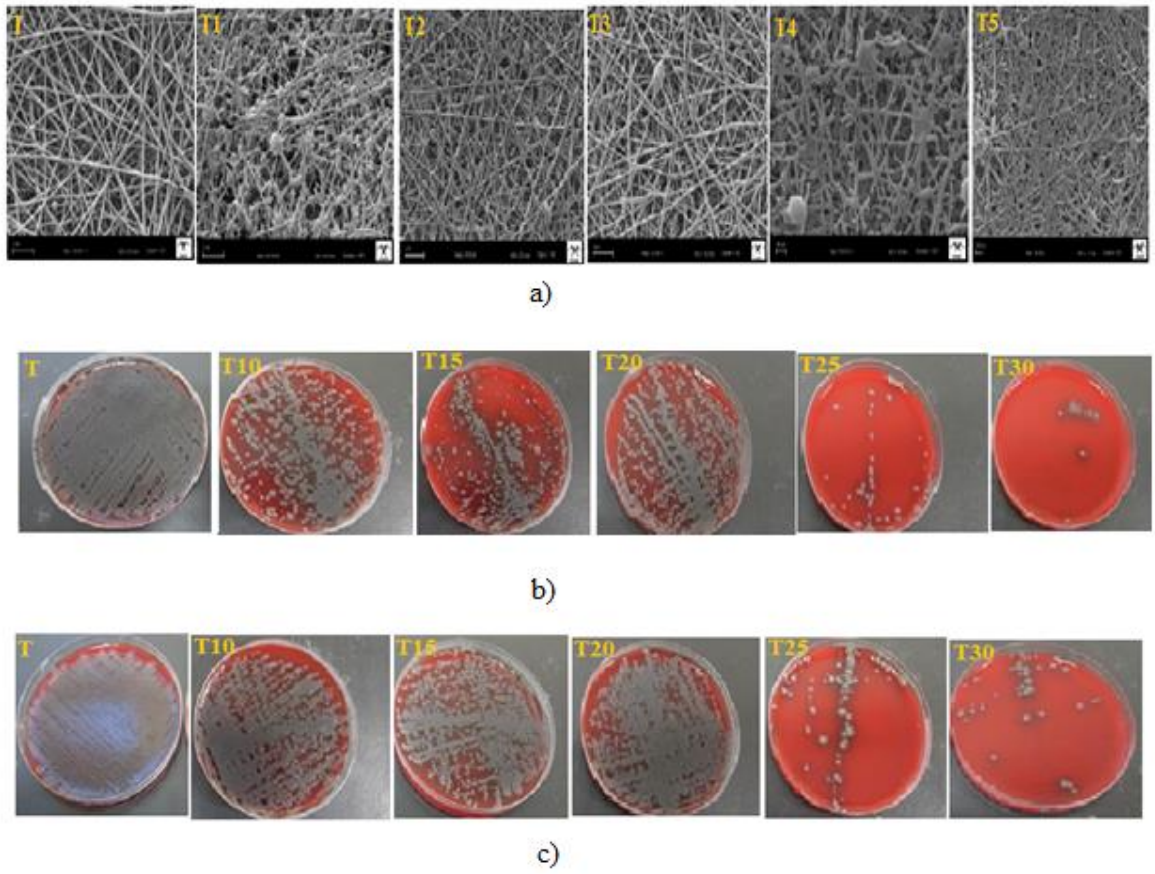

Fig 1 a) Sem images of 5 prepared samples, b) 3rd-day growth results of 5 prepared samples against staphylococcus aureus bacteria, c) 3rd-day growth results of 5 prepared samples against pseudomonas aeruginosa bacteria

The results of the received biomaterials at the medical institution micro organism Staphylococcus Aureus (gram +) and Pseudomonas Aeruginosa (gram-) have been tested with the aid of using in-vitro check withinside the Microbiology Laboratory. Bacterial boom becomes measured in synthetic surroundings breaking away the residing thing. First of all, biomaterials have been sterilized with the aid of using the recent method. At the give up of the first day, third day, and fifth day of the organized biomaterials, bacterial boom becomes found in sheep blood agars. Thus, colony counts on sheep blood agar have been counted for every sample. As a result, we cautiously tested the in-vitro check consequences of biomaterials and supplied the received data. Figure 1 (b) suggests the efficacy of 5 samples in opposition to the S. aureus microorganism. Figure 1 (c) suggests the effectiveness of 5 samples in opposition to

the P. aeruginosa microorganism. The consequences will make considerable contributions to the literature.

Material

Of the substances for use withinside the research: PVA (polyvinyl alcohol) Acn Chemical Mad.İnş tex. conf. Singing. ve Tic.A.Ş. Chitosan became obtained from Acros Organics. Acetic acid and different chemical compounds have been bought from Sigma Aldrich. Tetracycline hydrochloride and physiological saline have been furnished with the aid of using Mustafa Nevzat İlaç san. A collagen hemostatic agent became received from the Pahacel hemostat. P. aeruginosa and S. aureus microorganisms have been received from the microbiology laboratory of the Faculty of Medicine, Kahramanmaraş Sutcu Imam University. The media used for bacterial cultivation (sheep blood agars) have been received from Salubris A.Ş. In addition, the microbiology laboratory of Kahramanmaras Sutcu Imam University became used for the flame burner, tube spores, and bacterial germ cells used in the course of the research to decide antibacterial properties.

Methods

In the study, solutions to chitosan in several bureaucracies were first prepared and drawn via electrospinning, and nanofibers were received from PVA-supported chitosan. After obtaining nanofibers from chitosan, the acetic acid answer became become a prepared option to acquire the samples, and all parameters have been tried to be saved consistently for each sample.

For the education of biomaterials, one-of-a-type solutions had been prepared after which blended. The first solution weighs 10 g. As the solvent for the answer, 2% acetic acid became used. PVA and chitosan have been dissolved in a 2% acetic acid answer in a magnetic stirrer for twenty-4 hours at room temperature. Second, for 2 hours at room temperature, hemostatic collagen matrix and antibiotic tetracycline hydrochloride have been dissolved in saline (with the aid of using weight) in a magnetic stirrer. The solutions were blended. In all prepared solutions for nanofiber spinning, the total percentage of additives (PVA, chitosan, collagen, and tetracycline hydrochloride) withinside the solution is normally 10%. Furthermore, an normal saline answer of 3.5 g became used withinside the education of the second solution, and the number of solids withinside the solution became saved constantly. When making ready the physiological saline answer, the quantity of soluble salt and the NaCl content material of the answer have been taken into account, and 3.5 g of physiological saline answer have become

used after a few experiments. Finally, the samples were assigned codes for each prepared solution, which can be listed in Table 1 together with their respective ratios.

Table 1 Sample codes and rates

Biomaterial Code	Additive ratios in biomaterials		Collagen and Tetracycline Hydrochloride added to Chitosan	
	PVA (%)	Chitosan + Collagen + Tetracycline Hydrochloride (%)	Collagen (%)	Tetracycline hydrochloride (%)
T5	60	40	0	0
T10	60	40	10	10
T15	60	40	10	15
T20	60	40	10	20
T25	60	40	10	25
T30	60	40	10	30

Results

Scanning electron analysis results

During the tries to create a splendid nanofiber from chitosan, it turned into observed that the super ground turned acquired with the useful resource of PVA. The PVA-supported chitosan solution became a 2% acetic acid solution. The ground turned first fashioned from the chitosan-PVA answer thru electrospinning, after which the T manipulator code turned into indicated. Then, the 10% hemostatic collagen matrix and T10 sample with 10% tetracycline hydrochloride, T15 sample with 15% tetracycline hydrochloride, T20 sample with 20% tetracycline hydrochloride, T25 sample with 25% tetracycline hydrochloride, and T30 sample with 30% tetracycline hydrochloride have been done inside the prepared PVA-chitosan solution with the useful resource of the electrospinning method. SEMS Photographs had been taken to represent six surfaces, including a manipulator and 5 samples.

EM snapshots were acquired to symbolize the T-coded controlled ground prepared with the useful resource of the use of the electrospinning technique from a PVA-brought chitosan solution in 2% acetic acid (Figure 2). By studying the SEM pictures of the prepared T-coded controlled ground, it changed into decided that the morphological form had come to be easy and uniform, and non-forestall fiber formations have been placed at the ground.

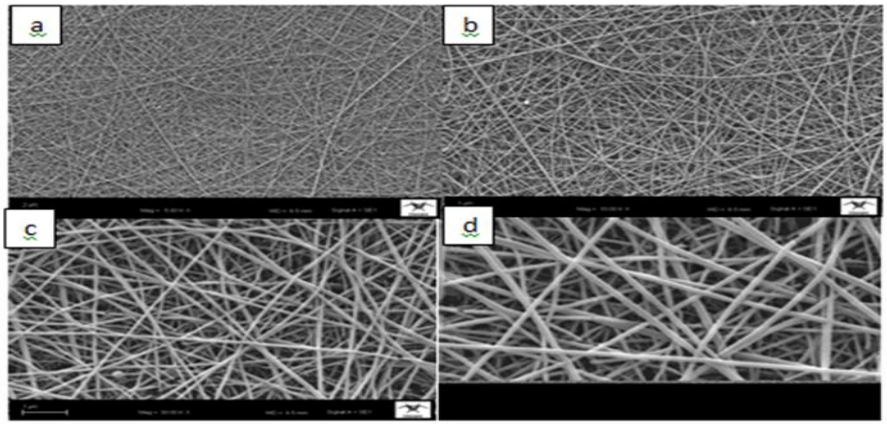

Fig 2 Sem İmages of T sample at different magnification ratios **a.** 5.00 kx magnification **b.** 10.00 kx Magnification **c.** 30.00 kx Magnification **d.** 50.00kx magnification

Due to the polycationic nature of chitosan, this is due to amine agencies in acidic solutions, it's miles more difficult to accumulate a fiber form with the useful resource of the electrospinning technique. The polycationic person of chitosan excessively will increase the ground tension of the solution and effects withinside the formation of droplets at the collection electrode all through the electrospinning process [15, 17, 18].

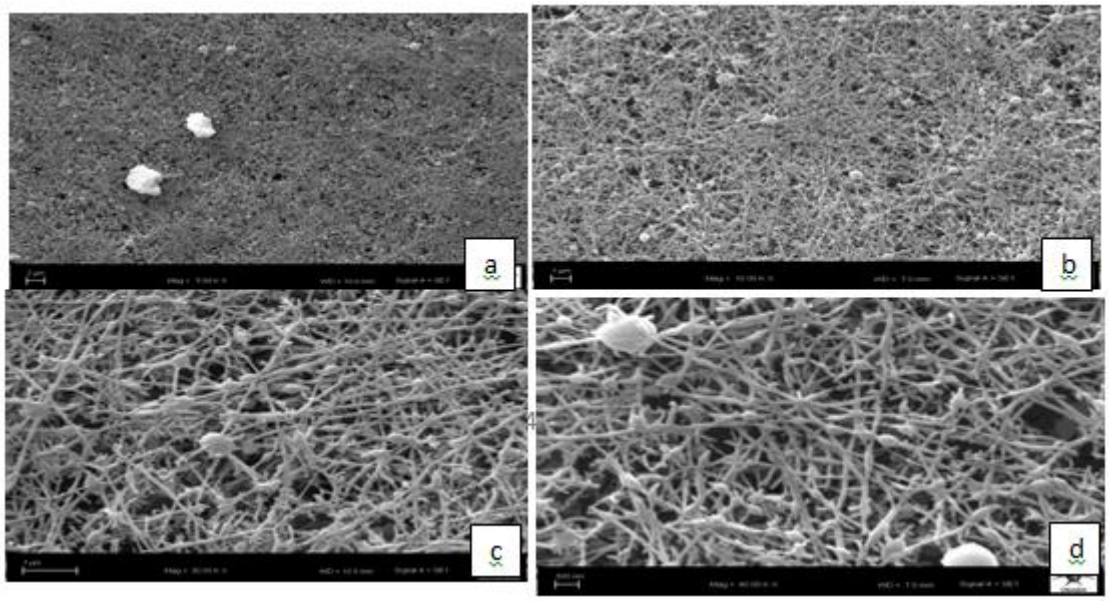

Fig 3 Sem images of t10 sample at different magnification ratios a. 5.00 kx magnification b. 10.00 kx magnification c. 30.00 kx magnification d. 40.00 kx magnification

SEM photos were acquired to symbolize the encoded T10 ground prepared thru electrospinning from a PVA-delivered chitosan solution in 2% acetic acid and a hemostatic collagen and tetracycline hydrochloride antibiotic solution in physiological saline (Figure 3). By analyzing

the SEM pictures of the prepared encoded T10 ground, it may be visible that the morphological form is not smooth. It can be seen that the fiber form is not formed. However, a beaded fiber form is formed, and similarly to the immoderate bead defects, the charged tablets make the fiber formation difficult.

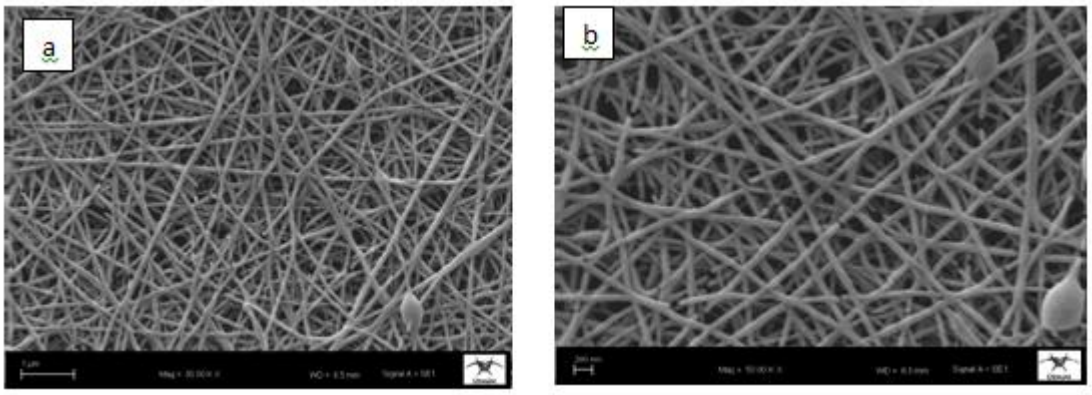

Fig 4 Sem images of t15 sample at different magnification ratios a. 30.00 kx magnification b. 50.00 kx magnification

SEM snapshots were received to symbolize the T15-encoded ground with a 15% tetracycline hydrochloride antibiotic (Figure 4). Examining the SEM snapshots of the produced encoded T15 ground, it may be visible that the morphological form is smoother than that of the T10 sample. It can be seen that the fiber form is formed, however, the pilling defect, which isn't uncommon in the electrospinning system, occurs due to the brought drugs, although very small.

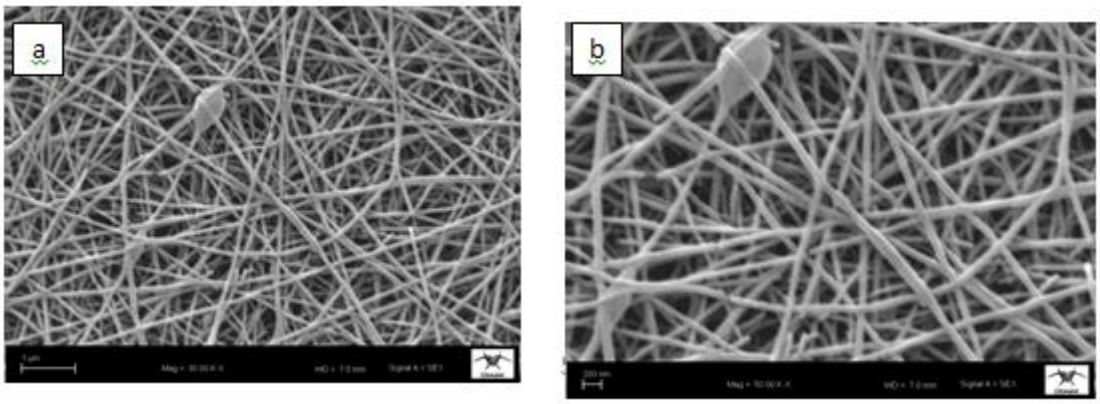

Fig 5 Sem images of t20 sample at different magnification ratios a. 30.00 kx magnification b. 40.00 kx magnification

SEM photographs were taken of the solution obtained via mixing PVA-introduced chitosan solution prepared in 2% acetic acid solvent with hemostatic collagen and tetracycline hydrochloride antibiotic solution prepared in physiological saline to indicate the T20-encoded

ground normal via the electrospinning method (Figure 5). By reading the SEM photos of the prepared T20 encoded ground, it's miles now seen that the morphological form is smoother than that of the T10 sample, but not smoother than that of the T15 sample. It can be seen that the fiber form is normal, however, the pilling defect, which is commonplace in the electrospinning system, takes location from region to region due to the introduced drugs.

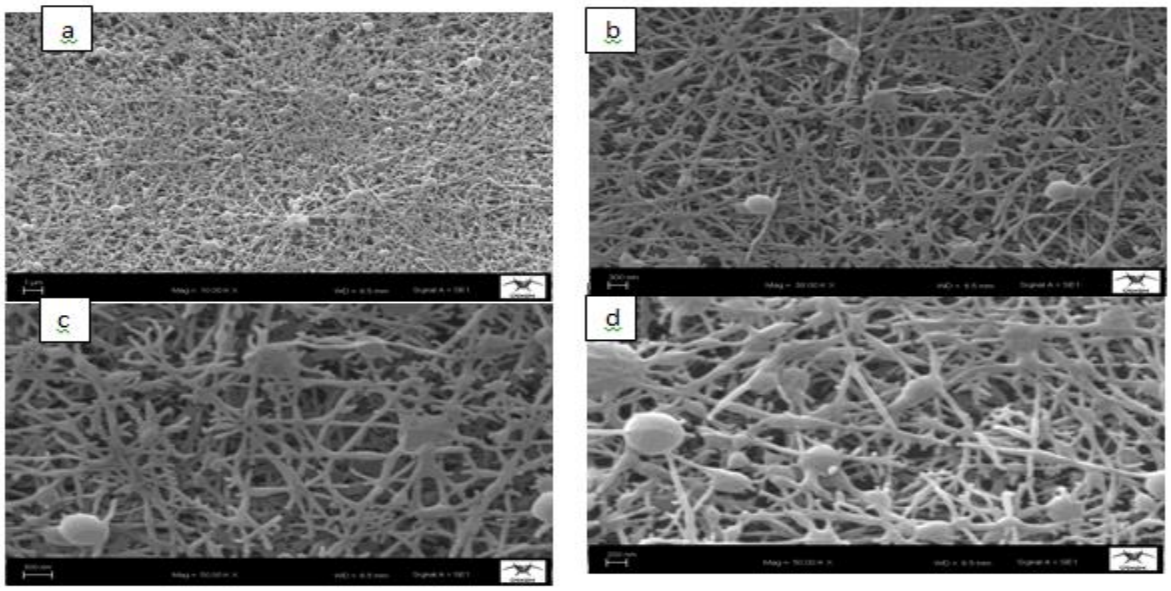

Fig 6 sem images of t25 sample at different magnification ratios a. 10.00 kx magnification b. 30.00 kx magnification c. 40.00 kx magnification d. 50.00 kx magnification

SEMS To constitute the encoded T25 surface, photos were acquired via way of means of using the electrospinning approach thru approach of mixing the PVA-added chitosan solution prepared in 2% acetic acid with hemostatic collagen and tetracycline hydrochloride antibiotic solutions prepared in saline (Figure 6). By examining the SEM photos of the prepared encoded T25 surface, it may be visible that the morphological form is not smooth. It can be seen that the fiber form is not formed; instead, the formation of reticular structures and bead defects is high, and the increase in the amount of charged pills complicates the fiber formation.

SEM pictures had been acquired to represent the encoded T30 ground prepared through a way of electrospinning from a PVA-delivered chitosan solution prepared in 2% acetic acid and a saline-prepared hemostatic collagen and tetracycline hydrochloride antibiotic solution (Figure 7).

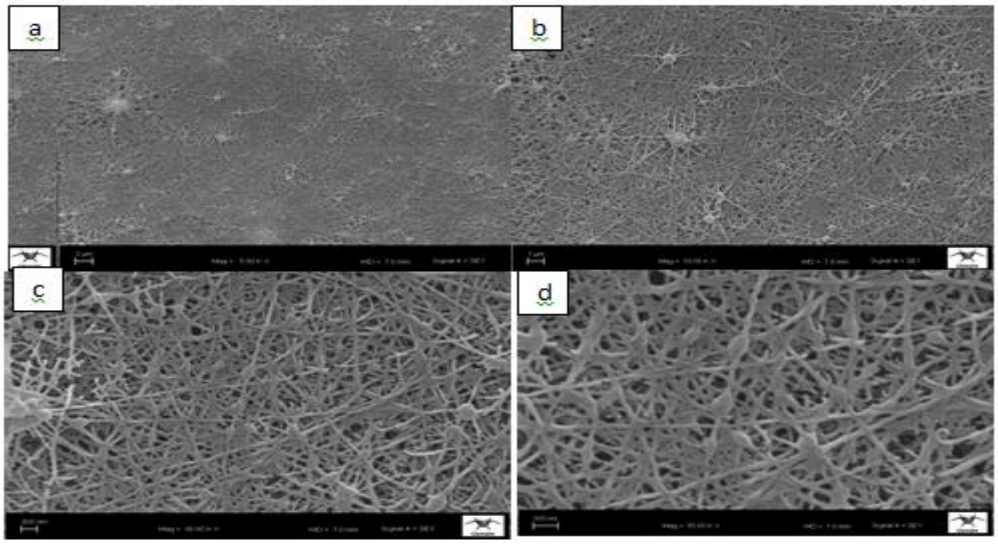

Fig 7 Sem images of t30 sample at different magnification ratios a. 50.00 kx magnification b. 10.00 kx magnification c. 30.00 kx magnification d. 50.00 kx magnification

By studying the SEM pictures of the prepared T30-encoded ground, it's miles viable to look that the morphological form isn't always smooth. It can be seen that the fiber form isn't always absolutely formed; instead, many net-like structures and bead defects are formed, and the charged pills make the fiber formation difficult. Table 2 shows the fiber and bead diameters are in keeping with the consequences of the assessment SEM.

Table 2. Fiber and bead diameters

Sample code	**Fiber diameter**		**Bead diameter**	
	Cover. Diameter (nm)	Standard deflection	Cover. Diameter (nm)	Standard deflection
T5	106,35	10,9172	None	None
T10	100,95	11,9361	217,20	79,4366
T15	74,4	12,5974	170,05	58,0816
T20	84,4	18,9999	234,00	99,7988
T25	94,05	19,0616	301,00	107,9645
T30	98,85	12,7455	182,00	79,8569

Colony count

Here, sheep blood agars are referred to as medium. The sheep blood agars are within the Petri dish. At the top of the incubation period, the samples are placed within the prepared tubes using sterile gloves, a flame burner, and a metal loop for colony counting in Petri dishes. It is based

on the principle that living cells form a colony, and with the resource of counting one's colonies, each living cell office work a colony, and the huge style of living cells in the fabric is calculated. For this purpose, a tremendous amount of the fabric to be remembered is taken and located into the lifestyle medium. The huge style of feasible cells in the fabric is calculated with the resource of counting the colonies in the Petri dish required for colony formation. In addition, consistent with the necessities of the microbiology laboratory, the problem is made consistent with the ground area of the Petri dish. For example, if the microorganisms have grown at the complete ground of the Petri dish, they will remember as 100 colonies. On the occasion that they've grown on half of the ground, they will remember as 50 colonies. On the occasion that they've grown in a ratio of 1 to 4, they will remember as 25 colonies. As for the productivity of the sample, a multiplication ratio of 1:4 approaches 25 colonies, and if the growth within the manipulating sample is 100, it approaches 75% of the microorganisms have been killed. However, to attain whole success, the multiplication rate must be between five and 10, i.e., 90–95% of the killed microorganisms. However, due to the fact vain cells cannot multiply and form colonies, best-stayed cells are counted in this method (Guerguen et al., 1990).

Results of bacteria cultivation in vitro

In this section, bacterial cultivation becomes become a success to investigate the effect of the biomaterial we prepared on Gramme-first-rate bacteria (S. aureus) and Gramme-bad bacteria (P. aeruginosa) inside the in vitro experiments we conducted [16, 19]. The information obtained from the seeding is given below. A manipulated planting become converted right into a one-of-a-type planting and photographed together.

As the result of planting the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline hydrochloride with S. Aureus bacteria solution with the useful resource of the usage of seek on the priority and manipulating agars at the 1st day of sowing, the give up result image of T10 Sample (Figure 8. a); even as 100 colonies had been customary on the pinnacle of factors cultivation, 90 colonies had been customary within the subject, and it changed into located that the biomaterial modified into effective at 10%. The give-up result of sowing the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline Hydrochloride with P. Aeruginosa microorganisms is: While 100 colonies had been shaped on the pinnacle of the priority, 100 colonies had been shaped within the subject, and it's miles now not effective.

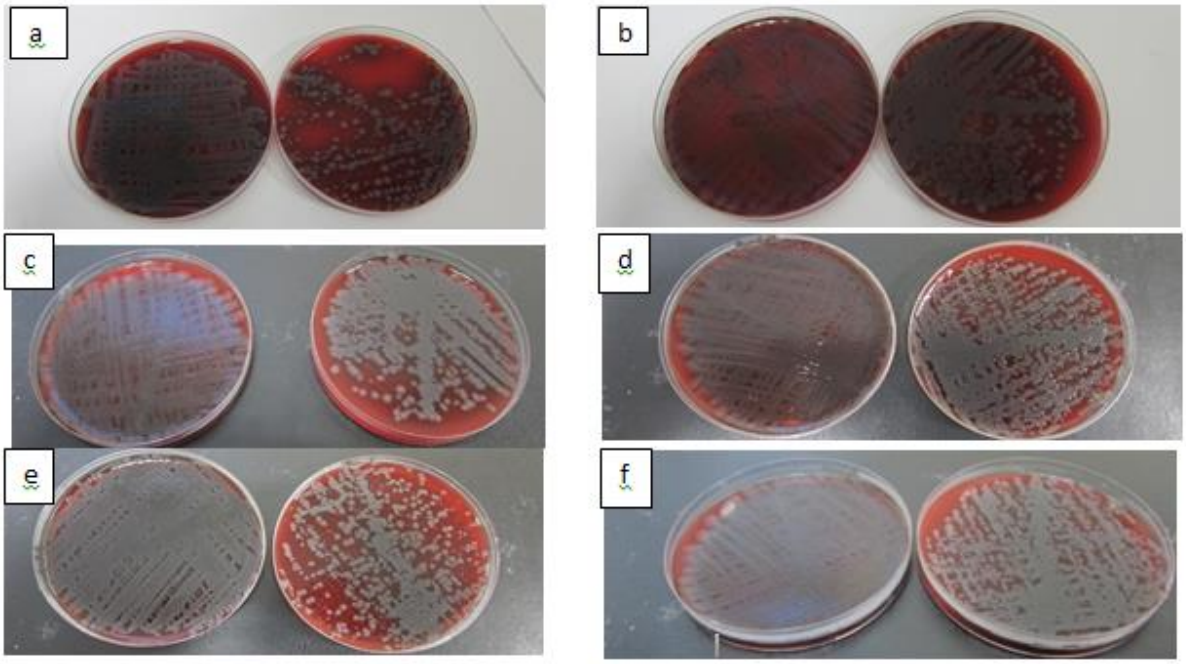

Fig 8 T10 sample **a.** image of control and subject (s. aureus) after cultivation 1st-day **b**. image of control and subject (p.aeruginosa) occurred after cultivation 1st day **c.** image of control and subject (s. aureus) occurred after cultivation 3rd-day **d.** image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day **e.** control and subject images (s. aureus) occurred after planting on day 5 **f.** image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

Planting the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline Hydrochloride with S. Aureus bacteria solution usage of looking at the trouble and manipulating agars led to 90 colonies being shaped inside the trouble, indicating that the biomaterial had very little electricity at 10%. As the result of planting the biomaterial containing 80% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with P.Aeruginosa bacteria solution with the useful resource of the usage of looking on the priority and manipulating agars at the 0.33 day of sowing, the give up result image of T10 Sample (Figure 8.d) suggests that even as 100 colonies had been customary inside the manipulative cultivation, 100 colonies had been customary inside the subject and it's miles now seen that the biomaterial has modified into now not effective. With the useful resource of the usage of seek on the priority and manipulation agars, they give up the result of sowing the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline hydrochloride with S. Aureus bacteria changed into While 100 colonies had been customary inside the control cultivation, 100 colonies had been customary inside the subject, and it's miles now seen that the biomaterial is now not effective. As the result of planting the biomaterial containing 80% chitosan, 10% collagen, and 10% Tetracycline

hydrochloride with P. Aeruginosa bacteria solution with the useful resource of the usage of looking on the priority and manipulating agars on the 5th day of sowing, give up result image of T10 Sample (Figure 8. f); While 100 colonies had been customary inside the manipulative cultivation, 100 colonies had been customary inside the subject, and it's miles now seen that the biomaterial is now not effective.

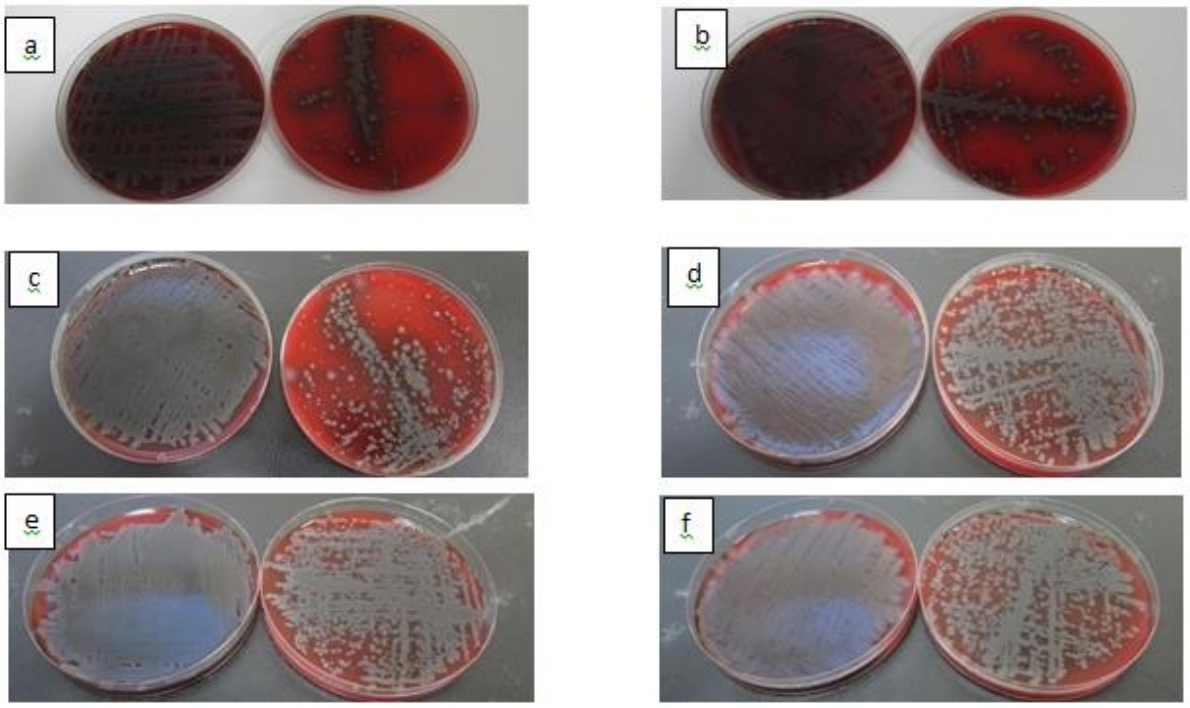

Fig 9 T15 sample **a**. image of control and subject (s. aureus) after cultivation 1st-day **b**. image of control and subject (p.aeruginosa) occurred after cultivation 1st day **c**. image of control and subject (s. aureus) occurred after cultivation 3rd-day **d.** image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day **e.** control and subject images (s. aureus) occurred after planting on day 5 **f.** image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

Sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with S. Aureus bacteria solution thru way of looking at the issue and handling agars on the primary day sowing quit result picture graph of T15 Sample (Figure 9. a), 100 colonies have been shaped in the viable planting, 40 colonies have been shaped inside the issue, and it changed into determined that the biomaterial has become effective. In the picture graph of T15 Sample 1st day sowing quit result (Figure 9. b), thru way of looking at the issue and handling agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with P. Aeruginosa bacteria changed into; While

100 colonies were normal in the viable planting, 60 colonies were normal inside the issue, and it changed into located that the biomaterial has become 40% effective. The 0.33 day of sowing quit result picture graph of T15 Sample (Figure 9. c), thru way of looking at the issue and handling agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with S. Aureus bacteria solution: While 100 colonies were normal in the viable planting, 80 colonies were normal inside the issue, and it has become seen that the biomaterial had emerged as very little effective at 20%. In the picture graph of the T15 Sample 0.33 day sowing quit result (Figure 9.d), thru way of looking at the issue and handling agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with P. Aeruginosa bacteria changed into; While 100 colonies were normal in the manipulate cultivation, 100 colonies were normal inside the issue, and it has become seen that the biomaterial had now emerged as now not effective. The T15 Sample, on the 5th day of sowing, quit result picture graph (Figure 9. e), thru way of looking on the issue and handling agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with S. Aureus bacteria solution: While 100 colonies were normal in the manipulate cultivation, 100 colonies were normal inside the issue, and it has become seen that the biomaterial had now emerged as now not effective. T15 Sample 5th day sowing quit result picture graph (Figure 9. f), looking at the issue and manipulate agars, the quit result of sowing the biomaterial containing 75% chitosan, 10% collagen, and 15% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution; While 100 colonies were normal withinside the manipulate planting, 100 colonies were normal withinside the issue and it emerges as seen that the biomaterial emerges as now not effective.

As a result of the cultivation of the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with S. Aureus bacteria solution through looking at the hassle and handling agars on a primary day, sowing cease result image of T20 Sample (Figure 10. a), whilst 100 colonies have been shaped inside the controlled planting, 60 colonies have been shaped inside the hassle, and it changed into observed that the biomaterial has become 40% effective. As the result of sowing the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution through the method of trying to find the hassle and handling agars inside the 1st-day sowing cease result image of T20 Sample (Figure 10. b), whilst 100 colonies were formed inside the controlled planting, 90

colonies were formed inside the hassle and it has come to be observed that the biomaterial turns into effective at 10%.

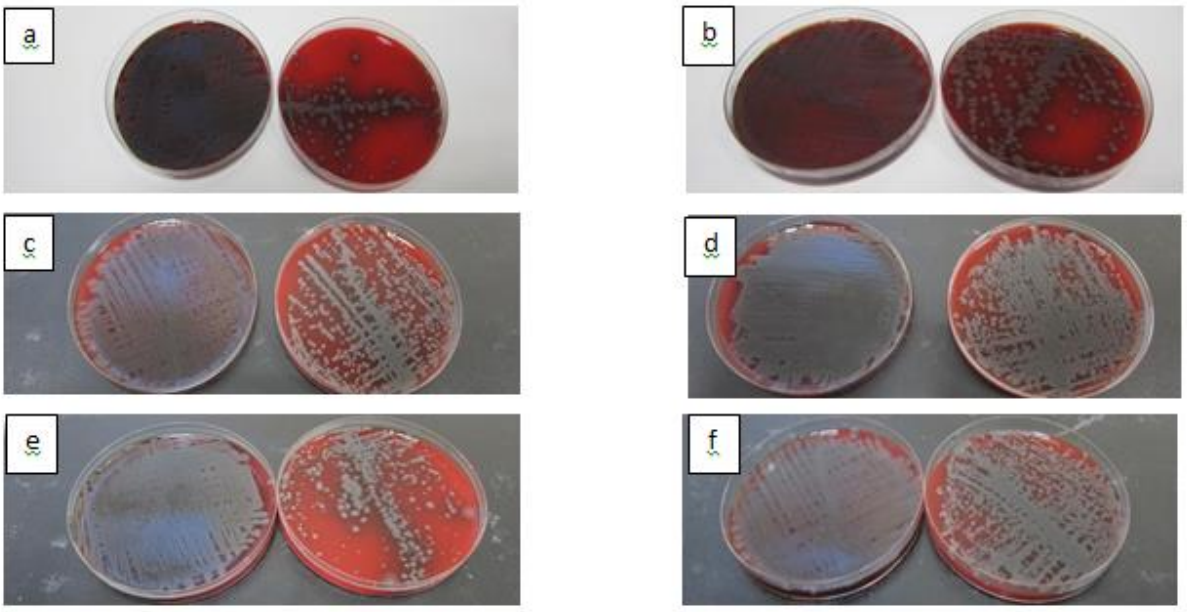

Fig 10 T20 sample **a.** image of control and subject (s. aureus) after cultivation 1st-day **b.** image of control and subject (p.aeruginosa) occurred after cultivation 1st day **c.** image of control and subject (s. aureus) occurred after cultivation 3rd-day **d.** image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day **e**. control and subject images (s. aureus) occurred after planting on day 5 **f.** image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

In the T20 Sample 0.33 day sowing cease result image (Figure 10. c), through the method of trying to find the hassle and handling agars, the cease result of sowing the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with S. Aureus microorganisms is: While 100 colonies were formed inside the controlled planting, 80 colonies were formed inside the hassle, and it has come to be seen that the biomaterial turns into very little strength at 20%. In the T20 Sample 0.33 day sowing cease result image (Figure 10.d), through the method of trying to find the hassle and handling agars, the cease result of sowing the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with P. Aeruginosa microorganisms is: While 100 colonies were formed inside the controlled cultivation, 100 colonies were formed inside the hassle, and it has come to be seen that the biomaterial has come to be now not effective Eighty colonies have been shaped inside the hassle due to the cultivation of the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with S. Aureus bacteria solution, through the method of looking at the hassle and handling agars at the 5th day of sowing, and it changed into observed that the

biomaterial turns into effective at 20%. In the image of the T20 Sample 5th day sowing cease result (Figure 10. f), through the method of trying to find the hassle and handling agars, the cease result of sowing the biomaterial containing 70% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution, whilst 100 colonies were formed inside the controlled cultivation, 90 colonies were formed inside the hassle, and it's miles now seen that the biomaterial has come to be now not effective.

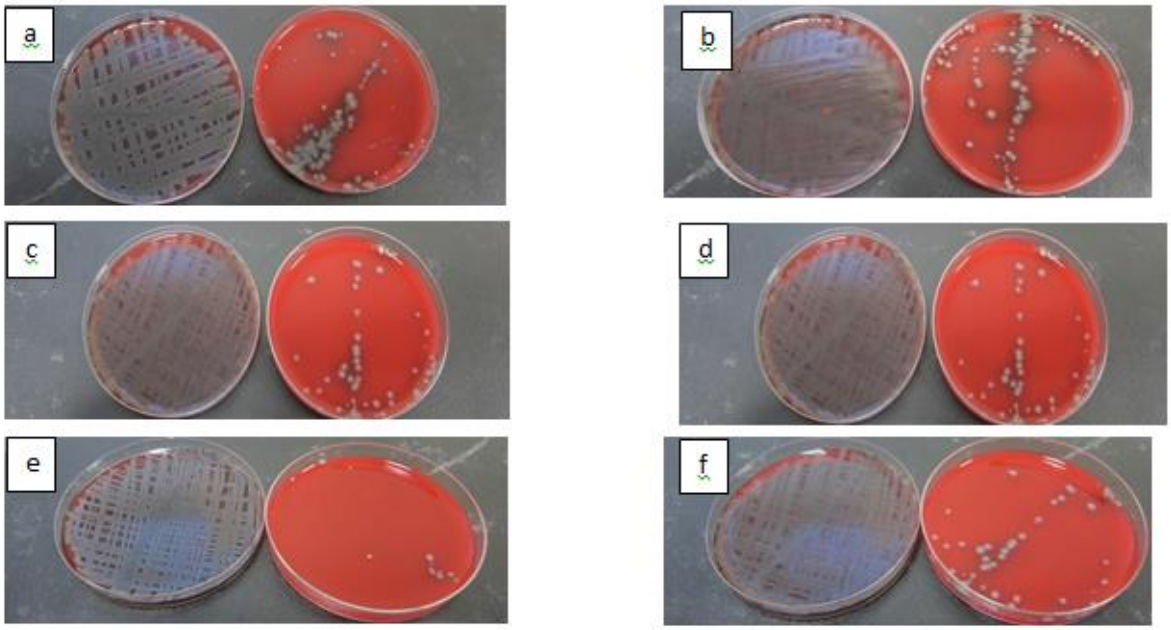

Fig 11 T25 sample **a**. image of control and subject (s. aureus) after cultivation 1st-day **b.** image of control and subject (p.aeruginosa) occurred after cultivation 1st day **c.** image of control and subject (s. aureus) occurred after cultivation 3rd-day **d**. image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day **e.** control and subject images (s. aureus) occurred after planting on day 5 **f.** image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

The final results of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with S. Aureus bacteria turned into as follows: even as one hundred colonies have been fashioned withinside the manage planting, 30 colonies have been fashioned withinside the venture, and it turned into determined that the biomaterial turned into 70% effective. With the useful resource of the usage of seek at the venture and manipulating agars, they give up the result of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with P. Aeruginosa bacteria turned into one hundred colonies have been formed withinside the manipulating planting, 30 colonies have been formed withinside the venture, and it turned into positioned that the biomaterial modified into 70% effective. As the result of sowing the biomaterial containing 65% chitosan, 10% collagen, and

25% Tetracycline Hydrochloride with S. Aureus bacteria solution with the useful resource of the usage of looking at the venture and manipulating agars at the 0.33 day of sowing, the give up result image of the T25 Sample (Figure 11. c) confirmed that even as one hundred colonies have been formed withinside the manipulating planting, 10 colonies have been formed withinside the venture, and it turned into positioned that the biomaterial has become 90 %effective. The T25 Sample on the 0.33 day of sowing give up the resulting image (Figure 11.d), looking at the venture and manipulating agars, they give up the result of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution; While one hundred colonies have been fashioned withinside the manipulating planting, 25 colonies have been fashioned withinside the venture, and it turned into determined that the biomaterial has become 75% effect. As a result of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with S. Aureus bacteria solution, with the useful resource of the usage of looking at the venture and manipulating agars at the 5th day of sowing, the give up result image of the T25 Sample (Figure 11. e) confirmed that even as one hundred colonies have been formed withinside the manipulating planting, 10 colonies have been formed withinside the venture and it turned into positioned that the biomaterial modified into 90% effective. The 5th day of sowing ended with an image (Figure 11. f) attempting to find the venture and manipulating agars. The give-up result of sowing the biomaterial containing 65% chitosan, 10% collagen, and 25% Tetracycline Hydrochloride with P. Aeruginosa bacteria turned into While one hundred colonies have been formed withinside the manage planting, 20 colonies have been formed withinside the venture, and it turned into positioned that the biomaterial modified into 80% effective.

The cultivation of the biomaterial containing 60% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with S. Aureus bacteria solution through a way of looking on the priority and handling agars inside the first-day sowing gives up result photograph of T30 Sample (Figure 12. a), at the same time as 100 colonies had been shaped inside the control planting, 10 colonies had been shaped inside the concern, and it changed into observed that the biomaterial changed into 90% efficient.

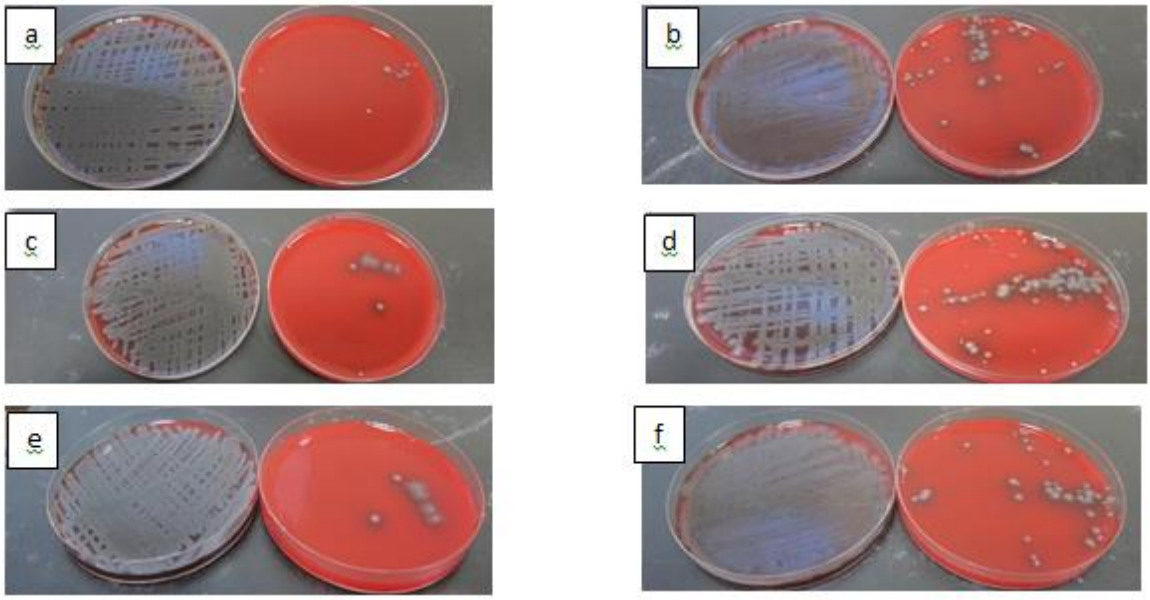

Fig 12 T25 sample a. image of control and subject (s. aureus) after cultivation 1st-day b. image of control and subject (p.aeruginosa) occurred after cultivation 1st day c. image of control and subject (s. aureus) occurred after cultivation 3rd-day d. image of control and subject (p.aeruginosa) occurred as a result of cultivation 3rd-day e. control and subject images (s. aureus) occurred after planting on day 5 f. image of control and subject (p.aeruginosa) occurred as a result of the cultivation 5th day

In the photograph of the T30 Sample 1st day sowing give-up result (Figure 12. b), through the way of looking at the priority and handling agars, the give-up result of sowing the biomaterial containing 60% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with P. Aeruginosa bacteria changed into; While 100 colonies were formed in the controlled planting, 25 colonies were formed in the concern, and it changed into positioned that the biomaterial modified into 75% effective. After sowing the biomaterial containing 60% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with S. Aureus bacteria solution at the 1/3 day of sowing, the give-up result photograph of the T30 Sample (Figure 12. c) found out that at the same time as 100 colonies had been shaped inside the achievable planting, ten colonies had been shaped inside the concern, and it changed into observed that the biomaterial modified into 90% effective. The 1/3 day sowing give-up result photograph of the T30 Sample (Figure 12.d), acquired by looking at the priority and handling agars, the give-up result of sowing the biomaterial containing 60% chitosan, 10% collagen, and 30% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution; at the same time as 100 colonies had been shaped inside the manipulate planting, 30 colonies had been shaped inside the concern, and it changed into observed that the biomaterial has become 70% effective. The cultivation of the biomaterial

containing 60% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with S. Aureus bacteria solution, through a way of looking at the priority and handling agars at the 5th day of sowing, found out that at the same time as 100 colonies had been shaped inside the control planting, ten colonies had been shaped inside the concern, and it changed into observed that the biomaterial In the photograph of the T30 Sample 5th day sowing give up the result (Figure 12. f), through the way of looking on the priority and control agars, they give up the result of sowing the biomaterial containing 60% chitosan, 10% collagen, and 20% Tetracycline Hydrochloride with P. Aeruginosa bacteria solution, at the same time as 100 colonies were formed inside the control planting, 25 colonies were formed inside the concern, and it changed into positioned that the biomaterial modified into 75% effective.

The efficacy chances obtained due to sowing on S. Aureus (Gram +) and P. Aeruginosa (Gram-) microorganisms of entire of 6 particular biomaterials produced together with the manipulating sample at the 1st day, 0.33 day, and 5th day, respectively, are given in Table three below.

In addition, the overall performance values obtained due to the primary day, 1/3 day, and 5th-day sowing of the produced biomaterials on S. Aureus (Gram +) and P. Aeruginosa (Gram-) microorganisms had been graphically drawn and people graphs had been established in Figure thirteen and Figure 14, respectively.

Table 3. Effective percentage of bacteria on biomaterial

Activity rates against S. Aureus (Gram +) bacteria (%)				**Activity rates against P. aeruginosa (Gram -) bacteria (%)**			
Sample Code	**First-day cultivation**	**Third-day cultivation**	**Fifth-day cultivation**	**Sample Code**	**First-day cultivation**	**Third-day cultivation**	**Fifth-day cultivation**
T5	0	0	0	T5	0	0	0
T10	10	10	0	T10	0	0	0
T15	60	20	0	T15	40	0	0
T20	40	20	20	T20	10	0	0
T25	70	90	90	T25	70	75	80
T30	90	90	90	T30	75	70	75

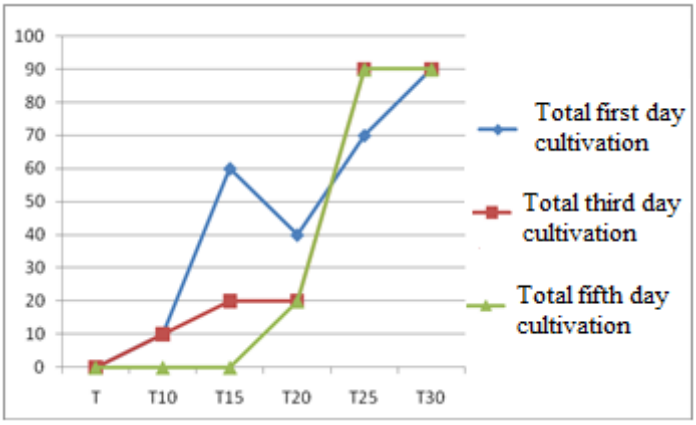

Fig 13 Efficacy of s. aureus (gram+) bacteria on biomaterial

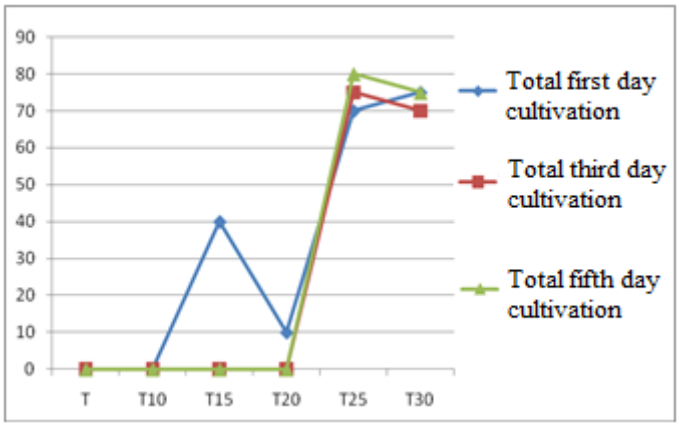

Fig 14 Efficacy of P. aeruginosa (Gram -) bacteria on biomaterial

Conclusion and Discussion

Tissue ground has become to be had thru the use of a mixture of polyvinyl alcohol (PVA) help polymer and Chitosan. Through the electrospinning technique, nanofiber biomaterials were obtained together with Collagen Hemostatic Matrix and Tetracycline hydrochloride antibiotic at precise rates into this biomaterial. To study the form of the obtained biomaterials, SEM analyses were performed and the results were visible. To study the overall performance of the biomaterial in vitro, the colonies common thru culturing microorganisms were counted.

To use the prepared samples as biomaterials, in vitro exams had been performed in the laboratory environment. The effectiveness of the prepared solutions on grammatical excessive pleasant and bad microorganisms changed into an investigation. Due to sowing on sheep blood agar with 0.5 McFarland bacterial solutions prepared with physiological saline, it changed into discovered that it has become effective at particular rates on gram excessive pleasant (S. aureus)

and gram bad microorganisms (P. aeruginosa). Biomaterials had been located to be extra effective in competition to gram-excessive pleasant (S. aureus) microorganisms than gram-bad (P. aeruginosa) microorganisms.

At the identical time, T15 and T20 coded samples had been slightly powerful towards gram excessive quality (S. Aureus) microorganisms, however enough has now not been found.

In particular, T25 and T30 coded samples with an immoderate tetracycline hydrochloride ratio are proven to be quite effective in competition with gram-excessive quality (S. aureus) microorganisms. However, the samples are not as effective as gram-excessive quality (S. Aureus) microorganisms in competition with gram-poor (P. aeruginosa) microorganisms. In addition, it become determined that the T25 coded sample has become extensively effective in competition to gram-excessive quality (S. Aureus) microorganisms, and there was no growth in charge of 90%, in particular on the 0.33 and 5th day of sowing. It become determined that the T30 coded sample has become extensively effective in competition to gram-excessive quality (S. Aureus) microorganisms and there was no growth in charge of 90%, in particular on the 1st day, 0.33 day, and 5th day of sowing.

It's been observed that the parabolic-delivered biomaterial is strong in competition to bacterial boom in some conditions, however, cannot prevent bacterial boom. However, T25 and T30 biomaterials had been found to have an inhibitory effect. In addition, at the identical time, because the biomaterial produced in Arkan's examination can be used for Band-Aids or open wounds, the biomaterials produced in this examination are biomaterials that can be used for each open wound and closed wounds.

In the continuation of this study, it's miles greater to bear in mind to deliver a biomaterial from honestly acquired biomaterials to relieve the affected man or woman for the duration of surgical remedy and provide comfortable use for surgeons. These developments, especially inside the health area and bio textile, provide essential opportunities to carry out that research.

As a result of the cultivation of gram-excessive quality (S. Aureus) and gram-poor (P. Aeruginosa) microorganisms via in-vitro tests, it changed into located that the nice biomaterial has become effective in competition with gram-excessive quality (S. Aureus) microorganisms of T25 and T30 samples. In the studies, it changed located that T25 biomaterial supplied 90% success in the competition to a boom at the 1/3 and 5th day of bacterial sowing. In addition, studies have verified that T30 biomaterial gives 90% success in the competition to a boom in 1st day, 1/3 day, and 5th-day bacterial sowing.

As a result, the T25 sample accomplished success at the 1/3 day and showed that it is a biomaterial that can be applied in open or closed wounds and not used a danger of infection. It is the concept that nanofiber structures are suitable and can be used as a superb nano and biodegradable biomaterial that can be used as a possibility in several fields of study.

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Data Availability statement
The authors confirm that data supporting the findings of this study are not available in the article.

Compliance with ethical standards

Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards

Authors' contributions
İbrahim ALTUN conceived and designed the study. All authors performed the experiments and contributed to the preparation of the manuscript.

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Araştırma makalesi /Research article

Akan, H., ve M.M. Balos, Karaköprü (Şanlıurfa)'da Gıda Olarak Tüketilen Yabani Bitkiler Üzerinde Etnobotanik Bir Araştırma. International Journal of Life Sciences and Biotechnology, 2023. 6(1): p. 61-81. DOI: 10.38001/ijlsb.1246913

Karaköprü (Şanlıurfa)'da Gıda Olarak Tüketilen Yabani Bitkiler Üzerinde Etnobotanik Bir Araştırma

Hasan Akan [1]*, Mehmet Maruf Balos[2]

ÖZET

Bu çalışmada, Şanlıurfa merkez ilçesi olan Karaköprü'de yaşayan yöre halkının, gıda olarak tükettiği yabani bitkiler belirlenmiş ve kullanım biçimleri tespit edilmiştir. Bu bitkiler, özellikle ilkbahar aylarında kırsal kesimde yaşayan insanlar tarafından toplanarak semt pazarlarına getirilmektedir. Bu bitkilerin bir kısmı çiğ veya pişirilerek taze tüketildiği gibi, bir kısmı dondurularak, kurutularak, salamura edilerek veya konserveye işlenerek de tüketilebilmektedir. Bu çalışmada, 50 kaynak kişi ile birebir görüşmeler yapılmış, okullardaki öğrencilerden de destek alınmış ve Etnobotanik bilgi formları düzenlenmiştir. Toplamda 28 farklı familyaya ait 57 yabani bitkinin gıda amaçlı olarak kullanıldığı tespit edilmiştir. Bu bitkilerden *Bellevalia pseudolongipes* türü endemiktir. Çalışmanın amacı, halkın yabani bitkilerden yararlanma biçimlerini araştırmak ve etnobotanik alanına katkı sağlamaktır.

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Geliş
2 Şubat 2023
Kabul
13 Mart 2023

ANAHTAR KELİMELER
Etnobotanik,
gıda bitkileri,
yabani bitkiler,
yenen otlar,
Şanlıurfa

An Ethnobotanical Investigation on Wild Edible Plant of Karaköprü (Şanlıurfa)

ABSTRACT

In this study, the wild plants consumed as food by the local people living in the center town, Karaköprü of Şanlıurfa were determined and their usage patterns were determined. These plants are collected by the people living in the countryside, especially in spring, and brought to the Daily local markets. Some of these plants can be consumed freshly by cooking raw or cooked, while others can be consumed by freezing, drying, pickling or canned. In this study, one-on-one interviews were held with 50 source people, support was received from students in schools and Ethnobotanical information forms were arranged. It was determined that 57 wild plants belonging to 28 different families were used for food purposes. Among these plants, *Bellevalia pseudolongipes* species is endemic. The aim of the studa is to investigate the ways people use wild plants in Karaköprü and contribute to ethnobotany.

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[1] Harran Üniversitesi, Fen Edebiyat Fakültesi, Biyoloji Bölümü, Şanlıurfa, Türkiye
[2] Karaköprü İlçe Milli Eğitim Müdürlüğü, Fatma Zehra Kız Anadolu İmam Hatip Lisesi, Şanlıurfa,Türkiye
*Sorumlu Yazar: Hasan Akan, E-Posta: hasanakan@gmail.com

Giriş

Anadolu toprakları pek çok medeniyete ev sahipliği yapmış ve zengin bir geleneksel bilgi mirası taşımaktadır [1]. Anadolu'da birçok yerel bitki asırlardır gıda için kullanılmaktadır. Bu açıdan, Anadolu'da bitkilerin geleneksel kullanımları özgün değer taşımaktadır. Bu bilgilerin gelecek nesillere aktarılabilmesi önem arz etmektedir [2]. Özellikle, son yıllarda doğal ve organik gıdalara karşı tüketicinin artan talebi bu bitkilere ilgiyi artırmıştır [3, 4].

Yenilebilir yabani bitkiler Anadolu'da yaklaşık 50.000 yıldır kullanılmaktadır. Mezopotamya, Eski Mısır, Hitit, Yunan, Roma, Selçuklu ve Osmanlı gibi uygarlıklar bu yabani bitkilerden birçok amaç için yararlanmış ve kendi beslenme kültürlerine dahil etmişlerdir [3].

Yabani bitkilerin kültür bitkilerine göre mineral bakımından daha zengin olduğu bilinmektedir (5). Yenebilir yabani bitkiler esansiyel yağlarca zengin olup kültür bitkilerinden daha yüksek miktarda antioksidan vitaminler içermektedir [6]. Ayrıca önemli miktarda kalsiyum, fosfor ve demir gibi mineralleri de içermektedirler [6, 7]. Gıda olarak kullanılan bu bitkiler, çiğ veya pişmiş olarak yenildiği gibi kurutularak, salamura halinde veya turşu şeklinde de tüketilmektedir [8]. Dünya genelinde gıda olarak kullanılan yabani bitki türlerinin ise 10.000'nin üzerinde olduğu rapor edilmiştir [9,10,11]. Anadolu'da gıda, çay, atıştırmalık, koku ve tat vericilerle beraber 1200 civarında doğal bitkiden gıda olarak yararlanılır [12].

Yapılan literatür taramasında gıda olarak tüketilen yabani bitkiler ile ilgili farklı bölge ve illerden birçok çalışmanın olduğu görülmüştür. Bu çalışmalar; Kayseri-Vatan köyünde yenen bazı yabani bitkiler [13], Tekirdağ ili halk ilaçları ve gıda olarak kullanılan yabani bitkiler [14], Balıkesir yöresinde yenen yabani meyveler ve etnobotanik özellikleri [15], Muğla-Bodrum yöresinin yenen yabani bitkileri [16], Batı ve Orta Anadolu'da (Türkiye) yabani yenilebilir bitkilerin kullanımı [5], Türkiye'nin Karadeniz Bölgesinde yenilebilir yabani bitkilerin insan beslenmesine katkısı [17], Ege Bölgesinde sebze olarak kullanılan yabani bitki türleri ve kullanım amaçları [18], Malatya-Akçadağ-Kürecik'te yabani yenilebilir bitkilerin kullanımı [19], Türkiye'nin yabani besin bitkileri ve ot yemekleri [20], Eskişehir ili Mihalıççık ilçesinde gıda olarak tüketilen yabani bitkiler ve bu bitkilerin tüketim biçimleri

[21]. Afyonkarahisar'da gıda olarak tüketilen yabani bitkiler ve tüketim biçimleri [22], İzmit'in yararlı ve yenilebilir bitkileri üzerine etnobotanik bir çalışma [23], Karaman'da yenilebilen yabani bitkiler [24], İzmir yerel pazarlarında satılan yabani yenilebilir bitkiler [25], Hakkari-Geçitli'de insan tüketimi amaçlı yabani gıda tesislerinin araştırılması [26], Elazığ ili insan tüketimi amaçlı yabani gıda bitkilerinin araştırılması [27], Marmara Adası'nın (Balıkesir-Türkiye) tıbbi ve yabani gıda bitkileri [28], Türkiye'den Meriç kasabasında kullanılan gıda bitkileri [29], Ege Bölgesi'nde yenilebilir yabani bitki türleri [30], Kastamonu-Tosya ilçesinin bazı yabani besin bitkileri [31], Bingöl'de insan tüketimi amaçlı yabani gıda bitkilerinin araştırması [32], Adana Karaisalı'da insan tüketimine yönelik yabani gıda araştırması [33], Bingöl-Karlıova'da yabani bitkilerin geleneksel gıda kullanımları [34], Balıkesir-Manyas'ta yenen yabani bitkiler ve bunların insan beslenmesinde geleneksel kullanımları [2], Batman ili ve Kozluk ilçesinin tıbbi ve yabani gıda bitkileri [35], Ağrı ilinde kullanılan yabani yenilebilir bitki türleri [4], Bitlis-Hizan'da insan tüketimi amaçlı yabani yenilebilir bitkiler üzerinde bir araştırma [36], Iğdır ilinin (Doğu Anadolu, Türkiye) yenilebilir yabani bitkileri hakkında geleneksel bilgiler [37], Çanakkae-Biga'da Yabani yenilebilir bitkilerin geleneksel bilgisi [38], Erzurum-Uzundere'de yenilebilir yabani bitkilerin gastronomik açıdan değerlendirilmesi [39], Mersin'in yenilebilir yabani bitkileri ve gastronomide kullanım şekilleri [40], Kahramanmaraş ili kentsel açık yeşil alanlardaki yenilebilir bitkilerin değerlendirilmesi [41], Ankara Gölbaşı'nda yabani bitkilerin kullanılış amaçları ve şekilleri üzerinde bir araştırma [42], Yalova ilinde yenen yabani ve çeşitli faydalı bitkiler [43], Antakya semt pazarlarındaki bazı doğal bitkilerin etnobotanik yönden araştırılması [44], Türkiye yenilebilir Apiaceae bitkiler, üzerine bir inceleme [45], Bingöl yerel pazarlarında satılan yabani bitkilerin etnobotanik yönden incelenmesi [46], Kelkit (Gümüşhane) ilçesinde doğal gıda bitkilerinin geleneksel kullanımları [47], Kahramanmaraş il merkezi semt pazarlarında satılan bitkiler hakkında etnobotanik araştırmalar [48], Türkiye'de Nevşehir'in güneyindeki yabani yenilebilir bitkiler [49] ve Gazipaşa (Antalya) ilçe pazarında satılan ve halk tarafından kullanılan bazı bitkiler ve kullanım amaçları [50] isimli çalışmalardır.

Güneydoğu Anadolu Bölgesi'nde yapılan çalışmaların çoğu kapsamlı etnobotanik çalışmalardır. Doğrudan gıda bitkilerin kullanımı üzerinde bazı çalışmalara rastlanmıştır.

Bunlar; Mardin-Yeşilli'de yenilebilir yabani bitkiler [51], Batman-Hasankeyf'te yabani yenilebilir bitkiler [52] ve Mardin ili'nin geleneksel yemeklerine katkıda bulunan yenilebilir yabani bitkiler [53] çalışmalarıdır.

Güneydoğu Anadolu Bölgesi'nde yapılan diğer etnobotanik çalışmalar; Kapari cinsinin GAP bölgesindeki işlenmesi, ihracatı ve son populasyon durumu [54], Şanlıurfa kent merkezindeki semt pazarlarında satılan bazı bitkiler ve kullanım amaçları [55], Şanlıurfa, Birecik ilçesi, Zeytinbahçe-Akarçay arasında kalan bölgenin etnobotanik özellikleri [56], Arat Dağı ve çevresinde (Birecik, Şanlıurfa) etnobotanik bir araştırma [57], GAP Bölgesi'nden toplanan meyan kökü taksonunun ihracat durumu, etnobotanik özellikleri ve tıbbi önemi [58], Şanlıurfa'da bazı odunsu bitkilerin etnobotaniği üzerine bir araştırma [59], Şanlıurfa'da el yapımı müzik aletleri üzerine etnobotanik bir araştırma [60], Birecik (Şanlıurfa) yöresindeki bazı baklagil bitkilerin etnobotanik özellikleri [61], Şanlıurfa, Kalecik Dağı ve çevresinde etnobotanik bir araştırma [62], Şanlıurfa'da tıbbi amaçlı kullanılan bitkiler [63], Kâhta (Adıyaman) merkezi ve Narince Köyü'nün etnobotanik açıdan araştırılması [64], Mardin, Savur yöresinde folklorik tıpta kullanılan Asteraceae familyasına ait bazı önemli bitkiler ve uygulama alanları [65], Tunceli ili Pertek ilçesinin yabani yenilebilir bitkileri [66], Şanlıurfa, Gölpınar mesire yeri florası ve etrafındaki köylerin etnobotanik özellikleri [67], Mardin, Savur'da halk hekimliğinde kullanılan Lamiaceae familyasına ait önemli bazı bitkiler ve kullanım alanları [68], Kültürlerin buluştuğu ipek yolu üzerinde bir şehir olan Midyat'ta (Türkiye) etnobotanik bir çalışma [69], Şanlıurfa, Tek Tek Dağları Milli Parkı eteklerindeki bazı köylerde etnobotanik bir çalışma [70], Şanlıurfa merkez ilçesi ve köylerinde etnobotanik bir araştırma [71], Mardin, Artuklu'da şifalı bitkilerin geleneksel kullanım alanları [72], Yaslıca beldesi ve Arıkök mahallesi (Şanlıurfa)'nin etnobotanik açıdan araştırılması [73], Mardin'in kırsal köylerindeki yerli halkın kullandıkları bazı bitkilerin etnobotanik açıdan değerlendirilmesi [74], Mardin merkez ilçe ve bağlı köylerde yabani bitkilerin geleneksel kullanımları [75], Araceae Familyasına Ait Bazı Taksonların Süt Pıhtılaştırma Özelliklerinin Araştırılması [76], Mardin ilinde yayılış gösteren Lamiaceae familyasına genel bakış [77] ve Mardin (Türkiye) Geofitleri üzerine etnobotanik bir araştırma [78] isimli çalışmalardır.

Şanlıurfa ve çevresinde yetişen yabani bitkiler hem insan beslenmesi için hem de ekonomik açıdan önemli yer tutmaktadır. Bu çalışmayla amacımız Şanlıurfa'nın merkez Karaköprü

ilçesinde halkın doğal bitkilerden yararlanma biçimlerini araştırmak ve etnobotanik alanına katkı sağlamaktır.

Materyal ve Metod

Bu çalışma, 2016-2017 yılları arasında, Şanlıurfa'ya bağlı merkez Karaköprü ilçesinde yapılmıştır. Şanlıurfa Karaköprü Ayşegül Kaman Anadolu Lisesi öğrencilerine yaklaşık 250 adet "etnobotanik bilgi formları (Ek 1) dağıtılmış ve öğrencilerin aileleri ile birlikte formları doldurmaları istenmiştir. Öğrencilerin aracılığıyla gelen formlar değerlendirildi, gelen bilgiler ışığında pilot çalışma yapılarak özellikle bitkiler konusunda daha deneyimli olduğuna inanılan aileler ziyaret edildi. Bilgi formlarında verilen cevaplardan yararlanılarak kaynak kişilere ulaşılmıştır (Tablo 1). Kaynak kişiler farklı yaş gruplardan oluşmuştur. Kaynak kişileri yerinde görebilmek için ev ziyaretleri yapılmıştır. Kaynak kişilerin belirttikleri bitkilerin yerel isimleri, toplandığı yer ve bilgiyi veren kişiye ait bilgiler kaydedilmiştir. Kaynak kişilerin verdiği bilgiler doğrultusunda, kaynak kişi veya bitkiyi tanıyan kişilerle beraber bitkilerin bulunduğu alanlara gidilerek bitki örnekleri toplanmıştır. Bitkilerin arazide fotoğrafları çekilmiş ve Ek 2'de verilmiştir.

Bitki örneklerinin teşhisinde, temel kaynak olarak "Flora of Turkey and the East Aegean Islands" [79] adlı eserlerden yararlanılmıştır.

Araştırmada elde edilen bitki örnekleri Harran Üniversitesi Fen-Edebiyat Fakültesi Herbaryumu (HARRAN)'unda saklanmaktadır.

Tablo 1 Araştırmada bilgi alınan kaynak kişilerin yaşları ve cinsiyetleri

Table 1 Ages and genders of the source persons from whom information was obtained in the study

Sıra No	Adı-Soyadı	Cinsiyeti	Yaşı	Yerleşim yeri
1	Abdullah K.	E	47	Şanlıurfa-Karaköprü
2	Adile K.	K	39	Şanlıurfa-Karaköprü
3	Adul T.	K	40	Şanlıurfa-Karaköprü
4	Ali K.	E	47	Şanlıurfa-Karaköprü
5	Ali K.	E	40	Şanlıurfa-Karaköprü
6	Aynur K.	K	36	Şanlıurfa-Karaköprü
7	Ayşe B.	K	57	Şanlıurfa-Karaköprü
8	Ayşe D.	K	41	Şanlıurfa-Karaköprü
9	Ayten K.	K	46	Şanlıurfa-Karaköprü
10	Edibe Y.	K	54	Şanlıurfa-Karaköprü
11	Emine B.	K	52	Şanlıurfa-Karaköprü

Tablo 1 Devam ediyor

Table 1 Cont.

12	Emine D.	K	68	Şanlıurfa-Karaköprü
13	Emine Y.	K	47	Şanlıurfa-Karaköprü
14	Emine Y.	K	80	Şanlıurfa-Karaköprü
15	Esra Y.	K	27	Şanlıurfa-Karaköprü
16	Fatma Ç.	K	60	Şanlıurfa-Karaköprü
17	Feride G.	K	72	Şanlıurfa-Karaköprü
18	Feride S.	K	41	Şanlıurfa-Karaköprü
19	Fettah K.	E	50	Şanlıurfa-Karaköprü
20	Fidan A.	K	36	Şanlıurfa-Karaköprü
21	Fikri D.	E	33	Şanlıurfa-Karaköprü
22	Gafur D.	E	52	Şanlıurfa-Karaköprü
23	Gürsel G.	K	50	Şanlıurfa-Karaköprü
24	Hacer C.	K	39	Şanlıurfa-Karaköprü
25	Halit T.	E	50	Şanlıurfa-Karaköprü
26	Hanım A.	K	45	Şanlıurfa-Karaköprü
27	Hanım G.	K	55	Şanlıurfa-Karaköprü
28	Harun B.	E	26	Şanlıurfa-Karaköprü
29	Hikmet E.	E	50	Şanlıurfa-Karaköprü
30	HülyaT.	K	42	Şanlıurfa-Karaköprü
31	İ. Halil B.	E	23	Şanlıurfa-Karaköprü
32	İslim A.	K	57	Şanlıurfa-Karaköprü
33	İslim K.	K	38	Şanlıurfa-Karaköprü
34	M. Arif N.	E	47	Şanlıurfa-Karaköprü
35	Medine H.	K	44	Şanlıurfa-Karaköprü
36	Mehmet K.	E	54	Şanlıurfa-Karaköprü
37	Mehmet U.	E	60	Şanlıurfa-Karaköprü
38	Müslüm S.	E	45	Şanlıurfa-Karaköprü
39	Nazime K.	K	49	Şanlıurfa-Karaköprü
40	R. Songül K.	K	47	Şanlıurfa-Karaköprü
41	Rümeysa A.	K	17	Şanlıurfa-Karaköprü
42	Sabiha D.	K	35	Şanlıurfa-Karaköprü
43	Selma S.	K	32	Şanlıurfa-Karaköprü
44	Serpil A.	K	36	Şanlıurfa-Karaköprü
45	Sevim F.	K	37	Şanlıurfa-Karaköprü
46	Şeriban K.	K	35	Şanlıurfa-Karaköprü
47	Şerife A.	K	33	Şanlıurfa-Karaköprü
48	Veysi E.	E	71	Şanlıurfa-Karaköprü
49	Zekiye T.	K	65	Şanlıurfa-Karaköprü
50	Zeynep A.	K	30	Şanlıurfa-Karaköprü

Bulgular

Etnobotanik çalışmalar sonucu elde edilen bitkilerin bağlı olduğu familyaya göre alfabetik sıraya göre Bulgular kısmında Tablo halinde verilmiştir (Tablo 2).

Tablo 2 Gıda amaçlı Yabani bitkilerin isimleri, kullanılan kısımları ve kullanım şekilleri

Table 2 Names, used parts and usage forms of wild plants for food purposes

Bitkinin Bilimsel adı	Familyası	Bitkinin Yöresel adı	Kullanılan kısmı	Kullanım şekli ve biçimi
Allium rotundum L.	Amaryllidaceae	Sirım, yabani sarımsak	tüm bitki	Haşlanır, kavrulur ve gözleme yapımında kullanılır
Allium noēanum Reut. ex Regel	Amaryllidaceae	Sirım	yaprak	İlkbaharda çiçek açmadan yaprakları toplanır, haşlanır ve pide yapımında kullanılır
Chenopodium album L.	Amaranthaceae	Sirken, kemberok	yaprak	Yaprakları salata yapımında kullanılır
Pistacia khinjuk Stocks	Anacardiaceae	Bezeki sakızı, Menengiç, bıttım	meyve	Meyveleri çerez olarak kullanılır
Bunium paucifolium DC.	Apiaceae	Heylok, ğeylok,	yumru	Yumrusu taze olarak yenir
Eryngium campestre L. var. *campestre*	Apiaceae	Boğa dikeni, kerengzer, çistok	yaprak	Çiğ olarak veya haşlanarak tüketilir
Ferula orientalis L.	Apiaceae	Çakşır otu	yaprak, kökler	Yaprakları haşlanarak tüketilir
Foeniculum vulgare Mill.	Apiaceae	Arapsaçı, yabani rezene	yaprak, tohum	Yaprakları kavrularak yenilir
Malabaila secacul (Mill.) Boiss. subsp. *secacul*	Apiaceae	Harik	taban yaprakları	Taze yaprakları salata olarak kullanılır
Arum dioscoridis Sm. var. *dioscoridis*	Araceae	Gavur pancarı, asalan, yılan yastığı, zilke araba, kari, kardi	yaprak	Yapraklarından Kardi denilen çorba yapılır. Ayrıca, yaprakları kurutularak da kullanılır.
Arum rupicola Boiss.	Araceae	Gavur pancarı, asalan, yılan yastığı, zilke araba, kardi	yaprak	*Arum dioscoridis* gibi işlemden geçirilir
Bellevalia pseudolongipes Karabacak & Yıldırım (Endemik)	Asparagaceae	akbandır	yaprak	Yaprakları toplanır, doğranır, haşlanır, pide yapımında kullanılır

Tablo 2 Devam ediyor

Table 2 Cont.

Muscari longipes Boiss.	Asparagaceae	Akbandır	yaprak	Yaprakları toplanır, doğranır, haşlanır, kavrulur pide yapımında kullanılır
Ornithogalum narbonense L.	Asparagaceae	Akbandır, gazrik	yaprak	Yaprakları kavrulur börek yapımında kullanılır veya taze olarak cacığa katılır
Ornithogalum sphaerocarpum A. Kern	Asparagaceae	Akbandır, gazrik	yaprak	Yaprakları kavrulur börek yapımında kullanılır veya taze olarak cacığa katılır
Achillea arabica Kotschy	Asteraceae	Civanperçemi-çiçega maran	çiçek	Çiçekleri sıcak suya konulur, çay olarak içilir
Centaurea iberica Trev. Ex Spreng.	Asteraceae	Çakırdiken, pancare tahl, cavbelok	toprak üstü kısımlar	Taze tüketilir, kavrulur, haşlanıp tüketilir veya salata yapımında kullanılır
Centaurea solsitialis L. subsp. *Solsitialis*	Asteraceae	Pıncar	taban yaprakları	Haşlanarak, kavrulur, gözlemesi ve yemeği yapılır
Cnicus benedictus L.	Asteraceae	Bostan out, şevketi bostan	tüm bitki	Kavrulur, haşlanıp tüketilir
Echinops heterophyllus P.H. Davis	Asteraceae	Topuz diken	topuz içi	Dikenler ayrılarak içi taze olarak yenir
Gundelia tournefortii L. var. *armata* Freyn & Sint.	Asteraceae	Kenger, kereng	taze toprak altı sürgün	Taze olarak yemeklere konur ve pişirilir, haşlanarak kavrulur veya taze tüketilir
Silybum marianum (L.) Gaertn. Subsp. *Marianum*	Asteraceae	Kerbeş	gövde	Gövdesi dikenlerinden ayrılarak taze olarak tüketilir
Taraxacum sintenisii Dahlst.	Asteraceae	Çitlik, karahindiba	yaprak	Taze olarak salata yapımında kullanılır
Tragopogon porrifolius L.subsp. *longirostris* (Sch.Bip.) Greuter.	Asteraceae	Yemlik, pore pire	toprak üstü kısım	Toprak üstü kısımları doğranır, haşlanıp kavrularak tüketilir veya taze olarak çiğ yenir
Anchusa azurea Mill.var. *azurea*	Boraginaceae	Guriz, sığırdili, mıjmıjok	toprak üstü kısımları	Toprak üstü kısımları pişirilerek yenir. Çiçeklerin balözü emilir
Anchusa leptophylla Roem. & Schult. Subsp. *Leptophylla*	Boraginaceae	Guriz, guhriz, mijmijok	taban yaprakları	Taze iken ilkbaharda yaprakları toplanır, haşlanır, pişirilir, kavrulması yapılır, çorbası da yapılmaktadır
Capsella bursa-pastoris (L.) Medik.	Brassicaceae	Nane cucıka, kuşyemi, şimlik, Mehapelkat	taban yaprakları	Haşlanıp kavrularak yemeği yapılır. Taze de tüketilir

Tablo 2 Devam ediyor

Table 2 Cont.

Lepidium draba L.	Brassicaceae	Kıneberk	taban yaprakları	Haşlanıp kavrularak yemeği yapılır, pilava katılır
Lepidium sativum L. subsp. *sativum*	Brassicaceae	Dejnik	taze taban yaprakları	Taze yaprakları salata olarak yenir
Nasturtium officinale R. Br.	Brassicaceae	Tere, tuzık, su teresi	tüm bitki	Çiğ olarak tüketilir
Sinapis alba L. subsp. *alba*	Brassicaceae	Ğardal, Hardal, harek, harık	toprak üstü kısım	Çiğ olarak tüketilir veya haşlanarak yemeği yapılır
Celtis australis L. subsp. *australis*	Cannabaceae	Çitlenbik, dağdağan	meyve	Meyveleri yenir
Silene conoidea L.	Caryophyllaceae	Şekerok	çiçek	Çiçekleri taze yenir
Capparis sicula Veill. subsp. *sicula*	Capparaceae	Kebere, keber	çiçek tomurcuku, meyvesi	Meyvesi ve tomurcukları salamura yapılır
Prosopis farcta (Banks & Sol.) J.F.Macbr.	Fabaceae	Çeti, hurnif, şermot	meyve	Olgun meyveleri taze olark tüketilir
Pisum sativum L. subsp. *elatius* (M.Bieb.) Aschers. & Graebn.var. *elatius*	Fabaceae	Yabani bezelye	tohum	Tohumları yenir
Vicia narbonensis L. var. *narbonensis*	Fabaceae	Colban, collık	tohum	Tohumları yenir
Crocus cancellatus Herb. subsp. *damascenus* (Herb.) B.Mathew	Iridaceae	Pivok	yumru	Yumrusu soyulup taze olarak tüketilir
Crocus pallasii Goldb. subsp. *turcicus* B.Mathew	Iridaceae	Pivok	yumru	Yumrusu soyulup taze olarak tüketilir
Iris persica L.	Iridaceae	Nevruz çiçeği, bılbızek	çiçek, yumru	Çiçeği ve yumrusu yenir
Ixiolirion tataricum (Pall.) Schult. & Schult.f.	Ixioliriaceae	Encurok, hiyarok	çiçek	Ciçeği, taze olarak, salata niyetine yenir
Erodium cicutarium (L.) L Hér. subsp. *cicutarium*	Geraniaceae	İğnecik, nikoldik, saat	taban yaprakları	Yaprakları kavrularak börek yapımında kullanılır
Geranium tuberosum L.	Geraniaceae	Yer elması	yumru	Yumrusu yenir

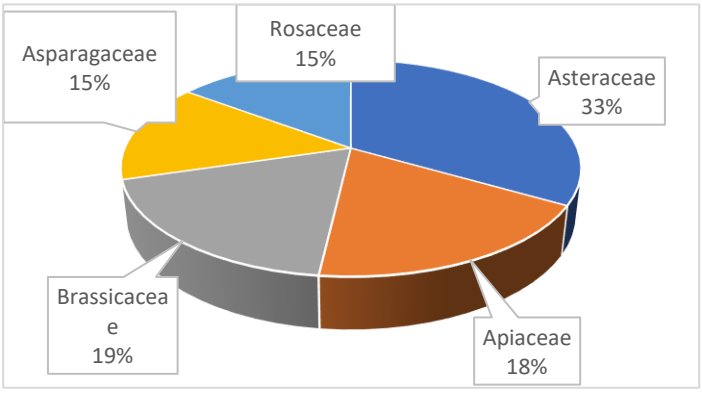
Tablo 2 Devam ediyor

Table 2 Cont.

Euphorbia chamaesyce L.	Euphorbiaceae	Siğil otu	toprak üstü kısımları	Topraküstü kısımları taze veya kurutularak yenir veya ekmek hamuruna karıştırılarak ekmek yapılır
Mentha longifolia (L.) L. subsp. *longifolia*	Lamiaceae	Yabani nane, pung	toprak üstü kısımları	Topraküstü kısımları taze olarak yenir
Salvia syriaca L.	Lamiaceae	Kunciya bej, gunpisik, sivenok	toprak üstü kısımları, tohum	Topraküstü kısımları çay olarak içilir. Ayrıca, tohumları yenir
Malva neglecta Wallr.	Malvaceae	Ebe gümeci, toğlık, kömeç, Kazan karası	toprak üstü kısmı	Toprak üstü kısımları kavrularak börek, pilavlı kömeç ve pide yapımında kullanılır, taze olarak da tüketilir
Platanus orientalis L.	Platanaceae	çınar	yaprak	Yaprakları haşlanır, kavrulur ve pide yapımında kullanılır, ayrıca sarma-dolma yapımında kullanılır
Polygonum cognatum Meissn.	Polygonaceae	Madımak, giyaye kerıka, tırşo	yaprak	Yaprakları kavrulur salata ve pilav yapımında kullanılır
Rumex acetosella L.	Polygonaceae	Kuzu kulağı	taban yaprakları	Yaprakları taze olarak salata ve cacık yapımında kullanılır
Portulaca oleracea L.	Portulacaceae	Semizotu, pırpırım	toprak üstü kısmı	Yaprakları taze olarak salata ve cacık yapımında kullanılır
Nigella unguicularis (Poir.) Spenn	Ranunculaceae	Çörek otu	tohum	Tohumları döğülerek balla karıştırılarak tüketilir.
Amygdalus communis L.	Rosaceae	Yabani badem	meyve	Meyvesi yenir
Crataegus monogyna Jacq. var. *monogyna*	Rosaceae	Aluç, Alıç	meyve	Meyveleri taze olarak yenir
Prunus mahaleb L. var. *mahaleb*	Rosaceae	Mahlep, yabani kiraz	meyve	Meyveleri taze olarak yenir
Rubus sanctus Schreb.	Rosaceae	Böğürtlen	meyve	Meyveleri taze olarak yenir
Eremurus spectabilis M. Bieb	Xanthorrhoeaceae	Çiriş otu	taban yaprakları	Yaprakları kavrularak börek yapımında kullanılır
Urtica pilulifera L.	Urticaceae	Isırgan otu, gezgezok	toprak üstü kısımları	Yapraklarından çorba yapılır, yaprakları kurutulup çayı içilir, haşlanıp kavrularak, börek yapımında kullanılır

Sonuç ve Tartışma

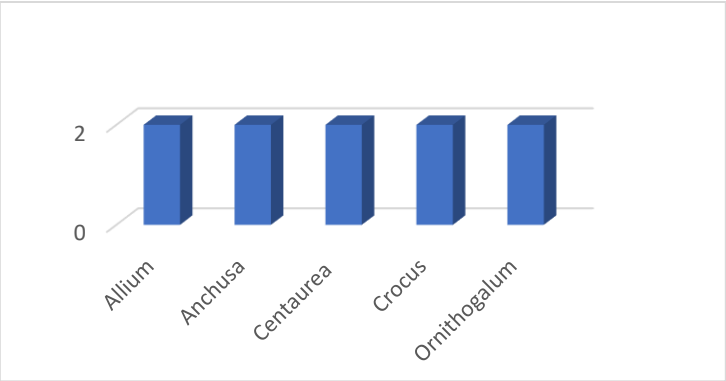
Bu çalışma sonucunda Şanlıurfa'nın Karaköprü ilçesinde 28 farklı familyaya ait 57 yabani bitki taksonunun gıda amacıyla kullanıldığı tespit edilmiştir (Tablo 2). Türkiye florasındaki takson sayısı bakımından en zengin familyalar Asteraceae, Fabaceae, Lamiaceae ve Brassicaceae'dir [79]. Ayrıca bu familyaya ait türler zengin protein, yağ ve mineral içerikleri nedeniyle Anadolu insanı tarafından gıda olarak kullanılmasında önemli rol oynamışlardır [80, 81]. Araştırma alanında kullanıldığı belirlenen bitkiler içerisinde en çok takson içeren ilk 5 familya Asteraceae %33, Brassicaceae %19, Apiaceae %18, Asparagaceae %15 ve Rosaceae %15'dir (Şekil 1).



Şekil 1 En fazla takson içeren familyalar

Fig 1 Families with the highest number of taxa

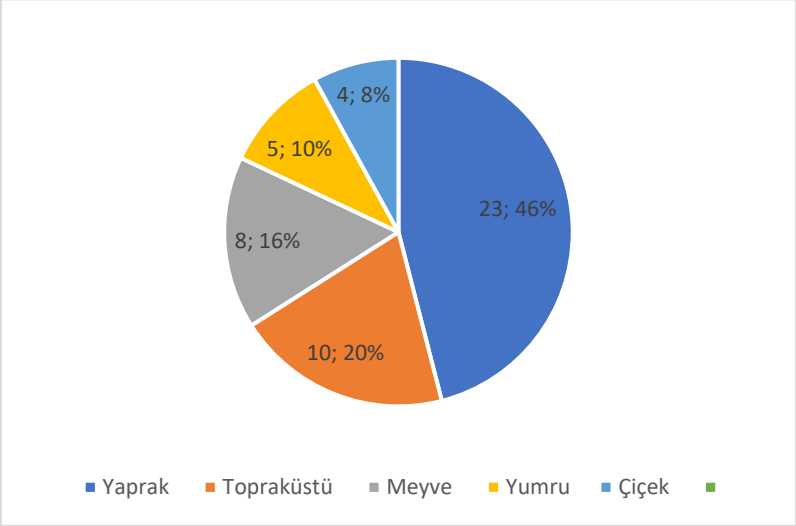
Çalışma alanında 51 farklı cinse ait bitkiler tespit edilmiş olup, ikişer tür ile en fazla takson içeren cinsler *Allium*, *Anchusa*, *Centaurea*, *Crocus* ve *Ornithogalum* olduğu tespit edilmiştir (Şekil 2).



Şekil 2 En fazla takson içeren cinsler

Fig 2 The genera with the highest number of taxa

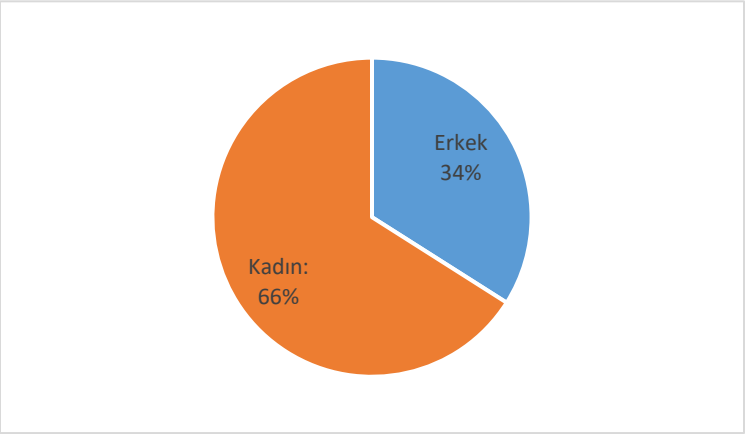
Araştırma alanında bitkilerin etnobotanik kullanımları açısından %23 oranında yapraklarının, %10 oranında toprak üstü kısımlarının, %8 oranında meyvelerinin, %5 oranında yumrularının, %4 oranında ise çiçeklerinin gıda olarak kullanıldığı tespit edilmiştir (Şekil 3). Yaprakların tüketim oranı bakımından yüksek olması bitkilerin çiğ veya sebze olarak tüketilmesinden kaynaklanmaktadır. Meyveler genelde atıştırmalık olarak kullanılır.



Şekil 3 Bitkilerin en çok kullanılan kısımları

Fig 3 The most used parts of plants

Çalışmada 50 kaynak kişi ile görüşmeler sağlanmıştır. Kaynak kişilerin dağılımı Şekil 4.'te verilmiştir. Kaynak kişilerin yaş aralığı;



Şekil 4 Kaynak kişilere ait erkek ve kadın dağılım grafikleri

Fig 4 Distribution graphs of men and women of the source persons

Araştırma alanında tespit edilen yabani bitkilerin tüketilme-değerlendirme durumuna durumları şu şekildedir;

Taze tüketilenler: Bu kategoride 30 takson kaydedilmiştir. Bitkilerin taze yaprakları, topraküstü kısımları, tohumları, meyveleri ve çiçekleri tüketilir. Bu bitkiler; *Bunium paucifolium, Malabaila secacul, Ornithogalum narbonense, Ornithogalum sphaerocarpum, Centaurea iberica, Echinops heterophyllus, Gundelia tournefortii* var. *armata, Silybum marianum, Taraxacum sintenisii, Tragopogon porrifolius*.subsp. *longirostris, Capsella bursa-pastoris, Lepidium sativum, Nasturtium officinale, Sinapis alba, Crocus pallasii, Iris persica, Ixiolirion tataricum, Geranium tuberosum, Euphorbia chamaesyce, Mentha longifolia, Malva neglecta, Rumex acetosella, Portulaca oleracea, Pistacia khinjuk, Celtis australis, Prosopis farcta, Amygdalus communis, Crataegus monogyna, Prunus mahaleb* ve *Rubus idaeus*'dur.

Kavrularak yemeği yapılanlar: Bu kategoride 20 takson kaydedilmiştir. Bu bitkiler; *Allium rotundum, Foeniculum vulgare, Muscari longipes, Bellevalia pseudolongipes, Ornithogalum narbonense, Ornithogalum sphaerocarpum, Centaurea iberica, Centaurea solsitialis, Cnicus benedictus, Gundelia tournefortii, Tragopogon porrifolius, Anchusa azurea, Capsella bursa-*

pastoris, Lepidium draba, Erodium cicutarium, Malva neglecta, Polygonum cognatum, Platanus orientalis, Eremurus spectabilis ve *Urtica pilulifera*'dır.

Börek veya pide içi olarak kullanılanlar: Bu kategoride 11 takson kaydedilmiştir. *Allium noēanum, Bellevalia pseudolongipes, Muscari longipes, Malva neglecta, Platanus orientalis, Ornithogalum narbonense, Ornithogalum sphaerocarpum, Erodium cicutarium, Malva neglecta, Eremurus spectabilis* ve *Urtica pilulifera*'dır.

Meyvesi tüketilenler: Bu kategoride 8 takson kaydedilmiştir. Bu bitkiler; *Pistacia khinjuk, Celtis australis, Capparis sicula, Prosopis farcta, Amygdalus communis, Crataegus monogyna, Prunus mahaleb* ve *Rubus idaeus*'dur.

Tohumu tüketilenler: Bu kategoride 5 takson kaydedilmiştir. Bu bitkiler; *Foeniculum vulgare, Pisum sativum, Vicia narbonensis, Salvia syriaca* ve *Nigella unguicularis*'dir.

Çorba şeklinde tüketilenler: *Urtica pilulifera* ve *Anchusa leptophylla*'dır.

Pırpırım (*Portulaca oleraceae*), Kenger (*Gundelia tournefortii* var. *armata*), akbandır (*Ornithogalum narbonense*), çakırdikeni (*Centaurea iberica*), kıneberk (*Lepidium draba*), dejnik (*Lepidium sativum* subsp. *sativum*) ve kömeç (*Malva neglecta*) bitkileri gıda olarak sıklıkla kullanılmaktadır.

Bellevalia pseudolongipes türü Şanlıurfa'da gıda amaçlı kullanılan endemik bir bitkidir. Bitkinin yaprakları ve taze sürgünleri pide yapımında kullanılır. Bitki populasyonu kayalık alanlarda yoğun bir nüfusa sahiptir ve kullanımı çok yoğun değildir.

Araştırma alanının yakın bölgelerde yapılan çalışmalar ile familya ve takson sayıları bakımından karşılaştırılması Tablo 3.'de verilmiştir.

Tablo 3. verilerine bakıldığında sadece gıda amaçlı kullanım üzerine yapılan çalışmalarda takson sayılarının yüksek olduğu görülmektedir. Bunda alana yoğunlaşma, bölgenin yüzölçümü, görüşülen kaynak kişiler, kırsalda yaşama gibi etkiler de belirleyici olmaktadır.

Güneydoğu Anadolu Bölgesi'nde yenilebilir yabani bitkilerle ilgili sınırlı sayıda etnobotanik çalışmalar yapılmıştır. Mardin ili, Yeşilli ilçesinde yenilebilir yabani bitkilerin belirlenmesi amacıyla yapılan çalışmada 74 adet yenilebilir yabani takson kayıt altına alınmıştır [51]. Batman ili Hasankeyf ilçesinde yenilebilir yabani bitkilerin belirlenmesi amacıyla yapılan çalışmada 86 adet yenilebilir yabani takson kayıt altına alınmıştır [52] ve Mardin ili'nin genelinde yapılan ve geleneksel yemeklerine katkıda bulunan yenilebilir yabani bitkiler

çalışmasında 92 adet yenilebilir takson kayıt altına alınmıştır [53]. Adıyaman ili Kahta ilçesi ve Narince köyünde [64] etnobotanik kullanımı olan 113 takson rapor edilmiş fakat yemeği yapılan veya gıda amaçlı kullanımlara değinilmemiştir.

Tablo 3 Çalışmamızın yakın civardaki diğer araştırmalarla karşılaştırılması

Table 3 Comparison of our study with other studies in the immediate vicinity

	Familya	**Gıda olarak kullanılan takson sayısı**
Araştırma alanı (Şanlıurfa, Karaköprü)	28	57
Arat Dağı [57]	15	33
Kalecik Dağı [62]	15	38
Zeytinbahçe-Akarçay [56]	22	56
Tek Tek Dağları [70]	19	37
Şanlıurfa, Merkez ilçeler [71]	47	56
Mardin, Yeşilli [51]	31	74
Batman, Hasankeyf [52]	32	86
Mardin yenen bitkiler [53]	34	92
Mardin, Midyat [69]	14	24
Mardin ili Geofitleri [78]	15	45

Araştırma alanı olan Şanlıurfa'da yenen yabani bitkilere ait doğrudan bir çalışmaya rastlanmamıştır. Yapılan çalışmalar genel etnobotanik çalışmalardır. Şanlıurfa Merkez ilçe ve bağlı köylerde yapılan etnobotanik çalışmada 134 bitki taksonu içerisinde 56 taksonun gıda amacıyla kullanıldığı tespit edilmiştir [71]; Şanlıurfa ili Birecik ilçesi Arat Dağı ve çevresindeki köylerde yapılan çalışmada 170 bitki taksonu içerisinde 33 taksonun gıda amacıyla kullanıldığı tespit edilmiştir [57], Birecik ilçesin Zeytinbahçe-Akarçay arası köylerde yapılan etnobotanik çalışmada 190 bitki taksonu içerisinde 56 bitkinin gıda amacıyla kullanıldığı tespit edilmiştir. Zeytinbahçe çalışmasında çay ve tıbbi amaçlı kullanılan bitkiler ile kültür bitkileri bu sayıya eklenmemiştir [56]. Şanlıurfa ili Tek Tek Dağlarındaki bazı köylerde yapılan etnobotanik çalışmada ise 120 taksonun 37'sinin gıda amacıyla kullanıldığı rapor edilmiştir [70]. Şanlıurfa ili, Karaköprü ilçesi sınırlarında yer alan Kalecik Dağı ve çevresindeki köylerde yapılan etnobotanik çalışmada 126 taksonun 38'inin gıda amacıyla kullanıldığı rapor edilmiştir [62].

Şanlıurfa ili başta olmak üzere Türkiye'de gıda olarak kullanılan bitkilerle ilgili yapılan etnobotanik çalışmalar karşılaştırıldığında aşağıdaki taksonlar ilk kez bu çalışmada

kaydedilmiştir: *Muscari longipes, Echinops heterophyllus, Euphorbia chamaesyce* ve *Nigella unguicularis.*

Euphorbia (sütleğen) türleri genellikle zehirli olarak bilinir ve doğrudan tüketilmezler. Bununla birlikte, *Euphorbia chamaesyce* türünün toprak üstü kısımları taze, kurutularak veya ekmek hamuruna katılarak pişirildikten sonra tüketilerek derideki siğilleri giderdiğine inanılmaktadır. Benzer kullanım Şanlıurfa'nın Bozova ilçesinde [82] *Euphorbia macroclada* Boiss. türünde görülmektedir. *E. macroclada* bitkisinin kök kısmı sadeyağ, bal mumu ve fıstık sakızı ile hazırlanan merhem kıvamındaki hülasanın tüketilmesi ile öksürüğe iyi geldiği rapor edilmektedir. Aynı çalışmada bu türün sütünün diş ağrısına iyi geldiği de rapor edilmiştir. Ayrıca *E. macroclada* türünün yakılmasıyla elde edilen külünün üzümlerin kurutulmasında kullanıldığı [52] rapor edilmiştir. Diğer yandan *Euphorbia helioscopia* subsp. *helioscopia* türünün tüketilmesi [5] ve pekmeze aroma vermede kullanımı [51], *Euphorbia craspedia* türünün [51, 53], *Euphorbia gaillardotii* Boiss. & Blanche türünün [53], *Euphorbia macrostegia* türünün [57, 62], *Euphorbia cheiradenia* Boiss. & Hohen. ve *E. falcata* L. subsp. *falcata* var. *galilaea* (Boiss.) Boiss. taksonlarının [71] pekmez aroması için kullanımı rapor edilmiştir. Diğer bir çalışmada ise [83] *Euphorbia helioscopia* L. 'nın kök suyu antelmintik ve müshil olarak kullanılırken, yaprak dekoksiyonu bağırsak kurtları düşürücü olarak sıtmayı önleme, ateş düşürücü ve deri ödemi tedavisi için kullanıldığı rapor edilmiştir.

Türkiye'deki yenilebilir yabani bitkiler genellikle doğadan toplanarak doğrudan tüketilmekte veya pazarlarda satılmaktadır [84]. Bu bitkilerin kullanımı bölgesel olmakla birlikte günümüzde ticareti yapılmaya başlanmıştır [85]. Bunda doğa odaklı yaşama olan ilgi de etkili olmaktadır. Endüstriyel ürünlerine alternatif yabani bitkiler çiğ veya işlenmiş olarak Türk mutfağında daha fazla yer almaya başlamıştır. Yenilebilir yabani bitkilerin iyi bir besin kaynağı olmasının yanı sıra önemli bir gelir kaynağı olabileceği belirtilmektedir [86].

Şanlıurfa'nın Karaköprü ilçesinde yabani bitkilerin gıda amaçlı kullanım kültürü özellikle kırsal alanlarda devam etmektedir. Bununla beraber, genç nüfusun çok azı yabani bitkilerin kullanımına ilgi duymaktadır. Bu nedenle binlerce yıllık bir tecrübenin sonucu olarak gelişen bu kültürel bilgi birikiminin kayıt altına almak zorunlu hale gelmiştir. Bu araştırmanın yenilebilen yabani bitkilerle ilgili kültürel mirasın korunması ve gelecek nesillere

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Abbreviations/Kısaltmalar

var.: Variety/Varyete, subsp.: Subspecies/Alttür, L.: Carl Linnaeus, Pierre Edmond Boissier: Boissier, DC.: de Candolle, M. Bieb.: Friedrich August Marschall von Bieberstein, Schult: Josef August Schultes, Roem.: Max Joseph Roemer, Sint.: Paul Ernst Emil Sintenis, Freyn: Josef Franz Freyn, Dahlst.: Gustav Adolf Hugo Dahlstedt, HARRAN: Harran University Faculty of Arts and Sciences Herbarium/Harran Üniversitesi Fen-Edebiyat Fakültesi Herbaryumu.

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Data Availability statement / Veri Kullanılabilirliği bildirimi

The author confirms that the data supporting this study are cited in the article.

Yazar, bu çalışmayı destekleyen verilere makalede atıfta bulunulduğunu onaylamaktadır.

Compliance with ethical standards / Etik standartlara uyum

Conflict of interest / Çıkar çatışması

The author declare no conflict of interest.

Yazar herhangi bir çıkar çatışması beyan etmemektedir.

Ethical standards / Etik standartlar

The study is proper with ethical standards.

Çalışma etik standartlara uygundur.

Authors' contributions / Yazar katkıları

During the study, Mehmet Maruf BALOS conducted field research, Hasan AKAN and Mehmet Maruf BALOS wrote the article.

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Research article

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Occurence of Antibiotic Resistant Bacteria Causing UTIs Among Children Under School Age in Soran City, North of Iraq

Hasan Akan[1]*, Rawezh Hakeem Mustafa[1]

ABSTRACT

The aim of this study to research the event of antibiotic resistant bacteria causing UTIs among children under six years old in Soran, North of Iraq. Totally, 200 urine samples were taken from children suffering from UTIs, in Ashti hospital and Childbirth hospitals between 2017 and 2018 years. It is determined that 70 bacterial uropathogens have been isolated while 130 samples showed negative culture, and the incidence of UTIs was significantly higher in little girls (57.1%) than in little boys. The majority of uropathogenic isolates have resisted ampicillin, ceftazidime, ceftriaxone and cefotaxime. Other antibiotics differently showed moderate susceptibilities. The DNA profile showed that only the isolate K 61 (*Klebsiella pneumoniae*) was bearing *qnrB.*

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Introduction

Urinary tract consists anatomically of lower urinary tracts include the bladder, urethra and upper urinary tract include the kidneys and ureters [1]. During urination the urinary system expels metabolic wastes from the blood and discharges it as urine to the outside [2]. UTI (Urinary tract infection) is characterized as the notable of microscopic organisms in urine along with symptoms of infection or a bacterial infection of the urinary bladder (cystitis), the kidneys (pyelonephritis), or both. UTIs are widely recognized in childhood [3, 4, 5]. By adolescent age, about 11% of girls and 7% of boys have had at least one episode of UTI, with recurrent infections reported in many cases [6-9].

UTIs are infections resulting from the existence and growth of microorganisms anywhere in urinary tract and may be one common bacterial of human infection [10-11]. Microorganisms that causes UTIs originate from the stool [12]. UTI may be present when

[1] Department of Biology, Faculty of Art & Science, University of Harran, Şanlıurfa, Turkey
*Sorumlu Yazar: Hasan Akan, E-Posta: hasanakan1972@gmail.com

microscopic organisms and white blood cells are available in the urine [13]. *Healthcare-associated Infections* is widely recognized inconvenience from hospitalized patients [14-15]. The spread of antimicrobial resistance among urinary pathogens has been expanding everywhere throughout the world [16-18]. Many strains of bacteria were developed that cause urinary tract infection usually resistant to various types of antimicrobial agents such as amoxicillin and ampicillin [19-21]. UTIs are generally known for all aggravations of urinary tracts, which contains kidney injury (pyelonephritis), bladder (cystitis) and the urethra (urethritis) [22-23].

The resistance of quinolone was correlated to mutations that prompt amino acid changes in the QRDRs inside the subunits which are incorporated in the synthesis of DNA [24].

The objective of this study was to find antibiotic resistance patterns of microorganisms grown most frequently in Soran, North Iraq, and to determine empirical treatment options according to the results obtained. The results of our study will support the technicians working in the governmental laboratories with new data about the drug of choice for new multi drug resistant strains repeatedly arisen.

Materials and Methods

This study was carried out in Soran city- North of Iraq. Most people live in villages and have rural lifestyle. They depend on domestic animals and ground water. Rural people there are generally poor and ignorant of the rules of health. In Soran most of families attend Ashti hospital and Childbirth hospitals. 200 patients of UTI intended to Ashti hospital and Childbirth hospitals laboratories. The period of sample collection was from July 2017 to March 2018. Samples had been taken before antibiotic use. At the firstly have written all information on a questionnaire list of each patient.

Ethics committee approval was obtained from the Ministry of Health General Directorate of Health-Erbil Committee for the study (19/06/2018/98). This study was conducted in accordance with the Declaration of Helsinki.

All reagents used in the current study were prepared as follows by Harley and Presccott [25]. The uropathogenic isolates were tested for antimicrobial susceptibility by the Kirby-Bauer disk diffusion method on Mueller Hinton agar plates by Merlino et al. [26]. The antibiotics

that was widely used in the current study for the treatment of UTIs are Cefotaxime, Meropenem, Ciprofloxacin, Ampicillin, Tobromycin, Trimethoprim-sulphamethoxazole, Gentamycin, Augmentin, Levofloxacin, Ceftazidime, Impenem, Azithromycin, Vancomycin and Ceftriaxone. The antibiotic discs which are used in identification of the isolates are Novobiocin, Bacitracin, Bacitracin and Rifampin.

MacFarland standard solution is utilized to standardize the density for the susceptibility test. So to preparations of turbidity standard by Cheesbrough [27]. The girl patients were asked their parents to clean their external genitalia. If the patient was under 2 years they used Pediatric urine collector according to the method of Morello et al. [28]. First isolation and purification of bacterial species were on suitable culture media (Nutrient agar, MacConkey agar and blood agar). The recommended procedure uses a sterilized calibrated metal loop to transfer 1µl of uncentrifuged urine specimen and was streaked on Nutrient agar, Blood agar and MacConkey agar, at one and same time as per the biochemical and bacteriological tests utilizing standard techniques by Vandepitte et al. [29], additionally after marking and streaking the plates were put in the incubater at 35-37°C for 18-24 hours. Identified isolated bacteria determined with the techniques described in Bergey's manual by Holt et al. [30].

Differentiation of isolated bacteria by CHROMagar^{TM} orientation, is known as chromogens in which depended on soluble colourless. When the target organism's enzyme cleaves the colourless chromogenic conjugate, the chromophore is released [26].

Identified isolates were presserved for long time according to Mcfaddin [31]. The susceptibility was tested by Kirby-Bauer disc diffusion technique [32]. Disc diffusion test was carried out according to [33].

Molecular method

In this study, specific primers (Metabion, Germany) were designed for amplification of *qnrA*, *qnrB*, *qnrS*, and bla_{kpc} genes. For isolation of nucleic acids from Bacteria, DNA extraction kit (Roche, USA) was used for purification of genomic DNA of the isolates. Details of primer sequences are shown in (Table 1). The method described by Sambrook and Russell [34] was used for gel electrophoresis.

Table 1 *qnrA, qnrB, qnrS,* and bla_{kpc} primer sequences

Isolates No.	Resistance Genes	Primer Sequence	Target size(bp)
E. coli E 7, E 51, E 57, E 59, E 62	*qnrA*	(F)5'-TCAGCAAGAGGATTTCTCA-3'	(R)5'-516
	qnrB	GGCAGCACTATTACTCCCA-3' GATCGTGAAAGCCAGAAAGG-3'	(F)5'- (R)5'-469
Klebsiella pneumoniae K 61	*qnrS*	ACGATGCCTGGTAGTTGTCC-3' ACGACATTCGTCAACTGCAA-3' TAAATTGGCACCCTGTAGGC-3'	(F)5'- (R)5'-714 (F)5'-
	bla_{kpc}	ATGTCACTGTATCGCCGTCT-3' TTTTCAGAGCCTTACTGCCC-3'	(R)5'- 893

Result and Discussion

Incidence of urinary tract infection

Our results showed that, out of 200 urine specimen gathered from patients complaining of signs and symptoms of UTIs attending Ashti hospital and Childbirth hospitals in Soran city, 70 (35%) were positive for bacterial infections while 130 (65%) samples showed culture negative. The study of Alsammani et al. [35] showed that 203 (66.7%) of children had urinary tract infection.

Incidence of the isolated uropathogens associated with UTIs

Uropathogens isolates from 200 samples, 70 strains of bacteria were belonging to 9 species (Table 2).

Table 2 Uropathogens obtained from urine culture

No.	**Isolated uropathogens**	**Number**	**Percentage**
1	*Escherichia coli*	27	38.6%
2	*Staphylococcus aureus*	13	18.6%
3	*Coagulase-negative Staphylococci*	8	11.4%
4	*Klebsiella pnuemoniae*	7	10%
5	*Enterococcus faecalis*	6	8.6%
6	*Streptococcus spp.*	4	5.7%
7	*Pseudomonas aeruginosa*	2	2.9%
8	*Staphylococcus saprophyticus*	2	2.9%
9	*Proteus vulgaris*	1	1.4%

Most common causative organism was *E. coli* (38.6%). This result was consistent the study by Sharma et al. [36] from Nepal and from Aligarh, India by Akram et al. [37] Studies by Islam et al. and Mantadakis et al. [38-39] showed *E. coli* as most common organism but with

varying proportionsut. According to Schlager (2001), in children, *Staphylococcus aureus* is uncommon without in-dwelling catheters or many other factors of infection after instrumentation of the urinary tract [40].

Incidence of UTIs in relation to sex and age groups

70 (35%) patients out of 200 showed to be urine culture positive (Fig. 1).

UTI is predominantly a disease of female. According to Ouno et al. (2013) and Ramazan et al. (2004), the length of male urethra provides a distance barrier that eliminates bacteria from the urinary bladder [41-42].

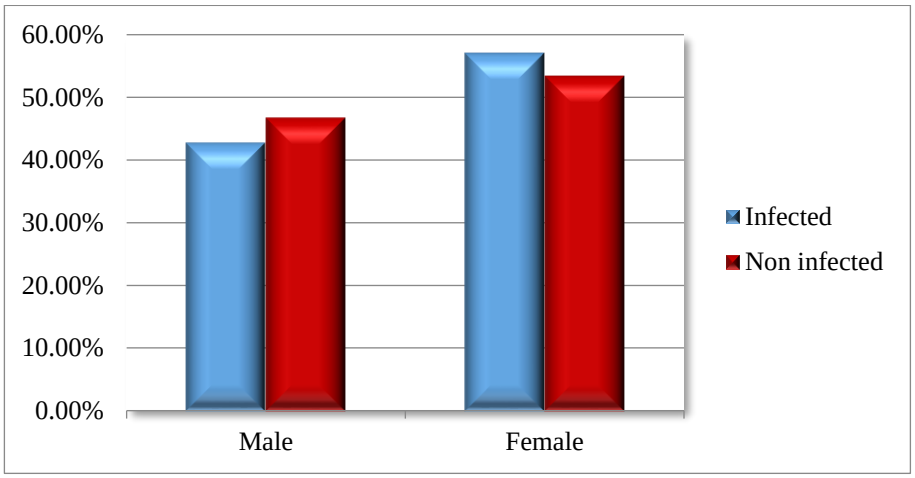

Fig. 1 Frequency of UTIs according to the gender

The majority of children 26 (37.1%) were among those less than two years old, and more than half of them were male 15 (57.7%) (Fig. 2). The same situation reported by Alsammani et al. [35]. The high incidence of UTIs in females under one year old might to be as a result of a distinct type of factors, such as the short and wide female urethra (3-4 cm length) and its proximity to the anus according to Kolawole et al.[43].

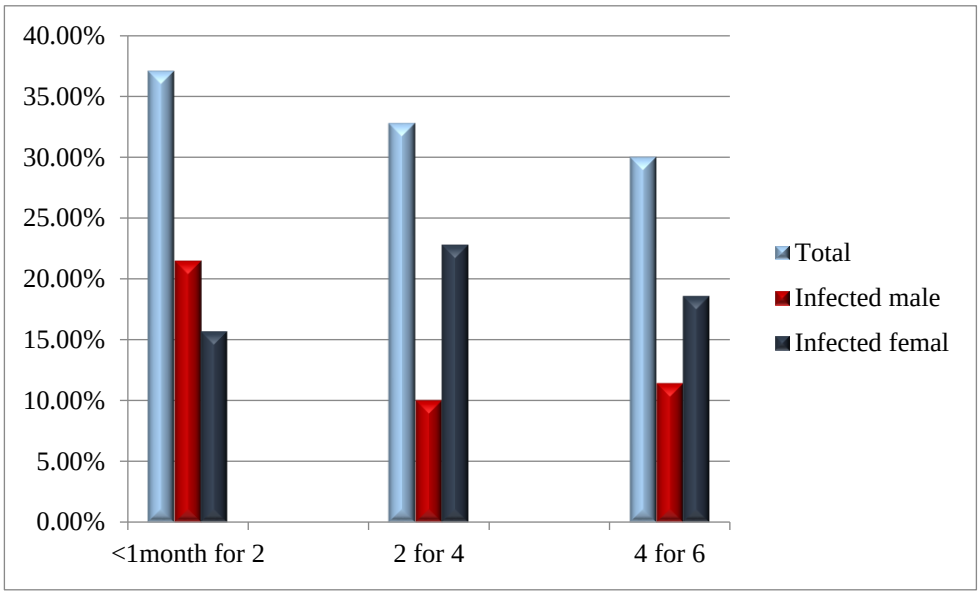

Fig. 2 Distribution of UTIs in relation to age groups

Incidence rate of UTIs according to the residence of the patients

In rural area, the incidence rate of UTIs was significantly high in comparison to urban The incidence rate of UTIs at urban was 34.3% and 65.7% in rural. The most probable explanation, is might to be due to poor diagnostic facilities available in the health centers in rural areas and also the lower socioeconomic, hygienic standards and the absence of a proper sewage system.

Identification of isolated bacteria

By comparing results of microscopic and cultural characteristics, biochemical characteristics and differential susceptibility to special antibiotics like: Rifampin and Bacitracin 10 µ to differentiate *Staphylococcus* from *Micrococcus*, Novobiocin 5µ to differentiate *Staphylococcus saprophyticus* from other staphylococci, Bacitracin susceptibility 0.04µ to differentiate *Streptococcus pyogenes* from other negative gram positive cocci. The microscopic examination showed that 37 (52.8%) of isolates were gram negative (*Escherichia coli, Klebsiella pnuemoniae, Pseudomonas aeruginosa and Proteus vulgaris)*. Also showed that there were 33 (48.2%) of isolated were gram positive (*Staphylococcus aureus,* coagulase-negative Staphylococci (CoNS), *Enterococcus faecalis, Staphylococcus saprophyticus* and *Streptococcus spp*).

Four species of isolates showed positive growth on MacConkey agar are belong to gram negative bacteria were isolated accordance to the growth on MacConkey agar. These were differentiated preliminarily into lactose fermenters and lactose non fermenters which include; *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris*.

Most of *Pseudomonas aeruginosa* isolates were easily recognized on different media due to their ability to produce green pigment confirmed with positive oxidase.

Gram positive bacteria

33 (48.2%) of isolates were gram positive show the characteristics used in the diagnoses and distribution cocci of gram positive, which were confirmed by Holt et al. [44]. and Harley and Prescott [25].

Differentiation of isolated bacteria by CHROMagar^{TM} orientation

This technology is supported the result of identification and making final diagnosis, in which bacterial pathogenes on this medium [26]. Bacterial pathogenes on this medium as the following; *Eschercia coli* is dark pink to reddish, *Enterococcus* is turquoise blue, *Klebsiella sp. is* metallic blue, *Proteus sp.* is brown halo, *Pseudomonas* is cream and translucent, *Staphylococcus aureus* is golden and small, *S.saprophyticus* is pink, opaque and small, *Streptococcus spp.* light blue-green.

Antimicrobial susceptibility

The results of antibiotic susceptibility are appeared in (Fig. 3a-n).

The results of antibiotic susceptibility are appeared in (Fig. 3a), most of our bacterial isolates were highly resistant to ampicillin, we found that 96.3% of *E. coli*, 85.7% of *Klebsiella pneumonia* and all others bacteria in our bacterial isolates were resistant to ampicillin.

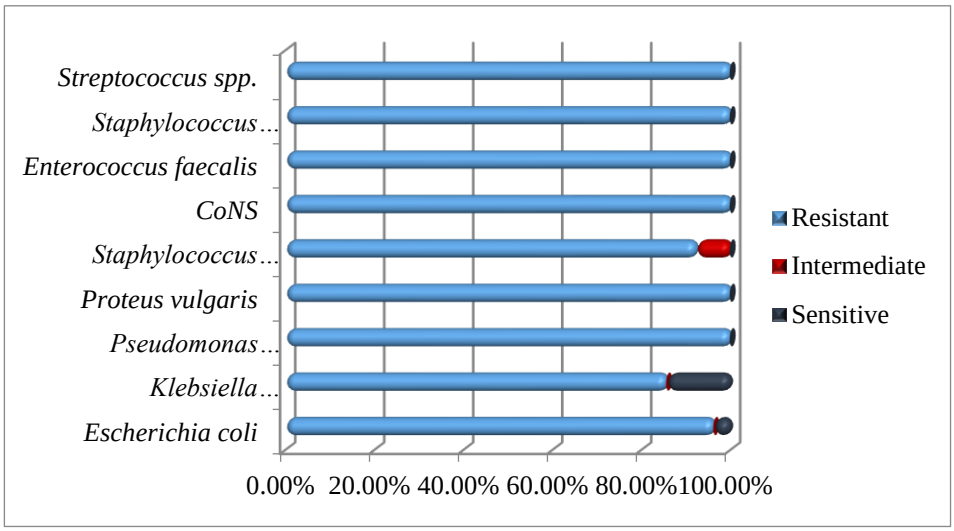

Fig. 3a Antimicrobial susceptibility to Ampicillin

Our result appeared that most of the isolated bacterial uropathogens are resistant to the ceftazidime and ceftriaxone (Fig. 3b).

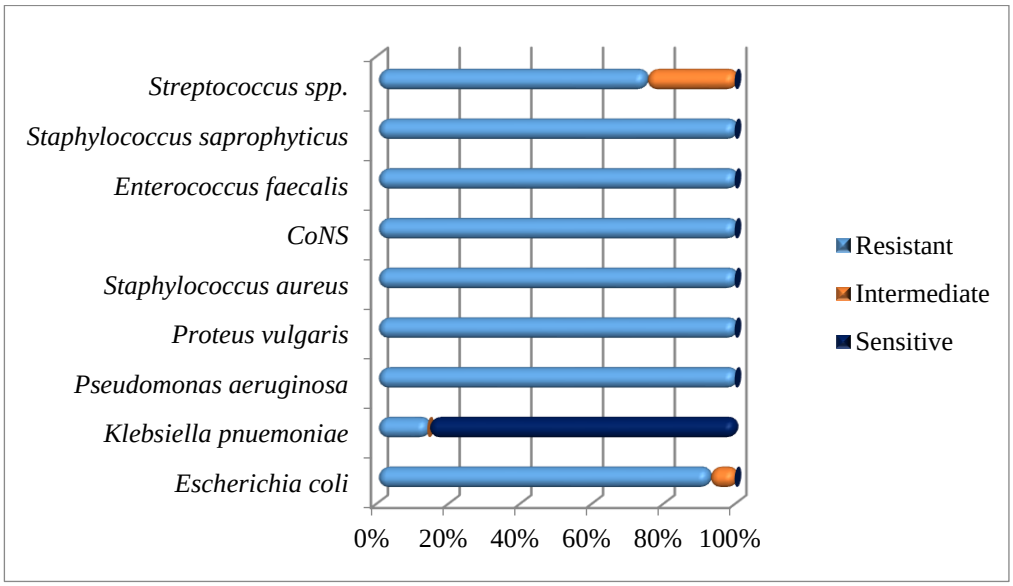

Fig. 3b Antimicrobial susceptibility to Ceftazidime

Most of isolates bacterial uropathogens were resistant to the ceftriaxone (Fig. 3c).

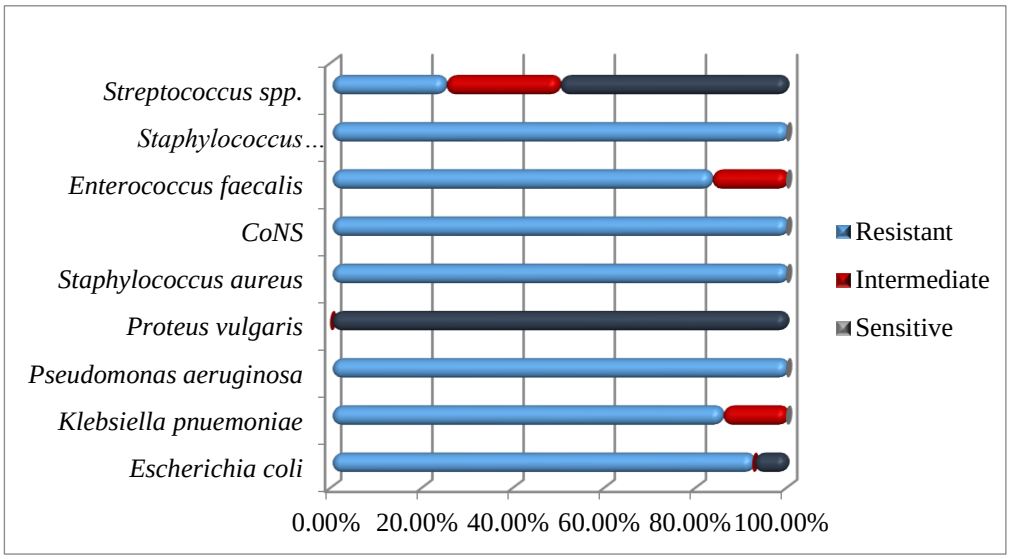

Fig. 3c Antimicrobial susceptibility to Ceftriaxone

As shown in the (Fig. 3d), 55.5% of *Escherichia coli*, 42.8% of *Klebsiella pneumoniae*, 84.6% of *Staphylococcus aureus*, 87.5% of CoNS and 50% of *Enterococcus faecalis* were resistant to azithromycin.

According to Zuckerman et al. (2009), Azithromycin inhibits protein synthesis due to reversibly ties to the bacterial ribosome [45].

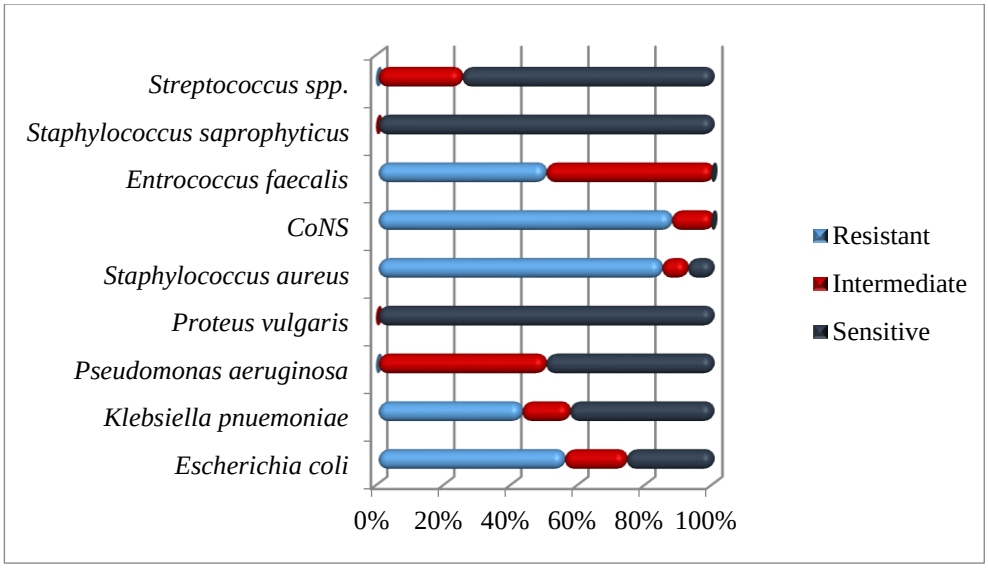

Fig. 3d Antimicrobial susceptibility to Azithromycin

The percentage of resistance to cefotaxime are shown in (Fig. 3e), Bacteria can resist these cefotaxime and ceftriaxone by production of β-lactamase especially CTX-M beta-lactamases class A [46].

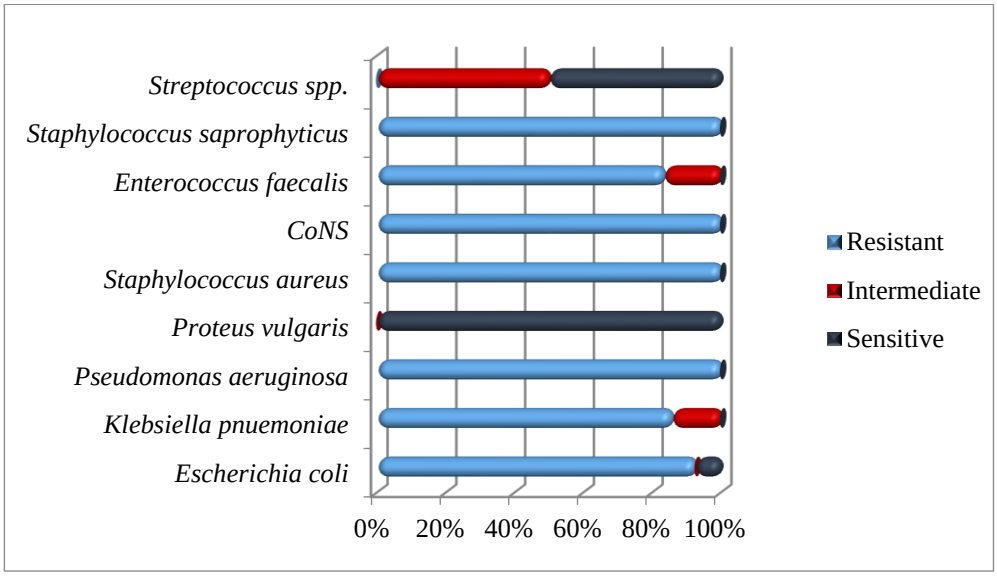

Fig. 3e Antimicrobial susceptibility to Cefotaxime

The results that the resistance percentage of isolates bacterial uropathogens for augmentin (amoxicillin-clavulanic acid) shown (Fig. 3f).

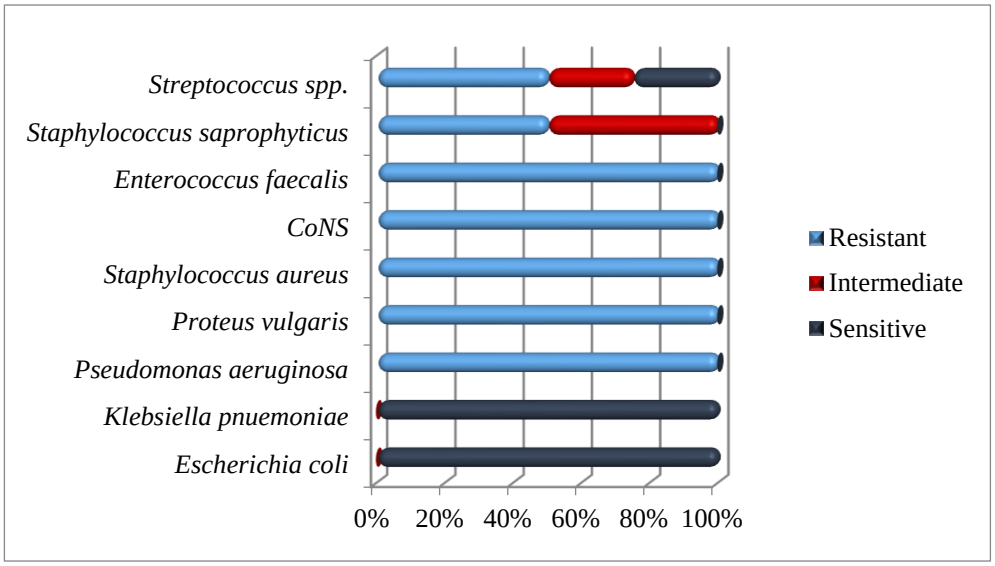

Fig. 3f Antimicrobial susceptibility to augmentin

In our study, the percentage of resistance to tobromycin are shown in and (Fig. 3g) for isolated bacterial uropathogens.

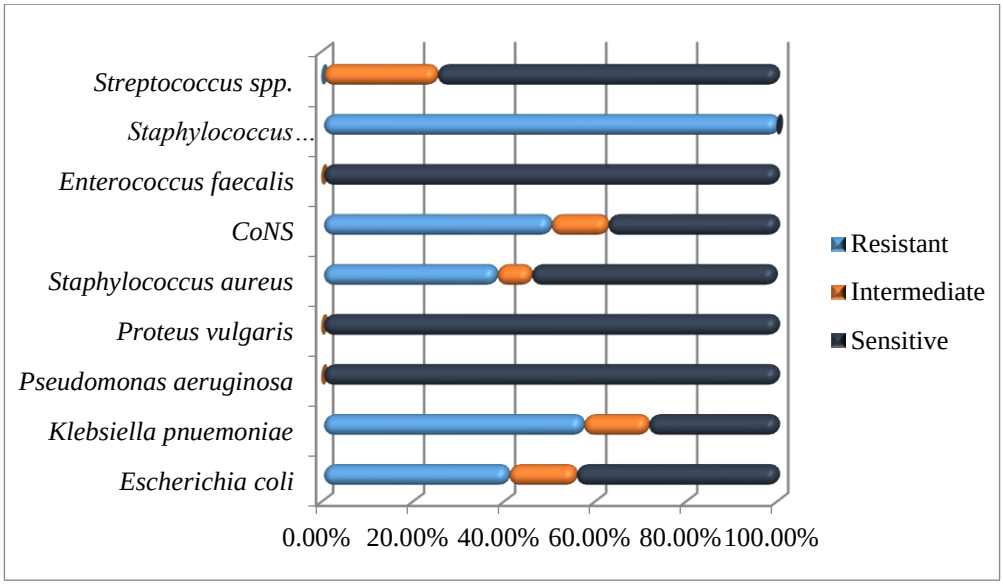

Fig. 3g Antimicrobial susceptibility to tobromycin

As shown in (Fig.3h), we found that 44.4% of *E. coli*, 28.6% of *Klebsiella pneumonia*, 15.4% % of *Staphylococcus aureus*, 12.5% of CoNS and 50% of *Staphylococcus saprophyticus* were resistant to ciprofloxacin. According to Diver and Wise (1986) Ciprofloxacin inhibit bacterial DNA synthesis and promote cleavage of DNA leading to bacterial cell death [47].

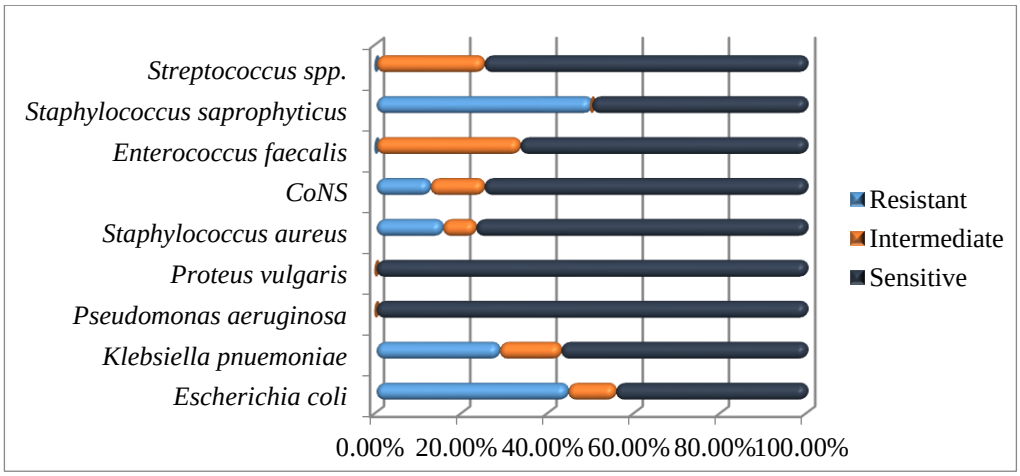

Fig. 3h Antimicrobial susceptibility to ciprofloxacin

In this study, the antibiotic susceptibility tests shown that 85.1% of *E. coli*, 100% of *Klebsiella pneumonia*, 100% of *Pseudomonas aeruginosa*, 100% of *Proteus vulgaris*, 76.9% of *Staphylococcus aureus*, 100% of CoNS, 66.66 % of *Enterococcus faecalis* and 100% of *Streptococcus spp.* were sensitive to meropenem (Fig.3i).

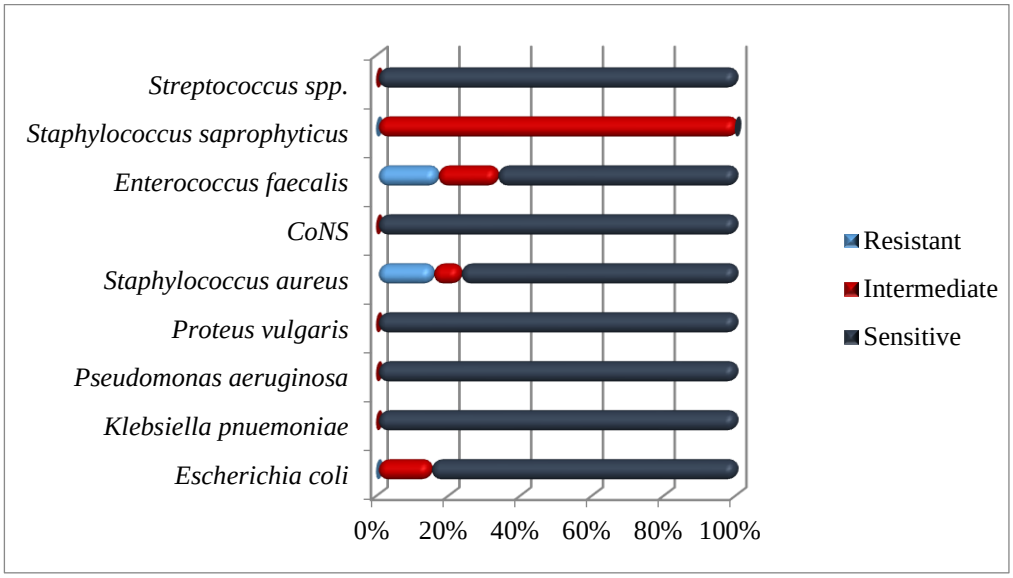

Fig. 3i Antimicrobial susceptibility to meropenem

Antimicrobial susceptibility to trimethoprim-sulphamethoxazole showed in the (Fig. 3j). High resistance to trimethoprim sulphamethoxazole seen in many other studies in different countries [10, 18].

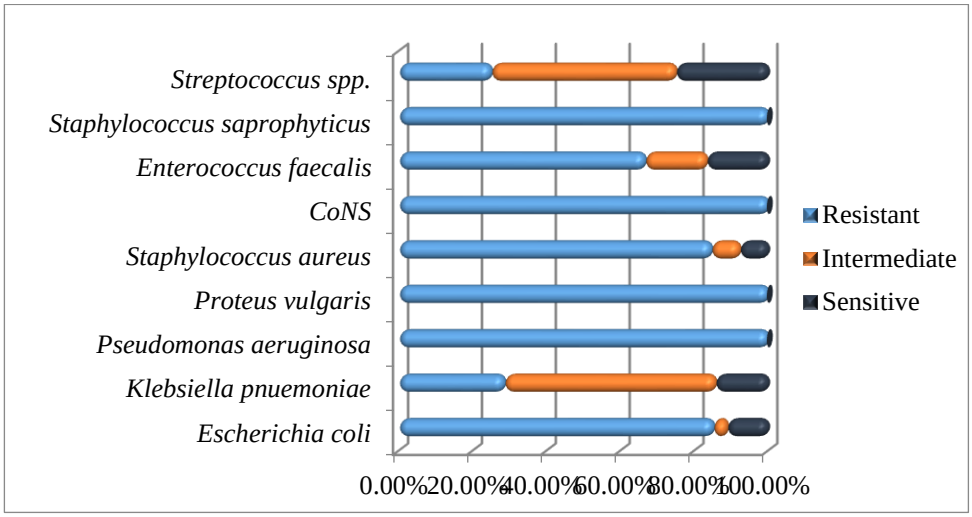

Fig. 3j Antimicrobial susceptibility to trimethoprim-sulphamethoxazole

Antimicrobial susceptibility to gentamycin showed at (Fig. 3k).

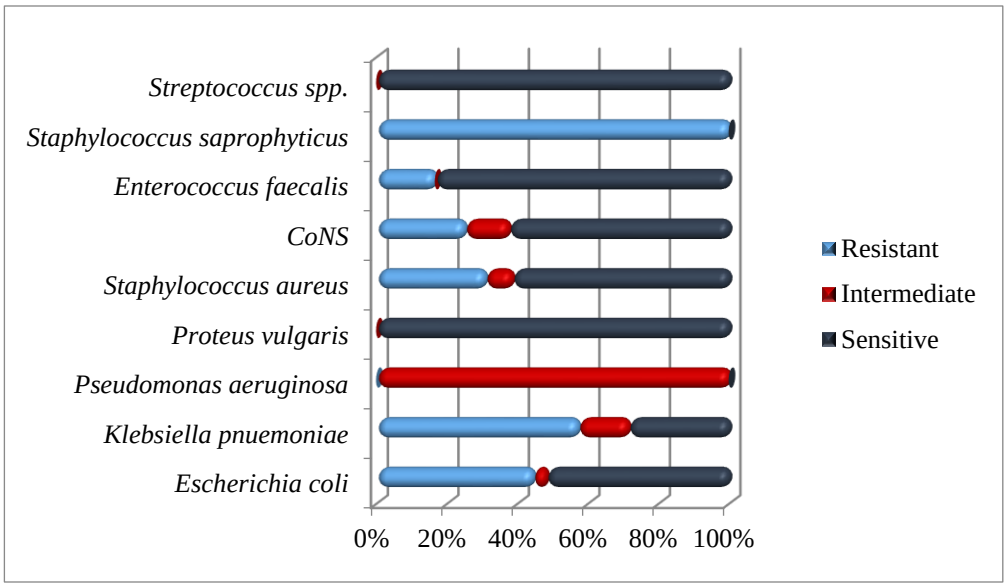

Fig. 3k Antimicrobial susceptibility to gentamycin

The results of present study showed that the sensitive percentage of isolates bacterial uropathogens for levofloxacin were shown (Fig. 3l). Levofloxacin is active against both Gram-positive and Gram-negative bacteria [48].

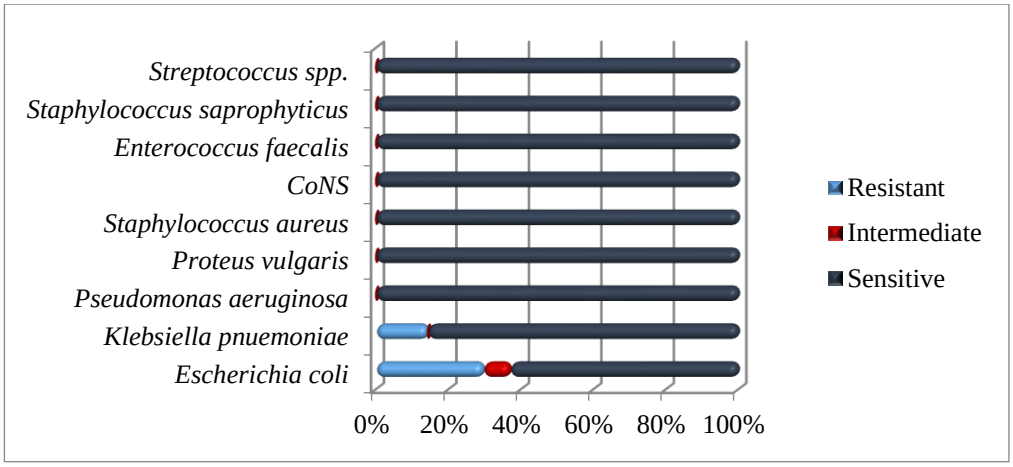

Fig. 3l Antimicrobial susceptibility to levofloxacin

In our results that the sensitive percentage of isolates bacterial uropathogens for impenem were shown at (Fig. 3m).

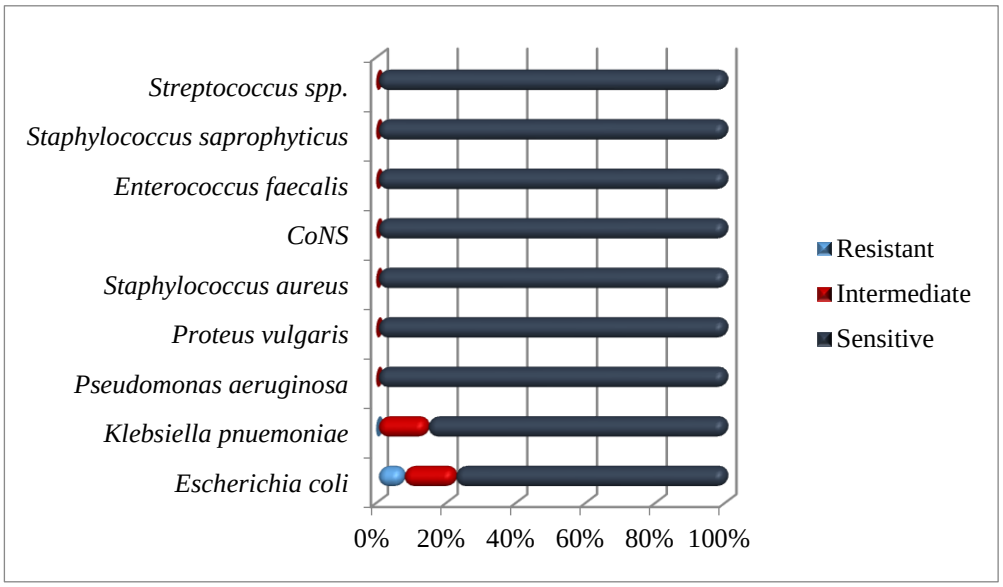

Fig. 3m Antimicrobial susceptibility to imipenem

Antimicrobial susceptibility to vancomycin showed in the (Fig. 3n). Vancomycin is active just against gram-positive organisms.

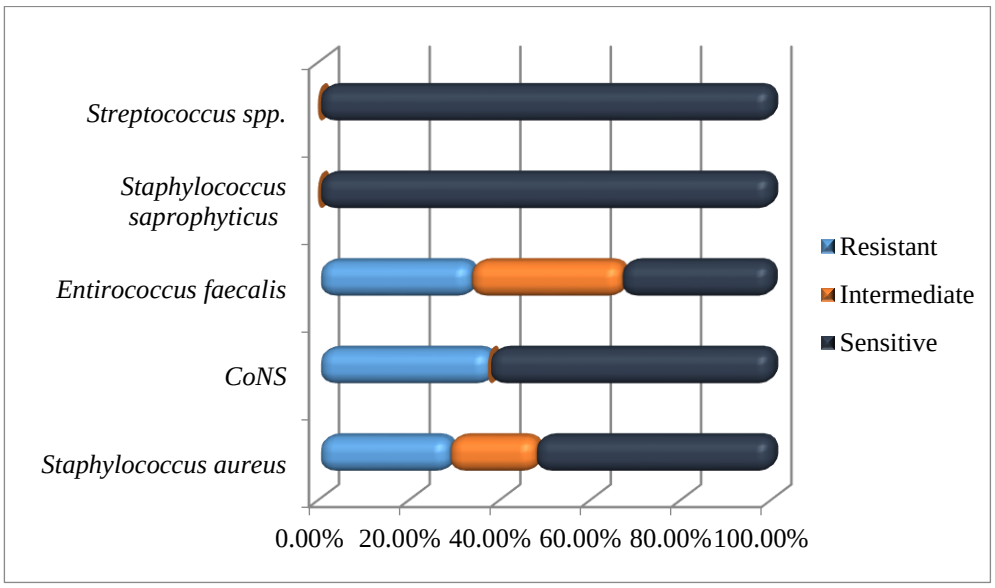

Fig. 3n Antimicrobial susceptibility to vancomycin

In our study and after we mentioned all previous susceptibility tests results to 14 different antibiotics which commonly used in hospitals, shown us that a higher prevalence rate of resistance to commonly prescribed antibiotic agent, all the bacterial uropathogens showed

the highest degree of resistance to ceftazidime, ceftriaxone, cefotaxime, trimethoprim-sulphamethoxazole, ampicillin and augmentin. This result agrees with results obtained by Sahilin et al. [49] in India, by AbdulRazzaq (2013) [18] in Mosul, and by Mezal and coworkers (2011) [50] in Basrah. while this drugs exhibited low resistant rate in another study outside Iraq such as Jha and Bapat [51] reported that all the organisms causing UTI were sensitive to ciprofloxacin. Hussein [52] reported that all the organisms causing UTI were sensitive to gentamycin and tobramycin was found to be least effective. In our study, isolates appeared high sensitivity to meropenem, and imipenem, also *levofloxacin* apeared high (more than 90%) sensitivity in all groups except of *Escherichia coli* (62.9%) were sensitive. Yasmeen et al. in Dhaka reported in their study, meropenem, and imipenem appeared high sensitivity, also *levofloxacin* appeared (47.9%) sensitivity of *Escherichia coli*. [53]. Kyoung reported that meropenem, and imipenem appeared very high (over 90%) sensitivity in all groups [54]. Abera and Kibret, in rural community of Ethiopia reported about the resistant and sensitivity of approximate resistance result to azithromycin of UTI causative agents [55].

Detection of quinolone and carbapenem genes

We have amplified four genes of carbapenem and quinolone risistance genes responsible in *Escherichia coli* and *Klebsiella pnuemoniae*, which antibiotics used more than others to treat of infection of bacteria. We have chosen six isolates to detecte the presence of resistance to carbapenem and quinolone genes by (RAPD-PCR), because both of them can share the same sequences antibiotic resistance and they may get them by gene transferring [56].

The results showed that out of five isolates of *Escherichia coli* and one of *K. pneumoniae*, no one of these isolates contained genes in their genomic DNA except of *Klebsiella pnuemoniae* (K 61) contained *qnrB* in its genomic DNA. *qnrA* was more prevalent until 2001, but since then, *qnrB* has predominated [57].

Also others had negative results for detected genes as mention because we have detected specific sizes (bp). Pathogenic bacteria carry another sequences to resist the same antibiotics, for example [58] in China reported in their study, they found that *Escherichia coli* were all resistant to meropenem and imipenem.

Conclusions and Suggestions

The main investigation directed to decide the pervasiveness of UTI. The impact of gender and age on its predominance, and their vulnerability profile to basic utilized anti-infection in the group of Soran city. The study also allows comparison of the situation in Soran city, North of Iraq with different areas outside and inside the state as well as in the country.

According to this study, we concluded, there were no control on getting the drugs and alot of it given to outpatients without physicians prescription from outside of hospital, when infection is occur in the any parts of body**'s** patient, they taking antibiotics without culturing and determination of antibiotic susceptibility for side of infection, this led to kill commensal bacteria and the emergence of different types of antibiotic resistant bacteria.

Abbreviations/Kısaltmalar

UTI: Urinary tract infection, Bp: Base pair, CFU: Colony forming unit, CLSI: Clinical and laboratory standards institute, E.coli: *Eschericia coli*, EMB: Eosin methylene blue agar, PBS: Phosphate buffered salin, PCR: Polymerase chain reaction, QRDRs: Quinolone-resistant determining region, RAPD: Random amplification of polymorphic DNA, VUR: Vesico-ureteral reflux.

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Data Availability statement
The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards
Conflict of interest
The author declare no conflict of interest.
Ethical standards
The study is proper with ethical standards.
Authors' contributions
During the study, Hasan Akan wrote the article and Rawezh Hakeem Mustafa conducted laboratory research.

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Derleme makale/ Review article

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Kanser Gelişiminde Ağır Metallerin Rolü

Nebiye Pelin Türker[1*]

ÖZET

Ağır metallere maruz kalma, insan popülasyonunda önemli sağlık sorunlarını temsil etmektedir. Bu elementler, olumsuz sağlık etkisi yaratma yeteneğine sahip olmasının yanı sıra karsinojenezde de etkilidirler. Pubmed gibi araştırma veritabanlarında, bu ağır metallerin neden olduğu çeşitli maruziyetler ve kanserlerle ilgili çalışmalar bulunmaktadır. Ancak, yine de bu konuda büyük ölçüde bilgi eksikliği vardır. Bu çalışma, maruz kalınan ağır metallere ve hangi vücut sistemlerinin hedeflendiğine ilişkin çalışmaları içermektedir.

MAKALE GEÇMİŞİ
Geliş
17 Eylül 2022
Kabul
18 Kasım 2022

ANAHTAR KELİMELER
Kanser,
ağır metal,
element

The Role of Heavy Metals in Cancer Development

ABSTRACT

Exposure to heavy metals represents significant health problems in the human population. These elements are effective in carcinogenesis as well as having the ability to produce adverse health effects. Research databases such as Pubmed contain studies of various exposures and cancers caused by these heavy metals. However, there is still a great lack of information on this subject. This study includes studies on exposure to heavy metals and which body systems are targeted.

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Giriş

Çevre ile biyolojik sistem arasındaki etkileşim, organizma sağlığı için çok önemli bir faktör olarak belirlenmiştir [1]. Pestisitlerin, boyaların, ağır metallerin, plastiklerin, deterjanların ve kozmetiklerin çeşitli amaçlarla gelişigüzel kullanımı, insan sağlığına ciddi tehditler oluşturabilecek ekolojik ve biyolojik sorunlar için yeterlidir. Ağır metaller olarak adlandırılan metaller ve metaloidler, yüksek atom ağırlığına ve sudan beş kat daha fazla yoğunluğa sahip elementlerdir. Farklı çevresel kompartmanlarda bulunmalarının yanı sıra canlı organizmalarda da eser miktarlarda bulunarak, çeşitli biyolojik süreçlerde önemli rol oynarlar [1]. Örneğin bakır (Cu) hemen hemen tüm dokularda bulunur ve çeşitli metabolik reaksiyonlar için gereklidir; demir (Fe), oksijen taşınmasında ve nükleik asit sentezinde hayati bir rol oynamaktadır, çinko (Zn) düzenleyici ve yapısal öneme

[1] Trakya Üniversitesi, Teknoloji Araştırma Geliştirme Uygulama ve Araştıra Merkezi, Edirne, Türkiye
*Corresponding Author: Nebiye Pelin Türker, e-mail: npelinturker@trakya.edu.tr

sahiptir. Benzer şekilde, molibden (Mo), sülfit oksidaz, ksantin oksidaz, aldehit oksidaz için bir kofaktör görevi görmektedir. Selenyum (Se), kobalt (Co) ve krom (Cr) sırasıyla antioksidan savunma sistemlerinde, B12 vitamini sentezinde ve glikoz metabolizmasında rol oynar [2]. Ağır metaller biyolojik öneme sahip olmanın yanı sıra endüstriyel, tarım, sağlık, kozmetik, ilaç gibi farklı sektörlerde ve evsel amaçlarla da çeşitli uygulamalara sahiptir. Ağır metaller, artan kullanımı ve yaygın dağılımı ile organizma sağlığını tehdit etmektedir. Bu metallere maruz kalma, insan popülasyonunda önemli sağlık sorunlarını temsil etmektedir. Araştırmalar, ağır metal maruziyetinin sinir, hematolojik ve kardiyovasküler sistemlerin gelişimine zarar verdiğini ve böbrek, akciğer, karaciğer, cilt ve mide kanseri dahil olmak üzere çok sayıda kanser riskini artırdığını göstermiştir [3]. Cr, Pb, As, Cd ve Hg gibi bazı metaller, Uluslararası Kanser Araştırmaları Ajansı tarafından kesin veya olası kanserojenler olarak sınıflandırılmaktadır [4]. İstatistiksel analizlerde, Türkiye'de Simav Ovası'nda bulunan köylerdeki karaciğer, mesane ve mide kanserlerinin çoğunun içme suyundaki yüksek As konsantrasyonları ile ilişkili olduğunu gösterilmiştir [5]. Toprak, meyve ve sebzelerin ağır metal kirliliği, Türkiye'de yüksek oranda gastroistestinal (GI) kanserlerine yol açmaktadır. Ağır metaller, E-kadherinin işlevini etkiyerek ve reaktif oksijen türleri (ROS) hasarını indüklemektedir. Bu metaller doğrudan veya dolaylı olarak ROS oluşumunu indüklemekte ve daha sonra gen regülasyonunu, sinyal iletimini ve hücre büyümesini değiştiren gastrik mukoza ve DNA lezyonlarına neden olmaktadır. Bu durum da karsinogeneze yol açmaktadır. Düşük ve orta miktarda ROS'un, istilacı patojenleri öldürdüğü ve yara iyileşmesini/doku onarımını indüklediği için faydalı olduğu bilinmektedir [2, 6]. Buna karşılık, yüksek konsantrasyonlarda ROS, hücre yapılarına, lipitlere, zarlara, proteinlere ve nükleik asitlere verilen hasara aracılık etmektedir. ROS'un endojen veya eksojen olarak kümülatif üretimi, çeşitli kanserlerde hücresel redoks dengesizliğini indüklemektedir [7]. Oksidatif stresi indükleyen, dolayısıyla GI yolunu doğrudan veya dolaylı olarak etkileyen çok sayıda dış tetikleyici bulunmaktadır [7]. Pb, Cd, Hg, Cr ve As dahil olmak üzere ağır metaller, ROS'un yaygın eksojen kaynaklarıdır ve doğrudan veya dolaylı olarak ROS oluşumunu indükleyebilmektedirler [7]. Ağır metaller, DNA hasar onarımını engellemekte veya yetersiz lezyon onarımına neden olmaktadırlar. Bu metaller diğer gen anormalliklerini indükleyebilmektedirler. Ek olarak, ağır metaller, tümör oluşumunu destekleyen proinflamatuar kemokin interlökin-8 (IL-8) ve mikroRNA'ların

ekspresyonunu indükleyebilmektedir [8, 9]. Metal iyonlarının tiroidde biriktiğine dair çalışmalar mevcuttur ve bazılarının tiroid bezinin işlevinde ve homeostatik mekanizmalarında önemli bir rol oynadığı bilinmektedir. Bazı metal iyonları endokrin bozucular olarak bilinirken diğerleri kanserojen olarak sınıflandırılmaktadır. Volkanik bölgeler de dahil olmak üzere toprakta ve içme suyunda yüksek metal seviyeleri bulunan bölgelerde daha yüksek tiroid kanseri insidans oranları bildirilmiş olsa da ağır metallerin tiroid üzerindeki etkisi de hala tam olarak anlaşılamamıştır [10]. Araştırma veritabanlarında, bu ağır metallerin neden olduğu çeşitli maruziyet kalıpları ve kanserlerle ilgili çok sayıda bilgi bulunmaktadır. Ancak, bu bilgiler bu noktada büyük ölçüde eksik kalmıştır, bu da bu araştırmanın konsolidasyonunu gerektirmektedir. Bu derleme, ağır metaller olan alüminyum, arsenik, berilyum, kadmiyum, kurşun, cıva ve nikelin insan sağlığı üzerindeki etkisiyle ilgili araştırmaları vurgulamaktadır. Araştırma PubMed aracılığıyla toplanmış ve bu metallere maruz kalma kaynakları, indüklenen kanser türleri ve terapötik önlemler hakkındaki mevcut bilgileri değerlendirmek için derlenmiştir. Ayrıca, ağır metaller ve kanser ile ilgili gelecekteki araştırma çabalarına rehberlik etmek için tasarlanmıştır.

Alüminyum

Alüminyum, çok sayıda maruz kalma yoluna sahip benzersiz bir ağır metaldir. Bu elemente maruz kalma, kontamine gıdalarda, daha güçlü bir bağışıklık tepkisi ortaya çıkarmak için aşılarda ve endüstriyel işlemlerde ve ticari ürünlerde kullanılan alüminyum tuzlarında belgelenmiştir [11].

Özel ticari ürünlerden belirli antasitler ve terlemeyi önleyici deodorantlar, alüminyum tuzlarını içermektedir [11]. Alüminyuma maruz kalma, meme dokusunda karsinojenez ile ilişkilendirilmiştir. Antiperspirant deodorantlarda kullanılan ve alüminyum tuzu olan $AlCl_3$'e maruz bırakılan fareler, meme bezi epitel hücrelerinde habis büyüme sergilemişlerdir [11]. Aynı sonuç, insan meme hücresi örnekleri üzerinde yapılan çalışmalarda da gözlenmiştir [12]. Bu ağır metalin ayrıca sarkomların gelişiminde de rolü olduğu bilinmektedir [13]. Ek olarak, bir hastada, alüminyum içeren ağır metal tuzlarının kronik maruziyetinin atipik bir nöroektodermal tümör gelişimi ile sonuçlandığı öne sürülmüştür [13]. İnsan meme hücrelerinin alüminyum elementine maruz bırakılması ile yapılan bir çalışmada, BRCA1 tümör baskılayıcı geninin mRNA konsantrasyonlarının azaldığı bulunmuştur [12]. Ayrıca bu çalışmada alüminyumun, östrojen reseptörleri için

bir agonist gibi davranan ve memede karsinogenez için bilinen bir riski temsil eden bir metaloöstrojen gibi davrandığı gözlemlenmiştir [12]. Başka bir çalışmada, insan göğüs hücrelerinin alüminyuma maruz bırakılmasının kontrolsüz büyümeyi indükleme potansiyeline sahip olduğunu belirlenmiştir. Alüminyum zehirlenmesini takiben yapılan standart bir tedavi de, şelatörlerin kullanılması olmuştur. Terapötik olarak kullanılan yaygın şelatör desferrioksamindir. Bu şelatörün, alüminyumun vücuttan atılmasında etkili olduğunu kanıtlanmıştır; bununla birlikte, kullanımıyla ilişkili bir takım toksik yan etkiler bulunmaktadır. Desferrioksaminin yerini alacak birkaç umut verici aday olmuştur, ancak şimdiye kadar hiçbiri bu kadar etkili olmamıştır. Vücudun başka bir bölgesinde analiz edilen mesane kanseri örneklerinde, analiz edilen ağır metaller arasında alüminyum seviyeleri, istatistiksel olarak daha yüksek bulunmuştur. Bu sonuç, alüminyumun mesanedeki malign büyümede en azından destekleyici bir rol oynadığını düşündürmüştür [14]. Alüminyumun toksik etkilerini sınırlamada en başarılı yöntem metale maruziyeti azaltmaktır. Bu maruziyetin azaltılmasının bir çözümü de, ters ozmoz filtrasyonunun kullanılmasıdır. Bu teknoloji, deneysel aşamada bakır madenciliği atıklarından önemli düzeyde alüminyum çıkarma yeteneğini göstermiştir [15].

Arsenik

Arsenik sitotoksik bir elementtir ve bu metale maruz kalmak insan sağlığı için ciddi riskler taşımaktadır [16]. Arsenik ile temas genellikle kontamine gıda ve su alımından, mesleki maruziyetten ve çevre kirliliğinden kaynaklanmaktadır [17]. Bu ağır metale çevresel maruziyetin bilinen bir kaynağı da, insan besin zincirine girme potansiyeline sahip kontamine toprakla temastır [18]. Bu ağır metal, çok çeşitli habis büyümelerde tespit edilmiştir. Yapılan araştırmalar arseniğin; akciğer, mesane ve cilt kanseri gelişimindeki rolünü güçlü bir şekilde desteklemektedir [19]. Bir araştırmada, kolon, mide, böbrek, akciğer ve nazofaringeal kanserlerin maruz kalma ve ölüm oranları arasında güçlü bir pozitif ilişki saptanmıştır [18]. Epidemiyolojik çalışmalar ayrıca arseniğe düşük düzeylerde kronik maruziyetin pankreas kanseri ve Hodgkin dışı lenfoma gelişimi arasında bir ilişki olduğunu öne sürmüştür [20, 21].

Bu ağır metal için iyi belgelenmiş kanserojen mekanizmalar, reaktif oksijen türlerinin (ROS) oluşumunu, epigenetik değişiklikleri ve dinamik DNA sisteminde hasarı içermektedir [17, 20]. Arsenik tarafından indüklenen epigenetik değişiklikler, tümü malign büyümeye neden olma potansiyeline sahip değişiklikler olan DNA metilasyonu,

histonlar ve miRNA durumundaki değişiklikleri içermektedir [17, 18] Başka bir çalışmada, bu toksik metalin akciğer epitel hücrelerine ek olarak makrofajlar için uygun olmayan büyüme döngülerini indükleyebileceği bulunmuştur [22]. Ayrıca, arsenik tarafından üretilen ROS'a maruz kalan makrofajların, potansiyel akciğer karsinogenezi ile korele olan M2 fazı yoluyla aktive ederek yanıt verdiği gözlenmiştir [22]. Arsenik, insan akciğer epitel hücrelerine karşı spesifik bir etki mekanizması sergilemiştir. Bu ağır metalin, p53 proteininin ekspresyonunu değiştirdiği belirlenmiş, bu da bir aşağı akış hedefi olan p21'in ekspresyonunun azalmasına neden olmuştur [23]. Arseniğin daha fazla incelenmesi, doğal bir antioksidan olan glutatyonun hücre içi konsantrasyonlarını azaltma yeteneğini ortaya çıkarmıştır [24]. Bu da, hücrenin oksidatif strese maruz bırakılmasıyla oluşan kanserojen aktivite potansiyelini taşımaktadır [24]. Bu ağır metal için önerilen ek bir kanserojen mekanizma, baz eksizyon onarımınıdır [25]. Yapılan çalışmada; DNA polimeraz beta enzimi baz eksizyon onarım sistemine dahil olarak arseniğin aktivitesini inhibe ettiği gözlenmiştir [25]. İnsan mesane hücrelerinde tümörijenik aktivite için başka bir yeni yol keşfedilmiştir. Bu çalışma da, kronik arsenik maruziyetinin morfoloji değişikliklerini indükleme ve proliferasyonu düzenleyen proteinler için gen ekspresyonunu değiştirme potansiyeline sahip olduğunu belirlenmiştir. Arseniği vücuttan atmanın en etkili yolu şelatör kullanımı olarak kalmıştır. Rac-2,3-dimerkaptopropanol, iki tiyol fonksiyonel grubu içermesinden dolayı, bu metal için öne çıkan bir şelatör olmuştur [26]. Bu noktada klinik veriler olmamasına rağmen, akut arsenik zehirlenmesi olan bir kişide 2,3-dimerkaptopropan-1-sülfonat kullanılmıştır [27]. Bu terapi, sınırlı yan etkilerle başarılı bir tedavi ile sonuçlanmıştır, bu da gelecekteki çalışmaların önemini ortaya koymaktadır [27]. In vitro analizler, arsenik trioksitin eritrolösemik hücre dizilerinde ve normal hemopoietik progenitör hücrelerde (HPC'ler), Stat5 aktivasyonunun inhibisyonu ve Bcl-X(L) hedef genlerinin ve glikoforinA'nın azaltılmış ekspresyonu gibi, bütünlüğü eritroid hücre hayatta kalması ve farklılaşması için gerekli olan eritroid transkripsiyon faktörleri Tal-1 ve GATA-1'in bölünmesine yol açan apoptotik mekanizmaların aktivasyonu; ve GATA-1 bütünlüğünü korumak için gerekli olan ısı şoku proteini 70'in azaltılmış ifadesinin arsenik toksisitesinin moleküler mekanizmasının birkaç yolunu içerdiğini göstermiştir [28]. İn vivo olarak, arsenite maruz kalma, farelerde patlama oluşturan birim eritroid (BFU-E) kolonilerinin oluşumunu ve eritroblastların daha ileri aşamalara farklılaşmasını da bozmuşturArsenik maruziyetini

takiben, bu metalin oksidatif stres gibi kanserojen etkilerini azaltmak için diyet antioksidanları önerilmiştir [29]. İnsan maruziyetini sınırlamak için yeni önleme yöntemleri geliştirilmelidir. Pirinç ve elma suyu iki yaygın maruziyet kaynağı olarak kabul edilmiştir [29]. Elma suyu için 5 μg/L arsenik güvenlik standartları, çocuklar tarafından yoğun şekilde tüketilmesi nedeniyle tavsiye edilmiştir [30]. Ayrıca yağmurlama sulama sisteminin kullanımının pirinçteki arsenik konsantrasyonunun çökelmesini indükleyerek, önemli ölçüde azaltma potansiyeline sahip olduğu da gözlenmiştir [29, 30].

Berilyum

Berilyum, endüstriyel süreçlerde ve teknoloji üretiminde kullanılan ağır bir metaldir. Bu element için birincil çevresel kirlilik kaynağının berilyumu toz halinde kullanan enerji santralleri olduğu düşünülmektedir [31]. Bu kontaminant için genel maruz kalma yönteminin inhalasyon olması nedeniyle, mevcut araştırmalar bunun akciğer karsinogenezindeki rolünü araştırmaktadır [31]. Akciğer kanserinde berilyumun rolünü destekleyen incelemeler bulunmaktadır. Yüksek konsantrasyonlarda berilyuma maruz kalan kişilerde akciğer kanseri riskinde artış gözlenmiştir, bu da bu elementin bazı kanserojen mekanizmaları indüklediğini düşündürmektedir [32]. Diş endüstrisinde berilyum kullanımı, maruziyet için ek mesleki risk oluşturmaktadır [32]. Koruyucu ekipman kullanımının bireylerde maruziyet düzeyini önemli ölçüde azalttığı belirlenmiştir [33]. Ek olarak, III. evre meme kanserli hastalarda yüksek berilyum konsantrasyonları tespit edilmiştir [34]. Berilyuma maruz kalma ayrıca osteosarkomların potansiyel gelişimi için bir risk olarak kabul edilmektedir. Berilyumun kanserojen mekanizmalarıyla ilgili çok fazla araştırma yapılmamıştır. Şu anda var olan literatürün çoğu, akciğerler üzerinedir. Örneğin, tanımlanan potansiyel olarak kanserojen bir mekanizma, akciğerlerdeki $CD4^+$ T hücrelerinden daha yüksek düzeyde tümör nekroz faktörü alfa (TNF-α) sitokin salgılanmasındaki rolüdür. Bu proteinlerin her ikisinin de enflamasyon sürecinde bir rolü vardır ve bunların yüksek varlığının kronik enflamasyonda etkili olduğundan şüphelenilmektedir [35]. Berilyum ayrıca genetik değişiklikleri indükleme potansiyeline sahiptir. Örneğin, bu ağır metalin, bilinen bir tümör baskılayıcı gen olan p16 genini metillediği ve inaktivasyonunu indüklediği gözlemlenmiştir. Şelatörler, berilyumu vücuttan atmak ve toksik etkilerini azaltmak için kullanılan yaygın tedavi biçimleridir. İlgili şelatörler, hayvanlarda test edildiğinde etkili

olduğu kanıtlanan 4,-dihidroksi-1,3-benzen disülfonik asit disodyum tuzu (Tiron) ve D-penisilamindir (DPA) [36]. Ayrıca, ağır metal zehirlenmesi yaşayan bir çocuğu kurtarmak için yapılan bir vaka çalışmasında mezo-2,3-dimerkaptosüksinik asit (DMSA) şelatör kullanılmıştır [37]. Bu sonuç, potansiyel klinik önemi orta koyarak daha fazla araştırma yapılması gerektiğini düşündürmüştür [37]. Bu metale maruziyeti, özellikle mesleki maruziyeti azaltmak için önemli çalışmalar bulunmaktadır.

Kadmiyum

Kadmiyum son derece toksik bir ağır metaldir ve çevresel bir kirletici olmasından dolayı önemli sağlık etkileri ile bulunmaktadır [38]. Kadmiyum kirliliği genellikle madencilik, metal araştırmaları, belirli pillerin geliştirilmesi ve pigmentlerde çökelmenin önlenmesi dahil olmak üzere bu elementi kullanan endüstrilerden kaynaklanan emisyonlardan kaynaklanmaktadır [39]. Toprak kirliliği, kadmiyum emisyonlarından kaynaklanan ciddi bir sorundur ve insan maruziyeti tipik olarak soluma, sigara içme, kontamine yiyecek ve su yoluyla ortaya çıkmaktadır [39, 40]. Diğer bir çevresel kontaminasyon kaynağı, belirli durumlarda güvenlik standartlarını aşan seviyelerde kadmiyum içerdiği gözlemlenen çöplüklerdir [41]. Ek olarak, bu metalin kontamine gıdalardan alınması, tipik bir maruziyet kaynağı olarak kaydedilmiştir [20, 41]. İnsanlar mesleki faaliyetlerden, sigara içmekten, Cd ile kontamine tozdan ve kontamine gıdaların yenmesinden kaynaklanan Cd'ye maruz kalmaktadır. Cd, diğer dokulara yaygın olarak dağılmakta ve uzun bir yarı ömre (yaklaşık 10 yıl) sahiptir, bu da yüksek toksisitesine katkıda bulunmaktadır [42, 43]. Kadmiyuma maruz kalma, meme, yemek borusu, mide, bağırsaklar, prostat, akciğerler ve testisler dahil olmak üzere birçok dokuda karsinojenez ile ilişkilendirilmiştir [39, 44-46]. Kadmiyum ayrıca safra kesesinin kanserleşme sürecinde önemli bir role sahiptir. Karsinogenez için bir risk faktörü olarak kabul edilen safra taşlarının bileşimi, bu tip kanserli hastalardan analiz edilmiştir. Diğer ağır metallere kıyasla istatistiksel olarak daha yüksek kadmiyum konsantrasyonları gözlenmiştir. Mesanenin malign büyümesinde de, kadmiyumun potansiyel bir rolü olduğu düşünülmektedir [47]. Kadmiyum ayrıca laboratuvar ortamında karaciğer hücreleri üzerinde kanserojen aktivite göstermiştir [44]. Ek olarak, malign gliomalı hastalarda artan kadmiyum konsantrasyonları tespit edilmiştir, bu da beyinde karsinogenezin potansiyel bir rolü olduğunu düşündürmüştür. Kadmiyumun kanserojen etki gösterdiği öne sürülen bir diğer organ da pankreastır. Bu metalin ayrıca kronik miyeloid ve lenfoblastik lösemi gelişimi ile de şüpheli bir ilişkisi

bulunmaktadır. Kontrollerle karşılaştırıldığında, bu lösemi formlarına sahip hastaların kan ve serum örneklerinde önemli ölçüde yüksek kadmiyum konsantrasyonları ve daha düşük magnezyum seviyeleri gösterdiği belirlenmiştir [48]. Bu metalle yapılan çalışmalarda, idrardaki artan kadmiyum konsantrasyonunun gastrointestinal kanser geliştirme riski ile güçlü bir şekilde ilişkili olduğu belirlenmiştir [14, 49].

Diğer ağır metallere benzer şekilde, kadmiyum ile ilişkili kanserojen mekanizmalar arasında ROS üretimi, epigenetik değişiklikler, DNA onarım süreçlerinin inhibe edilmesi ve apoptoz yer almaktadır [39, 45, 50]. Hem kronik hem de akut kadmiyum maruziyetinin, malign büyüme için artan bir risk oluşturan gen düzenlemesinde değişiklikleri indükleme yeteneğine sahip olduğu gösterilmiştir. Artmış ekspresyon sergileyen anahtar proteinler, hücre yıkımını düzenlemede yer alan bir protein olan DNAJB9'u ve metallotiyoneinleri içermektedir. Ayrıca transkripsiyonun düzenlenmesinde rol oynayan bir protein olan EGR-1'in ekspresyon seviyelerinde azalma tespit edilmiştir [44]. Kadmiyum zehirlenmesinin tedavisi için şu anda herhangi bir standart terapötik önlem bulunmamaktadır [51]. Bununla birlikte, bu metalin toksik etkilerini azaltan bileşikler geliştirmek için devam eden araştırmalar vardır. Örneğin, kadmiyum için seçici afiniteye sahip benzersiz peptoid ligandlar geliştirmek için araştırmalar yapılmıştır [51]. Ayrıca çoğu bitkide bulunan bileşikler olan flavonoidlerin antioksidan özelliklere sahip olduğu ve kadmiyum atomlarını şelatlayabildiği belirlenmiştir. Flavonoidlerin yapısının kadmiyum üzerindeki etkisi ile nasıl ilişkili olduğunu belirlemek için daha fazla çalışma önerilmektedir [52]. Kadmiyumun neden olduğu hasar için terapötik bir önlem olarak kök hücrelerin kullanımına ilişkin araştırmalar da mevcuttur. Bir çalışmada, sıçan testisleri zararlı seviyelerde kadmiyuma maruz bırakılmıştır [53]. Kemik iliği mezenkimal kök hücreleri ile tedavi üzerine, sıçan testislerinin apoptoz regülasyonu ile ilgili daha uygun protein seviyeleri gösterdiği gözlenmiştir. Ek olarak, bu hücrelerin hasarlı testis dokusunu restore ettiği belirlenmiş ve olası bir mekanizmanın mitokondriyal apoptoz ile ilişkili olduğu öne sürülmüştür [53].

Kurşun

Kurşun (Pb) toksik bir ağır metaldir ve maruz kalınması sağlık için önemli riskler oluşturmaktadır. 1995 yılında ticari olarak satılan benzinde kullanılması yasaklanmasına rağmen, kurşun hala havacılık yakıtına eklenmektedir [54]. Bu çevre kirliliği kaynağının yüksek düzeyde kurşun emisyonuna katkıda bulunduğu belirlenmiştir [54]. Ayrıca sigara

içenlerin, ek bir çevresel maruziyet kaynağını temsil eden yüksek kan kurşun seviyeleri içerdiği belirlenmiştir. Madencilik gibi belirli meslekler de kurşun maruziyetinde rol oynamaktadır [55].

Artmış kurşun maruziyetinin herhangi bir kanser türüyle ilişkili olup olmadığını belirlemek için çeşitli epidemiyolojik çalışmalar yapılmıştır. Pb'ye maruz kalma hem eritrositler hem de lökositler dahil olmak üzere kan hücrelerinin sayısını azaltmaktadır. Pb maruziyetine bağlı olarak kemik iliği üzerindeki toksik etkiler, insanlarda mesleki maruziyetler altında veya hayvan deneysel zehirlenme modellerinde daha düşük seviyelerde tanımlanmıştır [42]. Ek olarak, mevcut araştırmalar bu noktada kurşunun kanserde nedensel bir rolü olmadığını, ancak daha destekleyici bir rol oynayabileceğini göstermiştir [56]. Kadmiyum ve kurşun, glioma hastalarında önemli ölçüde yüksek konsantrasyonlarda saptanmış ve bu iki metalin bir araya getirilmesinin aşırı toksik etkiler üretebileceğini düşündürmüştür. Bir çalışma da, kurşuna maruz kalma ile böbrek kanseri gelişimi arasında güçlü bir ilişki belirlenmiştir [57]. Başka bir çalışma da, daha yüksek kan kurşun düzeyi olan hastalarda renal hücreli karsinom gelişme riskinin arttığı sonucuna varılmıştır[58]. Safra taşlarında istatistiksel olarak daha yüksek konsantrasyonlarda gözlenen kurşun, birkaç ağır metalden biridir [47]. Bu, bu metale maruz kalmanın safra kesesinde artan bir malign büyüme riskini temsil ettiğini göstermektedir [47]. Kurşuna maruz kalan işçiler üzerinde yapılan bir çalışmada, bu ağır metale maruz kalma ile akciğer dokusunda artan karsinojenez riski arasında pozitif korelasyon ve beyin, gırtlak ve mesane dokularında malign büyüme için marjinal pozitif korelasyon gözlenmiştir [59]. Ekzokrin pankreas kanseri olan bireylerde birkaç ek ağır metalle birlikte kurşunun yüksek seviyelerde tespit edildiği bildirilmiştir. Bu da, kanserin gelişiminde bilinmeyen bir mekanizma olduğunu düşündürmüştür [20].

Mevcut literatür, kurşunun kanserojen mekanizmaları hakkında kapsamlı bir anlayış sergilememiştir; ancak, makul mekanizmalar önerilmiştir [60]. Mevcut çalışmalarda kurşun elementinin, hücresel tümör düzenleme genlerini, DNA onarım sistemini bozarak ve DNA hasarını indükleyerek karsinojenik süreci desteklediği varsayılmıştır [61]. Fareler üzerinde yapılan bir çalışmada, kurşunun ROS oluşturma, kromozomal yapı ve diziyi değiştirmedeki rolünü destekleyen kanıtlar bulunmaktadır. Ayrıca kurşunun, bu sistemi düzenleyen bazı proteinlerde çinkonun yerini alarak transkripsiyon sürecini bozma potansiyeline sahip olduğu belirlenmiştir [61]. Epidemiyolojik bir çalışmada,

yüksek serum kalsiyum düzeylerinin renal hücreli karsinom gelişme riskinin daha düşük olmasıyla ilişkili olduğu belirlenmiş ve bu, anlamlılığı belirlemek için klinik bir araştırmaya ihtiyaç olduğu gösterilmiştir. Şelasyon tedavisi, kurşun zehirlenmesi olan bireyler için önerilen tedavi şeklidir [62]. Vücuttaki kurşun seviyelerini azaltmak için yaygın şelatörler arasında British, Anti-Lewisite, kalsiyum disodyum etilendiamintetraasetik asit, *D-penisilamin* ve Meso-2,3-dimerkaptosüksinik asit bulunmaktadır ve spesifik bir şelatörün kullanımı bireyin durumuna göre değişmektedir [62]. Daha az toksik tedavilerin etkinliği konusunda da araştırmalar yapılmıştır. Örneğin, klinik bir ortamda sarımsak verildiğinde, şiddetli olmayan kurşun zehirlenmesinde kandaki kurşun seviyelerini düşürdüğü ve semptomları hafiflettiği bulunmuştur [62]. Kandaki kurşun konsantrasyonunu düşük tutmak için en etkili strateji, maruziyetin önlenmesidir [57]. Bu, önemli düzeyde kurşun emisyonu üreten endüstrilerin ve çalışanların maruziyeti sınırlamak için güvenlik yönergelerine uymasını sağlamayı içermektedir [62]. Ayrıca kurşun kontaminasyon kaynaklarının tanımlanmasının ardından uzaklaştırma veya kaçınmanın bu ağır metale maruz kalmayı azaltmak için ideal çözüm olduğu öne sürülmüştür [62].

Civa

Civa, ciddi sağlık komplikasyonları potansiyeli olan başka bir toksik ağır metaldir. Bu element mineral formunda eser miktarlarda bulunabilmesine rağmen, çevrede bulunanların çoğu işlenmiş civadan kaynaklanan insan kaynaklı kirlilikten olmaktadır [63]. Civa, mesleki ve çevresel kontaminasyona neden olan geniş bir kullanım alanına sahip bir metaldir. Bu ağır metalin çeşitli kaynakları arasında termometreler, fosil yakıt emisyonları, diş dolguları, bazı piller ve yanan tıbbi atıklar bulunmaktadır [63]. Civa bileşikleri; buharlaşarak atmosfere girme veya toprak ve su sistemlerinden içeri girme potansiyeline sahiptir [64-66]. Metil civaya çevresel maruziyetin birincil yöntemi olarak büyük miktarlarda deniz ürünü tüketmek tanımlanmıştır [63]. Civanın ekosisteme girdiği yol henüz keşfedilmemiş olsa da, bu ağır metalin deniz kabukluları ve ton balıklarındaki biyolojik birikimi gözlemlenmiştir [63, 66, 67]. Şu anda nedensel bir rol belirlenmemiş olsa da, bu element için bir hedef olması nedeniyle civa maruziyetinin böbrek kanseri ile ilişkili olabileceği öne sürülmüştür [63]. Başka bir çalışmada, artan civa maruziyetinin karaciğer kanseri ile ilişkili olduğunu ve ayrıca mide kanserini indüklemek için karsinojenik potansiyele sahip olduğunu gözlemlenmiştir [68]. Civa, safra kesesi kanserli

hastalarda istatistiksel olarak daha yüksek konsantrasyonlarda bulunan ve safra taşlarında saptanan bir başka ağır metaldir [47]. Bu metal ile nedensel bir ilişki gözlenmemiş olmasına rağmen kanser gelişiminde bir rolü olduğu varsayılmıştır [47].

Bu çalışmada tartışılan diğer ağır metallerin çoğuna kıyasla civa, birkaç spesifik mekanizma yoluyla malign büyümeyi indükleme potansiyeline sahiptir. Bunlar, serbest radikal üretme yeteneğinin yanı sıra DNA moleküler yapısını ve DNA onarım sistemini bozmayı içermektedir [64]. Bununla birlikte, civanın ya bu metale özgü olan ya da çoğu ağır metalde gözlenmeyen birkaç kanserojen mekanizmaya sahip olduğu ileri sürülmüştür. Civanın kanserojenliğini artıran bir mekanizma, vücudun doğal bir antioksidan olan glutatyon konsantrasyonunu azaltmadaki rolü olduğu düşünülmüştür. Bu da, potansiyel olarak temel hücresel bileşenlerin oksidatif strese duyarlılığının artmasına katkıda bulunmaktadırr. Hücreler üzerindeki oksidatif stresin, karsinojenez ile ilişkili başka bir mekanizma olan lipid peroksidasyon oranlarını yükselttiği gösterilmiştir [63]. Civanın, diğer süreçlerin yanı sıra hücre bölünmesini bozabilen hücrelerdeki mikrotübülleri etkileyebileceği de öne sürülmüştür [64]. Şelatörlerin kullanımı, civanın vücuttan atılması için ortak terapötik stratejidir. Klinik ortamda en etkili iki şelatörün dimerkaptosüksinik asit (DMSA) ve dimerkaptopropan sülfonat (DMPS) olduğu belirlenmiştir [69, 70]. Test edilmemiş şelatörlerin civaya karşı etkinliklerinin araştırılması için de araştırmalar yapılmıştır. Örneğin, deferasiroks ve deferipron birleştirilerek sıçanlar üzerinde test edilmiştir [71]. Bu kombinasyonun civayı başarılı bir şekilde şelatlayabildiği ve civanın toksik etkilerini azalttığı gözlenmiştir [71]. Benzersiz bir deneysel şelatör, tiyol ile modifiye edilmiş nanogözenekli silika materyalidir [72]. Hayvanlar üzerinde yapılan testlerin ardından, bu materyalin, diğer birçok ağır metalle birlikte, marjinal toksisite ile civayı ortadan kaldırmak için muazzam bir potansiyel sergilediği gözlemlenmiştir [72]. Başka bir çalışmada; adli/koroniyal otopsiler sırasında tiroid örnekleri alınan, farklı klinikopatolojik koşullara sahip 1–104 yaşları arasındaki 115 kişiden formalinle sabitlenmiş parafine gömülü tiroid doku blokları elde edilmiştir. Bu doku bloklarından alınan yedi mikronluk kesitler, otometalografi kullanılarak hücre içi inorganik cıvanın saptanması için kullanılmış ve civanın varlığı, çoklu elementleri tespit edebilen lazer ablasyon-endüktif olarak eşleştirilmiş plazma kütle spektrometrisi kullanılarak doğrulanmıştır. Sonuç olarak; 1-29 yaşları arasındaki kişilerin %4'ünde, 30-59 yaşları arasında %9'unun ve 60-104 yaşları arasında %38'inin tiroid foliküler

hücrelerinde otometalografide civa bulunmuştur. Lazer ablasyon-endüktif olarak eşleştirilmiş plazma kütle spektrometrisi, otometalografi ile boyanan örneklerde civa varlığını doğrulanmış ve seçilen örneklerde ayrıca kadmiyum, kurşun, demir, nikel ve gümüşü tespit edilmiştir [73].

Nikel

Dünyanın çekirdeğinde bulunan ağır bir metal olan nikel, kanserojen özelliklerin daha iyi anlaşılması nedeniyle araştırmacıların dikkatini çekmiştir. Bu metal, çevresel veya mesleki bir ortamda maruz kaldığında toksik etkiler üretmektedir. Nikele potansiyel mesleki maruziyeti içeren birkaç endüstri, madencilik, metal alaşımları ve nikelin rafine edilmesini içermektedir [74, 75]. Bu ağır metalin kirliliği çevreye zarar vererek, balık gibi insan besin zincirine giren organizmalarda biyolojik olarak birikebilmektedir [76]. Kirlenmiş toprak, bu toksik metalle temas etmenin başka bir yöntemini temsil etmektedir [74].

Nikel maruziyeti ile ilişkili çeşitli birçok kanser vardır. Epidemiyolojik çalışmalar, akciğer, nazal ve sinüs dokularında maruziyet ile karsinogenez insidansı arasında önemli bir ilişki olduğunu ortaya koymuştur [18, 75, 77, 78]. Başka bir çalışmada, meme kanserli hastalarda yüksek serum nikel düzeylerinin istatistiksel olarak anlamlı olduğu belirlenmiş ve bu da, maruz kalmanın potansiyel olarak kanserojen sonuçları olduğunu düşündürmüştür. Bu ağır metale maruz kalma, akut miyeloid ve lenfoblastik lösemi gelişimi ile de ilişkilendirilmiştir. Bu lösemili çocuklarda idrarın daha yüksek seviyelerde nikel ve 8-hidroksi-2′-deoksiguanozin içerdiği belirlenmiştir [79]. Bu sonuçlar, bir etki mekanizması olarak oksidatif hasarı indükleyerek akut lösemide nikelin bir rolü olduğunu ortaya koymuştur [79]. Araştırma ayrıca ekzokrin pankreas kanseri olan kişilerde yüksek nikel konsantrasyonlarının varlığını da ortaya çıkarmıştır [18]. Mevcut başka ağır metaller olmasına rağmen, bu bulgular nikelin kanserojen etkisi olduğunu düşündürmektedir [18].

Nikel'in kronik inflamasyondaki rolü hayvanlarda ve insan hücre örneklerinde araştırılmıştır. Maruz kalmanın, inflamasyon düzeylerinin korunmasında rolü olan SQSTM1 ve TNF proteinlerinin ekspresyonunu artırdığı ve karsinogenezi indüklediği sonucuna varılmıştır [78]. Diğer ağır metaller gibi, nikel de DNA metilasyonundaki değişiklikler gibi epigenetik değişiklikleri indükleme potansiyeline sahiptir. Örneğin, nikel iyonlarının (Ni^{+2}) histon H3K4'ün tri-metilasyonunu indükleme potansiyeline sahip

olduğu gözlenmiştir [80]. Bu süreç, nikel için başka bir kanserojen mekanizma öneren uygun olmayan transkripsiyonel aktivasyon ile ilişkilendirilmiştir [80]. Diğer ağır metallerle karşılaştırıldığında, nikel içeren şelatörlerin kullanımı belirgin şekilde farklıdır. Sodyum dietilditiyokarbamatın nikel karbonile yanıt olarak etkili bir şelatör olduğu kanıtlanmıştır. Örneğin etilen diamin tetraasetik asidin (EDTA), Arundo donax L.'de kirlenmiş topraktan nikel alımını indüklediği gözlemlenmiştir [81]. CaNa(2) EDTA'nın nikel klorürün neden olduğu hasarı tersine çevirdiği ve metali *Cirrhinus mrigala*'dan uzaklaştırdığı da belirlenmiştir [82].

Sonuç

Ağır metaller vücuda içme suyu, yiyecek (sindirim sistemi), hava (solunum sistemi) ve bazen de deriden maruz kalma dahil olmak üzere farklı yollardan girmektedir ve insan vücudunda birikmektedirler. Toksik metallerin biyobirikimi, vücudun doku ve organları üzerinde çeşitli toksik etkilere yol açmaktadır. Metal toksisitesinin akut veya kronik belirtileri olabilmektedir. Ağır metaller, büyüme, çoğalma, farklılaşma, hasar onarım süreçleri ve apoptoz gibi hücresel olayları bozmaktadırlar. Toksik metaller ayrıca gen ekspresyonunu etkileyebilecek epigenetik değişiklikleri de teşvik edebilmektedir. Bu metallerin etki mekanizmalarının aydınlatılması; ROS üretimi, antioksidan savunmanın zayıflaması, enzim inaktivasyonu ve oksidatif stres dahil olmak üzere toksisiteyi indüklemek için benzer yollar ortaya koymaktadır. Örneğin; Cr, Cd ve As gibi bazı toksik metaller genomik kararsızlığa neden olmaktadırlar. Oksidatif stresin indüklenmesini takiben DNA onarımındaki kusurlar ve bu metallerin DNA hasarı, kanserojenliklerinin nedeni olarak kabul edilmektedir. Bu noktada mevcut olan bu araştırma, gelecekteki çalışmalar için çeşitli vurgu alanlarını aydınlatmıştır. Kanserojen mekanizmaların rafine bir şekilde anlaşılmasının gerekli olduğu açıktır. Bu, belirli ağır metaller için kişiselleştirilmiş terapötiklerin veya maruziyete yönelik önlemlerin oluşturulmasına yardımcı olmaktadır. Devam eden bilgi konsolidasyonu, ilerlemeye devam eden bir diğer önemli faktördür. Ağır metallere maruz kalmayla ilişkili riskler konusunda farkındalığı artırmak için yüksek riskli alanlarda da etkili eğitim programlarına ihtiyaç duyulmaktadır. Ayrıca ağır metalleri izlemek için özel biyobelirteçler geliştirmek bu alanda büyük bir başarı olacaktır.

Kısaltmalar

Al: Alüminyum, As: Arsenik, Pb: Kurşun, Ni: Nikel, Be: Berilyum, Cd: Kadmiyum, Hg: Civa, DMSA: Dimerkaptosüksinik asit, DMPS: Dimerkaptopropan sülfonat, DNA: Deoksiribonükleik asit, ROS: Reaktif oksijen türleri, TNF: Tümör nekroz faktör, GI: Gastrointestinal sistem

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The author confirms that the data supporting this study are cited in the article.

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Compliance with ethical standards / Etik standartlara uyum

Conflict of interest / Çıkar çatışması

The author declare no conflict of interest.

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Ethical standards / Etik standartlar

The study is proper with ethical standards.

Çalışma etik standartlara uygundur.

Authors' contributions / Yazar katkıları

Nebiye Pelin TURKER designed and writed the study.

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Ailevi Akdeniz Ateşi Hastalığının Tedavisinde Kolşisinin Yeri ve Potansiyel Tıbbi Bitkiler

Tuba Serbetci[1], Basak Ceylan[2*]

ÖZET

Ailevi Akdeniz Ateşi; otozomal resesif genin kalıtımıyla, doğuştan gelen ve immün sistemi etkileyen bir otoinflamatuvar hastalıktır. Hastalık, 16. kromozomdaki *MEFV* geninde oluşan mutasyon sonucu gözlenir ve tekrarlayan ateş ve serözit ataklarla seyreder. *Colchicum autumnale* L. isimli tıbbi bitkinin tohumlarından elde edilen kolşisin isimli alkaloit günümüzde ailevi akdeniz ateşi tedavisinde yaygın olarak kullanılmakta ve etkin bir rol oynamaktadır. Yakın zamanlı bazı araştırmalar *Andrographis paniculata* Nees., *Eleutherococcus senticosus* Maxim., *Schizandra chinensis* Bail., and *Glycyrrhiza glabra* L. bitki kombinasyonun ailevi akdeniz ateşi hastalarında güvenli ve etkili olduğuna dair anlamlı veriler sunmaktadır. *Cannabis sativa* L. ise dirençli ailevi akdeniz ateşi olgularında alternatif bir terapötik seçenek olarak değerlendirilmektedir. Bu çalışmada ailevi akdeniz ateşi tedavisinde kullanılan doğal kökenli bir ilaç etkin maddesi olan kolşisinin detaylı incelenmesi ile bunun yanı sıra diğer potansiyel tıbbi bitki türlerinin değerlendirilmesi amaçlanmaktadır.

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ANAHTAR KELİMELER
Ailevi Akdeniz Ateşi,
AAA tedavisi,
kolşisin,
tıbbi bitkiler

The Role of Colchicine and Potential Medicinal Plants in the Treatment of Familial Mediterranean Fever

ABSTRACT

Familial Mediterranean Fever; it is a congenital autoinflammatory disease that effects the immune system, inheriting an autosomal recessive gene. The disease is observed as a result of mutation in the *MEFV* gene on the 16th chromosome and progresses with recurrent episodes of fever and serositis. The alkaloid named colchicine, obtained from the seeds of the medicinal plant *Colchicum autumnale* L., is widely used and plays an active role in the treatment of familial mediterranean fever today. Some recent studies provide meaningful data that the combination of herbs *Andrographis paniculata* Nees., *Eleutherococcus senticosus* Maxim., *Schizandra chinensis* Bail., and *Glycyrrhiza glabra* L. is safe and effective in patients with familial mediterranean fever. *Cannabis sativa* L. is evaluated as an alternative therapeutic option in resistant familial mediterranean fever cases. In this study, it is aimed to examine in detail colchicine, a natural origin drug substance used in the treatment of familial mediterranean fever, as well as to evaluate other potential medicinal plant species.

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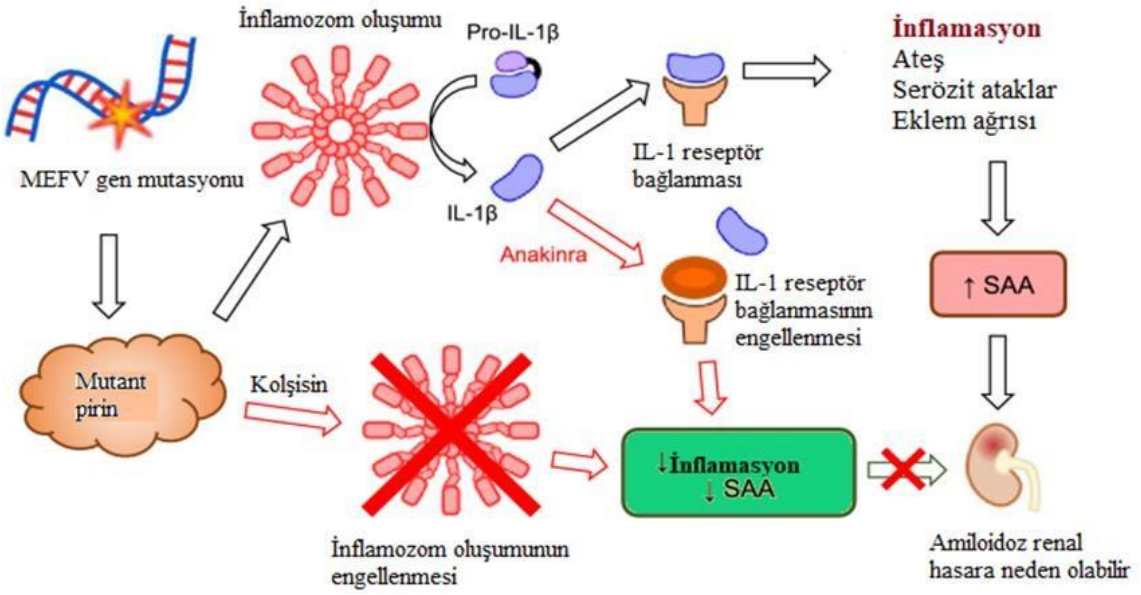
[1] Çukurova Üniversitesi, Eczacılık Fakültesi, Farmakognozi Anabilim Dalı, Adana, Türkiye
[2] Çukurova Üniversitesi, Eczacılık Fakültesi, Adana, Türkiye
[*]Corresponding Author: Başak Ceylan, E-mail: basakceylan70@gmail.com

Giriş

Ailevi Akdeniz Ateşi (AAA), tekrarlayan ateş ve poliserözit ataklar ile karakterize monogenik otoinflamatuvar bir hastalıktır [1]. Türkiye'deki AAA hastalarının %70'inin Doğu Anadolu ve Karadeniz Bölgesi, %24'ünün İç Anadolu ve küçük bir kısmının Ege Bölgesi kökenli olduğu belirtilmiştir [2, 3].

AAA'nın esas olarak miyeloid hücre kökeninden eksprese edilen pirin proteinini kodlayan MEFV geninde oluşan mutasyonlar sonucunda oluştuğu ve pirin proteininin proinflamatuvar sitokinlerin salgılanmasını artırarak inflamasyonu düzenlediği bildirilmektedir [4 , 5]. Mutasyon sonucu pirin proteini, Nacht bölgesi –, lösince zengin ve pirin bölgesi içeren protein 3 (NALp3)'teki spesifik bölgesine bağlanarak inflamozom kompleksi oluşturur [5-7]. Bu kompleks, kaspaz-1'i aktive ederek İnterlökin-1β (IL-1β) aktivasyonu ve lökosit apoptozunun inhibisyonu sonucu kontrolsüz inflamasyon ve atakların ortaya çıkmasına neden olur [2, 5, 6]. Ataklar sırasında Tümör Nekroz alfa (TNF-α,) IL-6 ve IL-8, remisyon döneminde ise TNF-α ve serum amiloid A (SAA) düzeylerinin arttığı, SAA protein düzeyinin artmasıyla da amiloidoz gözlendiği belirtilmiştir (Şekil 1) [5,8].



Şekil 1 AAA'nın patojenezi, kolşisin ve anakinra'nın etki mekanizması [9]

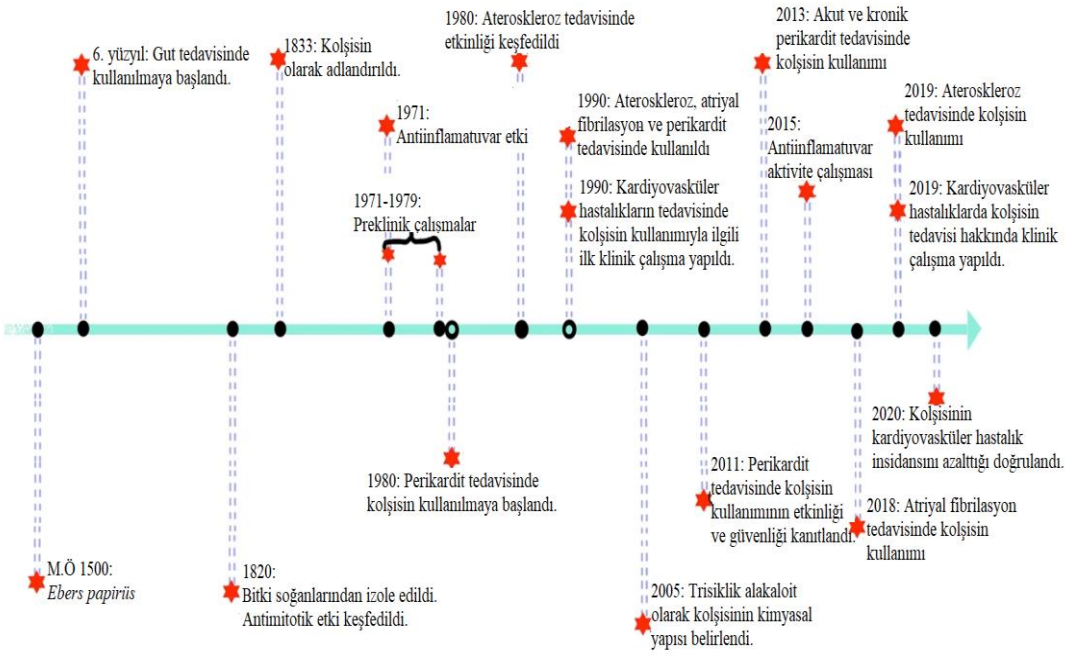
Fig 1 Pathogenesis of AAA, mechanism of action of colchicine and anakinra [9]

AAA tedavisinde yaşam kalitesinin artırılması, atakların sıklığı, süresi, şiddeti ile inflamasyonun azaltılması ve amiloidozun önlenmesi hedeflenmektedir [10]. Kolşisin ise atakların azaltılmasında, amiloidozun önlemesinde ve tam remisyonun sağlanmasında etkili bir ilaçtır [10]. Şu an AAA'nın geçerli tek tedavisi kolşisin olduğu için kolşisine alternatif kullanılabilecek yeni non-toksik antiinflamatuvar ilaçların araştırılması pratik açıdan önemlidir. Bu çalışmada, AAA tedavisinde kolşisin alkaloitinin ve diğer potansiyel tıbbi bitkilerin güncel olarak kullanımı incelenmiştir.

Kolşisin

Kolşisin, *Colchicum autumnale* L. (Colchicaceae, çiğdem) ve *Gloriosa superba* L. (Liliaceae, ateş lalesi) bitkilerinden elde edilen zehirli bir alkaloittir. [11, 12].

Kolşisinin MÖ 1500 yılına ait eski Mısır'ın önemli tıbbi belgelerinden *Ebers Papyrus*'ta eklem ağrılarında kullanılan bir bitkisel ilaç olarak tanımlandığı bildirilmektedir. *C. autumnale* soğanlarının gut tedavisinde kullanımının MS 550 yılına kadar uzandığı kayıtlıdır (Şekil 2) [13, 14]. Kolşisinin ilk olarak Pelletier ve Caventou tarafından 1820 yılında *C. autumnale* soğanlarından izole edildiği kayıtlıdır [11, 12].



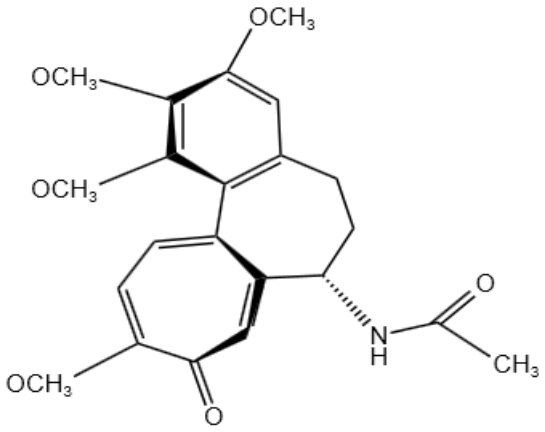
Şekil 2 Tıp tarihinde kolşisinin zaman çizelgesi [15].

Fig 2 Timeline of colchicine in medicinal history [15].

Goldfinger, ilk olarak 1972'de günde 1-3 mg kolşisin ile tedavi edilen beş AAA hastasında etkili semptomatik iyileşme olduğunu belirtmiş ve kolşisin AAA

profilaksisinde tercih edilmeye başlanmıştır [12, 16]. 2009'da ise ABD Gıda ve İlaç İdaresi, AAA ve diğer inflamatuvar (gut, artrit vb.) hastalıkların tedavisi için kolşisin kullanımını onaylamıştır [16, 17, 18].

Kolşisin, bir trimetoksifenil halkası, yedinci konumunda asetamid bulunan yedi karbonlu bir halka ve bir tropolonik halkadan oluşan trisiklik bir alkaloittir (Şekil 3) [12, 16].



Şekil 3 Kolşisinin kimyasal yapısı

Fig 3 Chemical structure of colchicine

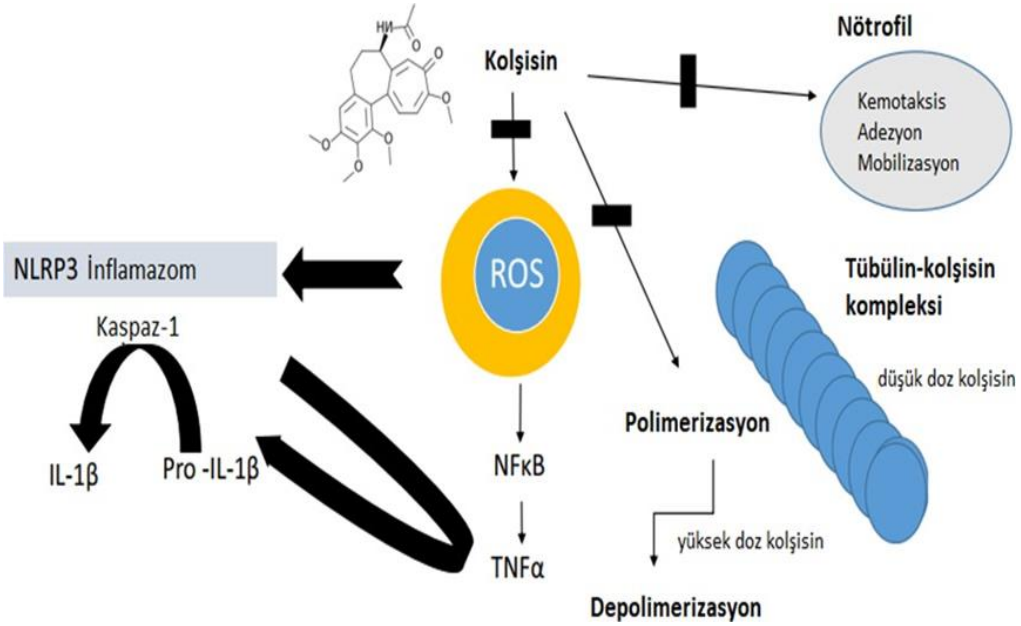
Kolşisinin antiinflamatuvar etki mekanizması

Kolşisinin kaspaz-1 aktivasyonundan ve IL-1β salgılanmasından sorumlu olan NALp3 inflamozomlarını ve lökotrien oluşumunu baskıladığı, profilaktik tedavide kaspaz-1 veya nükleer faktör kappa B (NF-κB)'ye bağlı hücre içi sinyal yolaklarını inhibe ederek inflamatuvar yanıtı düzenlendiği bildirilmiştir [17, 18]. Ayrıca makrofajlar aracılığıyla TNF-α sentezini inhibe edip salgılanmasını düzenlediği [19] ve IL-1β salgılanmasını inhibe ettiği kayıtlıdır [18]. Ek olarak; inflamasyon oluşumunda nötrofillerin kemotaksis, adhezyon vb. etkilerini düzenlediği görülmüştür [17, 18]. Antiinflamatuvar etkiyi oluşturan yolaklar (Şekil 4):

- Makrofajlar tarafından TNF-α sentezinin inhibisyonu [20];
- Siklooksijenaz-2 (COX-2) aktivitesi inhibisyonu, proinflamatuvar ajanların, (prostaglandin E2 ve tromboksan A2) sentezinin inhibisyonu ile gut ve AAA'da ödem ve ağrının azalması [12, 21, 22];
- Nötröfil ve monositlerde bulunan NALp3 inflamozom aktivasyonunu ve IL-1β

aktivasyonunun engellenmesi [21, 22];

- Endotelyal ve nötrofilik adhezyonun inhibisyonu olarak sıralanabilir [12, 21]. Gut ve AAA atakları üzerindeki profilaktik etkinin bu son iki mekanizmaya bağlı olduğu düşünülmektedir [12].



Şekil 4 Antiinflamatuvar etkiyi oluşturan yolaklar [20]

Fig 4 The pathways that produce the anti-inflammatory effect [20]

Kolşisinin tübülin üzerindeki yüksek afiniteli bağlanma bölgelerine geri dönüşümsüz bağlanarak tübülinin hücre iskeletindeki mikrotübüllerin (+) uçlarına yerleşimini önlediği, hücre bölünmesinde mitotik iğ ipliklerinin oluşumu dahil olmak üzere yapısal kararsızlık oluşturarak hücre bölünmesini engellediği belirtilmiştir. Aynı zamanda akut faz reaktanları ve NALp3 ile etkileşime girerek inflamasyon oluşumunu eş zamanlı inhibe ederek AAA üzerinde etki gösterdiği bildirilmektedir. [16].

AAA tedavisinde kolşisinin yeri

Kolşisin, AAA tedavisinin temelini oluşturur. Hastaların %60-75'inde tam remisyon, %15-30'unda anlamlı iyileşme ve %5-10'unda ise tedaviye yanıt alınmadığı belirtilmektedir [23]. Tedaviye yanıt vermeyen hastaların ise tedaviye uyum göstermediği veya dozun yetersiz olduğu bildirilmiştir [24].

Bazı AAA hastalarında ataklar görülmeden de inflamasyon oluşabileceğinden proteinüri ve inflamasyon ölçütlerinin takibi için SAA düzeyi belirlenmelidir, belirlenemediği

durumlarda C-reaktif protein (CRP), eritrosit sedimentasyon hızı (ESH) ve fibrinojen değerlerinin takip edilmesi gerektiği kabul edilmiştir [21].

Yetişkinlerde kolşisin dozu günde 1 mg'dır ancak, atakların kontrol altına alınamadığı hastalarda klinik remisyon gözlenene kadar 2 mg'a yükseltilebileceği ve toksisite riski açısından kısa süreli, yüksek doz kolşisin verilmesinin uygun görülebileceği bildirilmiştir. [12, 24].

Tekrarlanan dozlarla ilgili yapılan çalışmalarda; 15 gün boyunca 1 mg/gün dozunda oral kolşisin verilen sağlıklı gönüllülerde, 5 gün içinde 0,7-1,4 ng/mL plazma düzeyinde kararlı durum konsantrasyonunun oluştuğu belirtilmiştir. AAA'lı hastalarda ise 0,15-1,9 mg/mL veya 0,5 ve 2 mg/gün arasındaki dozlarda ortalama kararlı durum konsantrasyonunun 2,0 ± 0,4 ng/mL olduğu belirtilmiştir [25].

Çocuklarda kolşisin tedavisinin güvenli ve etkili olduğu bildirilmiştir [21]. Tedavinin başlangıç dozu ise vücut ağırlığına veya yüzey alanına göre ayarlanmakta olup minimum doz, 2 yaşına kadar günde yaklaşık 0,25 mg'dır ve 6-7 yaşlarında 1 mg'lık tam günlük doza yükseltilebileceği, vücut yüzey alanı 1 m^2'den fazla olan çocuklarda ise dozun yetişkin dozuna yükseltilmesi gerekliliği kayıtlıdır [24].

Amiloidoz gelişen hastalarda ve amiloidoza bağlı böbrek yetmezliğinde, amiloidozun önlenmesi veya ilerlemesinin durdurulması amacıyla kolşisinin 2 mg/gün dozunda verilmesi, şiddetli böbrek yetmezliği olgularında ise dozun azaltılarak kullanılması gerektiği bildirilmiştir. [21, 24].

Glomerüler filtrasyon hızı (GFH); 60 mL/dk/m^2 olduğunda normal doz, 20-60 mL/dk/m^2'ye düştüğünde normal dozun %65-75'i ve 20 mL/dk/m^2'ye düştüğünde ise normal dozun %50'sinin kullanılmasının uygun olduğu kayıtlıdır [2, 21].

Oral kolşisine dirençli AAA hastalarında ek olarak 1 mg/hafta dozunda intravenöz (i.v) kolşisin enjeksiyonlarının etkili olduğu gösterilmiş, kolşisin bolusunun ilaç absorpsiyonundaki sorunları önlediği ve farmakolojik etkinin oluşması için gerekli serum konsantrasyonunu sağladığı düşünülmüştür [24, 26].

Bunların sonucunda; kolşisinin akut atakları azaltmada ve renal amiloidozun profilaksisinde etkili olarak, AAA tedavisinde önemli bir rolünün olduğu gösterilmiştir [17]. Komplikasyonların tekrarlanmaması ve etkilerin devamlılığı açısından tedavinin yaşam boyu sürdürülmesi gerektiği bildirilmektedir [21, 24]. İlacın kesilmesi durumunda, çoğu hastada birkaç gün içinde hastalığın nüksettiği belirtilmiştir [12].

10 mg kolşisin dozunun toksisiteye neden olduğu gösterilmiştir [24]. Dozun alınmasından 2-5 saat sonra ateş, bulantı, kusma ve karın ağrısı gibi semptomların görüldüğü belirtilmiştir [11, 24]. 24-72 saat sonra ise karaciğer hasarı, böbrek yetmezliği, aritmi ve kalp yetmezliği gözlendiği kayıtlıdır [17, 24, 27].

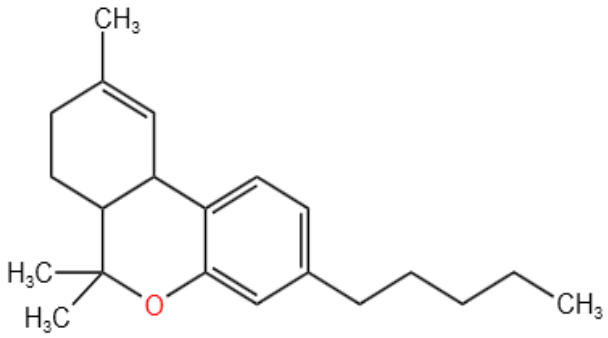
AAA Tedavisinde Tıbbi Bitkilerin Yeri

Otoimmün hastalıkların immunopatojenezinde çoklu mekanizmaların etkili olduğu, proinflamatuvar sitokinlerin inflamasyon oluşumunda rol oynadığı ve inflamasyon yolaklarının NF-κB ve NALp3 tarafından düzenlenmekte olduğu gözlenmiştir. Tıbbi bitkilerin ise inflamozom aktivasyonunu inhibe etmeleri sonucu oluşan immünomodülatör ve antiinflamatuvar aktiviteleri dolayısıyla kronik inflamatuvar hastalıkların tedavisinde etkili oldukları bildirilmiştir [28, 29].

İzleyen bölümde, AAA tedavisinde potansiyel olabilecek tıbbi bitkilerin etki mekanizmaları ve klinik verileri tartışılmaktadır.

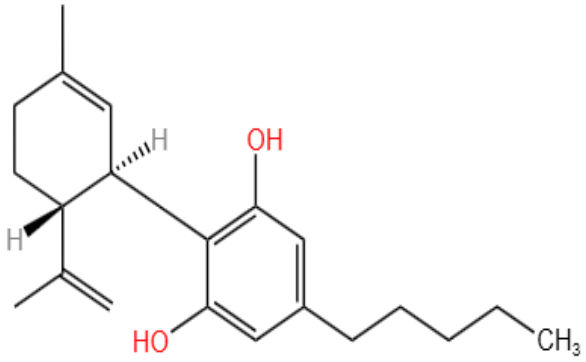
***Cannabis sativa* L.**

Cannabinacaea familyasından *Cannabis sativa* L. (kenevir) otsu bir bitki olup Orta Asya'da yetiştirilmektedir ve aktif bileşenleri kannabinoitler, flavonoitler ve alkaloitlerdir [30]. Bitkiden izole edilen terpenofenolik yapıdaki bileşikler "kannabinoitler" olarak adlandırılır. Bunlar 11 genel tipte sınıflandırılabilir; Δ9-tetrahidrokannabinol (Δ9-THC, Şekil 5), kannabidiol (CBD, Şekil 6), kannabinodiol (CBND), kannabinol (CBN) ve çeşitleridir [32]. Bunlar arasında psikoaktif etki açısından en önemli bileşik Δ9-THC'dir [30].



Şekil 5 Tetrahidrokannabinolün kimyasal yapısı

Fig 5 Chemical structure of tetrahydrocannabinol



Şekil 6 Kannabidiolün kimyasal yapısı

Fig 6 Chemical structure of cannabidiol

Kannabinoidlerin kanser, kronik ağrı ve fibromiyalji gibi belirli endikasyonlarda onaylanmış nabiximols, dronabinol ve kannabidiol isminde müstahzarlarının bulunduğu belirtilmiştir. Türkiye İlaç ve Tıbbi Cihaz kurumunun "Yurtdışı İlaç Listesinde" nabiximols ve kannabidiolun bulunduğu bildirilmiştir [31].

Kannabinoitlerin antiinflamatuvar ve immünomodülatör etkilerini araştırmak üzere yapılan çalışmalarda inflamasyon sürecini düzenledikleri bildirilmektedir. *İn vitro* bir çalışmada THC'nin kaspaz-1 ile pro-IL-1β aktivasyonunu ve sitokin üretimini inhibe ettiği gösterilmiştir. *İn vivo* bir diğer çalışmada ise, CBD tedavisinin uygulandığı makrofaj hücrelerinin NALp3 aktivitesini inhibe ettiği ve NF-κB sinyal yolunu baskıladığı belirtilmiştir. Preklinik çalışmalarda ise CBD'nin IL-1β ve TNF-α gibi proinflamatuvar sitokinlerin üretimini azalttığı gözlenmiştir [33, 34].

Otoimmün hastalıkların patogenezinde rol oynayan lenfosit ve makrofaj hücrelerinde de kannabinoitlerin immünomodülatör aktivite gösterdiği rapor edilmiştir [33]. Kannabinoitlerin bu etkilerinden dolayı otoimmün ve inflamatuvar hastalıkların (Crohn's, inflamatuvar barsak hastalığı, Multipl Skleroz, romatoid artrit vb.) tedavisinde kullanılabileceği belirtilmektedir [33, 35].

AAA tedavisinde Cannabis sativa

Dirençli AAA hastalarında tıbbi *Cannabis* tedavisi ile iyileşme görülen vaka çalışmaları aşağıda rapor edilmiştir [35].

Vaka 1

30 yaşında bir erkeğe 5 yaşında Tel Hashomer kriterlerine göre AAA teşhisi konulmuştur. Günde 2-3 kez, 0,5 mg kolşisin almasına rağmen her ay meydana gelen ve 3-4 gün devam eden ateş, karın ağrısı, plöritik göğüs ağrısı ve artralji atakları gözlenmiştir. Günde 2-3 kez non-steroidal antiinflamatuvar ilaçlar (NSAİİ) (diklofenak potasyum), acil serviste intramüsküler diklofenak sodyum ve intravenöz enjeksiyonlar ile tedavi uygulanmıştır [35].

Tıbbi *Cannabis* tedavisi, IMCA (İsrail Tıbbi *Cannabis* Ajansı) tarafından onaylanan iki farklı sigara şeklinde uygulanmıştır. Hastaya gündüz; Ella (%60 *C. sativa* + %40 *C. indica*; %10 THC + %2 CBD), akşam; Roma (%60 *C. indica* + %40 *C. sativa*, %20 THC + %4 CBD) sigaralarının verildiği belirtilmiştir [35]. Tedavinin sonunda hastanın eklem, göğüs ve karın ağrısı azalmış, vücut sıcaklığının 38°C'nin altına düşmesiyle birlikte atakların şiddetinde ve yaşam kalitesinde önemli bir iyileşme gözlenmiştir. Ayda 20 g tıbbi *Cannabis* tüketimi sonucunda herhangi bir yan etki gözlenmediği, sonuç olarak hastanın tedavi sürecinde sadece tıbbi *Cannabis* tercih etmek istediği bildirilmiştir [35].

Vaka 2

23 yaşında erkek birey günde 3-4 tablet kolşisin kullanmasına rağmen 15 yıldır dirençli AAA hastasıdır. Yaklaşık 2 haftada bir tekrarlayan, 2-3 gün süren plöritik göğüs ağrısı ve 40°C'ye kadar ateş gözlenmiştir. Evde oral NSAİİ'lar, proton pompa inhibitörleri (PPİ) ve gerektiğinde antiemetiklerle tedavi edilmiştir [35]. Böbrek fonksiyonu, laboratuvar test sonuçları ve idrar analizi normaldir, ancak ataklar sırasında CRP düzeyi yükselmiştir. Bunlardan dolayı, subkütan 100 mg/gün anakinra tedavisi başlanmıştır, ataklar sırasındaki ateş ve göğüs ağrısında minimal azalma gözlenmiştir [35]. Hasta, IMCA ruhsatı olmayan karaborsadan satın aldığı tıbbı *Cannabis*'in %18 THC ve %1 CBD içeren (%70 *C. indica* + %30 *C. sativa*) sigara türünü genellikle akşamları ayda 20-30 gram tüketerek kullanmaya başlamıştır [35].

Tıbbi *Cannabis* kullanımından sonra, ataklar sırasındaki ateş ve plöritik göğüs ağrısının şiddetinde önemli bir iyileşme bildirilmiştir, ataklar arasındaki süre bir aya kadar uzamıştır. Tedaviden önce, atak sırasında 37 mg olan CRP düzeyinin 1 ay sonra 6,09 mg'a düştüğü belirtilmiştir [35].

Tıbbi *Cannabis* üzerinde plasebo kontrollü yapılan bir diğer klinik araştırmada 50 mg'lık THC kapsülleri ve THC'nin etkili olmadığı durumlarda analjezik etkiyi sağlamak için

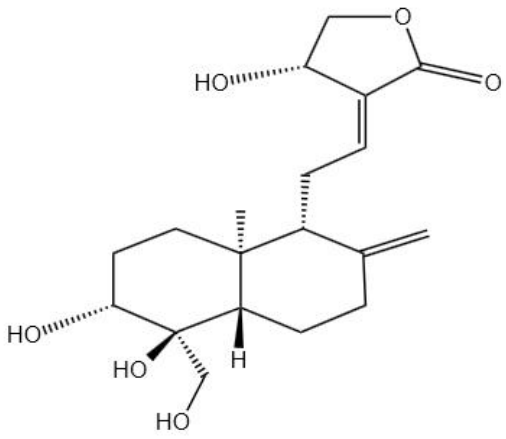
morfin sülfat tabletleri kullanılmıştır. Aktif ve plasebo tabletler arasında; antiinflamatuvar aktiviteyi saptayabilmek için yapılan laboratuvar testlerinde anlamlı bir fark gözlenmemiştir ve analjezik aktivite için yapılan VAS (VAS: 0 = ağrı yok, 10 = aşırı ağrı) skorlarının benzer olduğu belirtilmiştir [36]. Ancak plasebo tabletlerinin kullanıldığı haftada daha fazla morfin tableti tüketilmiştir, bununla birlikte THC kapsüllerinin kullanıldığı haftalarda inflamasyon atakları gözlenmediği kayıt edilmiştir [36].

Sonuç olarak, vaka raporları incelendiğinde tıbbi *Cannabis*'in AAA ataklarının şiddeti ve sıklığının azalmasındaki etkisinin endokannabinoid sistem (ECS) ve IL-1β üzerindeki inhibitör etkisinden dolayı olduğu düşünülmüş, tıbbi *Cannabis*'in dirençli AAA olgularını tedavi etmek için terapötik bir seçenek olabileceği bildirilmiştir [35].

THC'nin yasal olarak kullanımı için farmasötik preparatlarının hazırlanarak klinik etkilerinin araştırılmasının gerekli olduğu belirtilmektedir [36].

***Andrographis paniculata* (Burm.f.) Wall.**

Acanthaceae familyasından *Andrographis paniculata* (Burm.f.) Wall., (Hint ekinezyası) Hindistan'da Ayurvedik ve Geleneksel tıp sistemlerinde yeri olan önemli bir tıbbi bitkidir [37]. Diyabet, karaciğer bozukluğu ve nörodermatitte tedavi edici özellikleri olup antiinflamatuvar ve antipiretik kullanımları mevcuttur [38, 39]. *A. paniculata* türünün antiinflamatuvar aktiviteden sorumlu bileşenlerinin yaprak ve köklerde bulunan diterpenoit yapısındaki andrografolit (Şekil 7) ve türevleri olduğu düşünülmektedir [40].



Şekil 7 Andrografolit'in kimyasal yapısı.

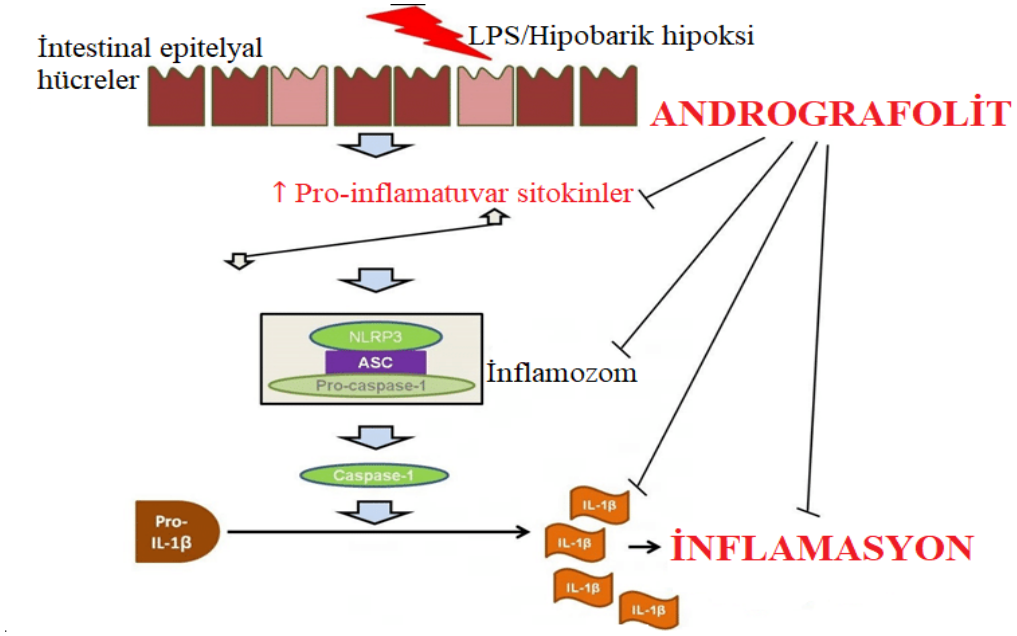
Fig 7 Chemical structure of Andrografolit.

A. paniculata, farmakolojik aktivite, etki mekanizmaları, etkinlik ve güvenlilik yönünden kapsamlı şekilde incelenmiş, andrografolit ve türevlerinin lökosit üretimi ve lenf

sisteminin aktivasyonu gibi immün sistem fonksiyonlarını düzenlediği bildirilmiştir [37]. Bitki, immünomodülatör fonksiyonlarından dolayı birçok hastalığın profilaksi ve tedavisinde ideal bir fitoterapötik olarak düşünülebileceği belirtilmektedir [37].

Andrografolit, neoandrografolit ve dehidroandrografolitin, sıçanlarda bakteriyel endotoksinler ve hemolitik *Streptococcus* gibi ateşi indükleyen ajanlar tarafından oluşan ateşi azalttığı gösterilmiştir [41]. 30, 100 ve 300 mg/kg andrografolitin rektal vücut sıcaklığını azalttığı belirtilmiş, andrografolitin analjezik aktivitesi aspirine oranla düşükken, antipiretik aktivite incelendiğinde 300 mg/kg andrografolitin aynı miktarda aspirin kadar etkili olduğu bildirilmiştir. Andrografolit ve türevleri arasında kimyasal kaynaklı inflamasyona karşı en belirgin antiinflamatuvar etkiyi andrografolitin gösterdiği tespit edilmiştir [37].

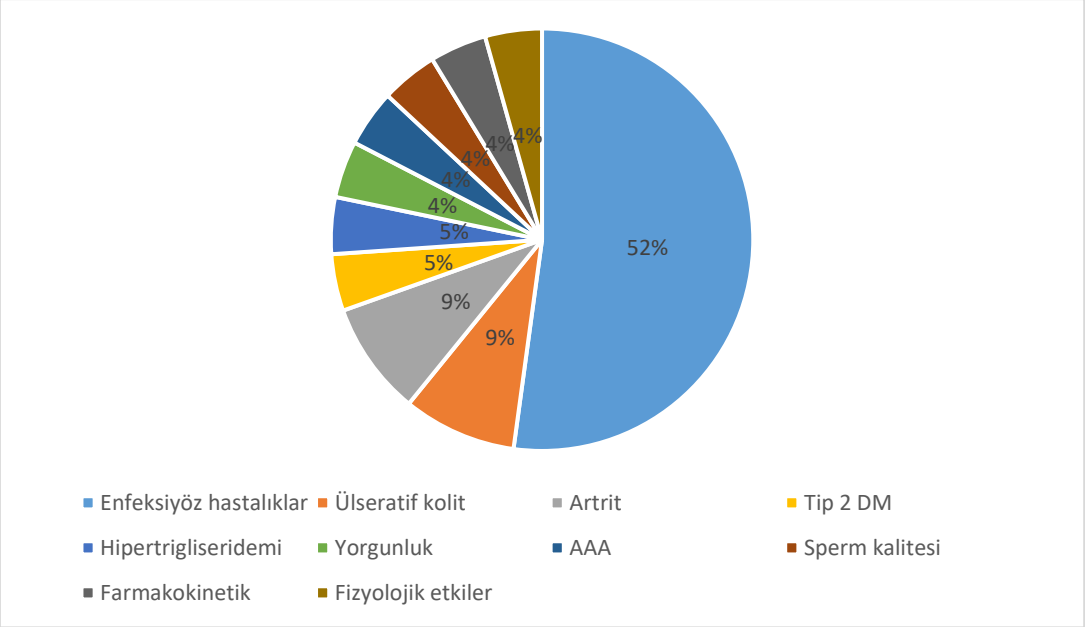
Andrografolitin inflamatuvar hastalıklarda, antiinflamatuvar etki mekanizmasının NF-κB aktivitesinin inhibisyonu üzerinden gerçekleştiği, ayrıca interferon-γ (IFN- γ) ve TNF-α salgılanmasını artırdığı kayıtlıdır [29, 42]. Diğer bir mekanizma incelendiğinde, mitojenle aktive olan protein kinaz (MAPK) yolaklarını inhibe ederek otoimmün artrit tedavisinde koruyucu etkisi olduğu gösterilmiştir [29]. Ayrıca hem *in vitro* hem de *in vivo* ortamda yapılan çalışmalarda lipopolisakkarit (LPS) ile indüklenen bağırsak hücrelerinde proinflamatuvar sitokin üretimini azalttığı ve NALp3 inflamozomlarını inhibe ettiği bildirilmektedir (Şekil 8). Bunun sonucunda, intestinal inflamasyonda etkili olduğu ve ilaç olarak kullanılabileceği belirtilmiştir [43]. *A. paniculata* hakkında yapılan kontrollü klinik araştırmaların yaklaşık %52'sinin bitki ekstresinin ve aktif bileşiklerinin viral enfeksiyonlardaki etkilerini araştırmak için yapıldığı belirtilmiştir. Diğer kontrollü klinik araştırmaların ise sağlıklı gönüllülerde *A. paniculata* tedavisi, fizyolojik etkileri, semen kalitesi, farmakokinetiği, ülseratif kolit, artrit ağrısı, hipertrigliseridemi, yorgunluk, tip 2 diabetes mellitus ve AAA hakkında yapıldığı gösterilmiştir (Şekil 9) [44].



Şekil 8 Andrografolitin antiinflamatuvar etki mekanizması [43]

Fig 8 Antiinflammatory mechanism of action of andrographolide [43]

A. paniculata'nın gözlenen antiinflamatuvar, antipiretik ve analjezik aktivitelerinden dolayı modern tıpta AAA'da kullanımı mevcuttur [45] ve türün immünomodülatör fonksiyonlarından dolayı birçok hastalığın profilaksi ve tedavisinde ideal bir fitoterapötik olarak değerlendirilebileceği bildirilmektedir [37].



Şekil 9 *A. paniculata*'nın kontrollü klinik çalışma yüzdeleri [44]

Fig 9 Percentage of controlled clinical studies of *A. paniculata* [44]

***Andrographis paniculata* Nees., *Eleutherococcus senticosus* Maxim., *Schisandra chinensis* (Turcz.) Bail., ve *Glycyrrhiza glabra* L. kombinasyonu**

AAA'lı çocuklarda yapılan çift kör, plasebo kontrollü klinik bir araştırmada içeriğinde *Andrographis paniculata* ekstresi (50 mg), *Glycyrrhiza glabra* ekstresi (10 mg), *Eleutherococcus senticosus* ekstresi (10 mg), *Schisandra chinensis* ektresi (100 mg) içeren İmmunoGuard tabletlerinin profilaktik ve tedavi edici bir ajan olarak etkinliği değerlendirilerek serözit atakların gelişmesi, süresi, sıklığı, şiddeti, vücut sıcaklığı artışı, karın ve göğüs ağrıları üzerine etkisi incelenmiştir [46].

Tablet içeriğinde, *Andrographis paniculata* harici yer alan diğer tıbbi bitkilerin biyolojik aktiviteleri aşağıda incelenmiştir. *G. glabra* L. (Fabaceae, meyan) türünün köklerinin 4000 yıldır tıbbi amaçlarla kullanıldığı kayıtlıdır [47]. Türkiye'de analjezik, antitüssif ve diüretik etkilerinden dolayı etnobotanik kullanımı kayıtlıdır. Ayrıca halk arasında astım, bronşit ve gastrointestinal sistem hastalıklarında kullanıldığı bildirilmiştir [48, 49]. Bitkinin köklerinde glisirizin, glisirizik asit, glabridin ve liquiritin maddelerinin bulunduğu bildirilmiştir [50].

Bileşimindeki glisirizin ve glisirizik asidin, COX aktivitesi, prostaglandin üretimi, fosfolipaz A2 aktivitesi ve trombosit agregasyonunu inhibe ederek antiinflamatuvar etki gösterdiği, glisirizin ve glabridinin ise inflamasyon bölgesinde nötrofiller aracılığıyla reaktif oksijen türlerinin (ROS) oluşumunu inhibe ettiği kayıtlıdır [47]. Bu etkilere ilave olarak immünomodülatör, antiviral, antimikrobiyal ve antioksidan aktivitelerinin bulunduğu da gösterilmiştir [50]. Araliaceae familyasından *E. senticosus* Maxim. (Sibirya Ginsengi) Türk Farmakopesinde yer almaktadır. Farmakopede köklerinin antiinflamatuvar, antiviral, antioksidan ve antitümör aktivitelerinin olduğu belirtilmektedir [51].

E. senticosus, adaptojen bir bitki olarak sadece vücuda bazı besinsel molekülleri sağlamakla kalmaz aynı zamanda vücudun immün sistemini destekleyip fizyolojik, biyokimyasal veya immünolojik hasarın iyileştirilmesinde yardımcı rol oynar [52].

İmmünomodülatör etkisinin yanı sıra türün bileşimindeki β-sitosterol aktif maddesinin aspirin benzeri antiinflamatuvar ve antipiretik aktivite gösterdiği bildirilmiştir [52, 53]. Antiinflamatuvar etki mekanizmasının NF-κB aktivitesinin inhibisyonu ile gerçekleştiği belirtilmiştir [54].

S. chinensis (Turcz.) Bail. (Schisandraceae, şizandra üzümü), türü de Türk farmakopesinde yer alır ve drog olarak meyvelerinin kullanıldığı belirtilmektedir [51].

Bitkinin antiinflamatuvar, antimikrobiyal, hepatoprotektif ve immünomodülatör etkilerinin olduğu rapor edilmiştir [55-57]. Bitkinin meyvelerinden izole edilen ana etkin madde şizandrindir [58]. Şizandrin, nitrik oksit (NO) ve prostaglandin E2 (PGE2) salgılanmasını ve NF-κB aktivitesini inhibe etmekte, ayrıca COX-2 ekspresyonunu azaltmaktadır [58, 59]. Bitki ekstrelerinin antiinflamatuvar aktivitesi, inflamasyon modellerinde incelendiğine düşük ateş ve lökosit infiltrasyonunda azalma bildirilmiştir [59].

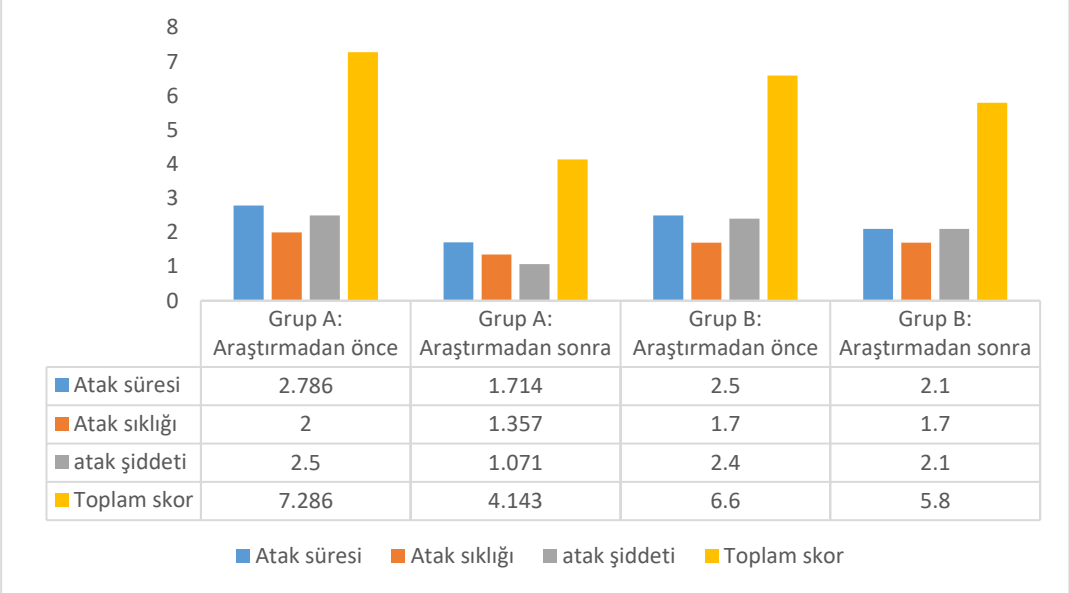
Bahsi geçen tıbbi bitki türlerini içeren, İsveç Bitkisel Enstitüsü'nün İyi Üretim Uygulamaları'na (GMP) uygun hazırlanmış Immunoguard tabletlerinin AAA'lı çocuk hastalarda etkinliğini araştırmak üzere üretildiği bildirilmiştir. Her bir tablet, (370 mg İmmunoGuard), 4 mg andrografolit üzerinden standardize edilmiş, *A. paniculata* ekstresi (50 mg), glisirizin (>0,6 mg) üzerinden standardize edilmiş *G. glabra* ekstresi (10 mg), eloterozit E (>0.8 mg) üzerinden standardize edilmiş *E. senticosus* ekstresi (10 mg), şizandrin (>0.8 mg) üzerinden standardize edilmiş *S. chinensis* ektresi (100 mg) içermektedir [46]. Araştırmada hastalar, Grup A ve Grup B olmak üzere 2 gruba ayrılmıştır. 14 hastadan oluşan Grup A'ya Immunoguard tabletleri verilirken; 10 hastadan oluşan Grup B'ye plasebo tabletleri verilmiştir [46].

ImmunoGuard'ın biyoaktif bileşeni, antiinflamatuvar ve analjezik etkinliği gösterilmiş olan andrografolittir. ImmunoGuard'ın TNF-α üretiminin aktivasyonu AAA hastaları üzerindeki etkisi ile ilişkilendirilebilir [42]. Andrografolite ek olarak eloterozit E, şizandrin ile glisirizin içermektedir ve bu bileşenlerin antiinflamatuar aktivitelerinin olduğu bilinmektedir. Bu bileşenlerin, AAA'da ImmunoGuard'ın antiinflamatuvar aktivitesine de katkısının olabileceği düşünülmektedir [46].

Araştırma sonrasında hastaların öz değerlendirmesinde Grup A'nın atak şiddeti Grup B'ye göre anlamlı bir şekilde daha düşük olup atakların sıklığı azalmıştır. Ayrıca hastalar karın ve göğüs ağrısının rahatladığını, ateş, artrit, miyalji ve erizipel benzeri eritem gibi semptomlarının azaldığını ifade etmişlerdir [46].

Hastalarda yapılan laboratuvar analizleri sonucunda; ImmunoGuard'ın ESH ve CRP düzeylerinin tedavi öncesine göre azaldığı belirtilmiştir. Bundan dolayı, ImmunoGuard'ın AAA'nın profilaksisi ve tedavisinde etkili olduğunun gösterildiği kayıtlıdır [46].

Hastaların klinik değerlendirmesinde atakların sıklık, süre ve şiddet oranlarının tedavi öncesine göre azaldığının gözlendiği bildirilmiştir (Şekil 10) [46].



Şekil 10 AAA'lı çocuk hastalarda Immunoguard (Grup A) ve plasebo (Grup B) tabletlerinin atakların süresi, sıklığı ve şiddeti üzerine etkisi [46]

Fig 10 Effect of Immunoguard (Group A) and placebo (Group B) tablets on duration, frequency and severity of attacks in pediatric patients with FMF [46]

ImmunoGuard üzerinde yapılan bir diğer araştırmada, AAA hastalarının akut atak ve ataksız dönemlerinde kan plazmasında NO üretimine ImmunoGuard'ın etkisi değerlendirilmiş, AAA hastalarının kanındaki NO seviyesinin ataklar sırasında önemli ölçüde azaldığı kaydedilmiştir[42]. ImmunoGuard tedavisi ile hastaların kanındaki NO düzeyinin ataksız dönemlerde artışı, atak dönemlerinde ise IL-6 düzeylerinde azalma gözlendiği kayıtlıdır. ImmunoGuard'ın NO ve IL-6 üzerinde düzenleyici aktivitesi, tedavi edilen hastaların yaşadığı atakların şiddetindeki azalma ile ilişkilendirildiği bildirilmektedir [42].

Sonuç olarak yapılan klinik araştırmalar, ImmunoGuard'ın AAA'lı hastaların profilaksisi ve tedavisinde etkili ve güvenli bir fitoterapötik olduğunu göstermektedir.

***Bupleurum falcatum* L., *Pinellia ternata* (Thunb.) Makino, *Scutellaria baicalensis* Georgi., *Zizyphus jujuba* Miller, *Panax ginseng* C. A. Meyer., *Glycyrrhiza uralensis* Fischer., *Zingiber officinale* Roscoe. kombinasyonu**

Sho-saiko-to, Japonya'da reçeteli kullanımı resmi olarak onaylanan, yedi bitki ekstresi içeren bir bitkisel ilaçtır. *Bupleurum falcatum* L., *Panax ginseng* C. A. Meyer, *Scutellaria*

baicalensis Georgi. ve *Glycyrrhiza uralensis* Fischer. kökleri, *Pinellia ternata* (Thunb.) Makino yumruları, *Ziziphus jujuba* Miller. meyveleri, ve *Zingiber officinale* Roscoe. rizomları ile hazırlanan ekstreleri içerdiği bildirilmiştir [60].*B. falcatum* (Apiaceae, tavşan kulağı*)* türünün Türkiye'de çok yıllık bir bitki olarak yetiştiği belirtilmiştir [61]. Konya bölgesinden toplanan *B. falcatum* türlerinin analizi yapıldığında 210,57 mg/g total fenolik madde içerdiği belirtilmiştir [62]. Bitki köklerinin, Çin ve Japonya'da uzun yıllardır geleneksel ilaç olarak kullanıldığı belirtilmiştir [63]. Bitki köklerinin antiinflamatuvar, antipiretik, immünomodülatör ve antimikrobiyal etki gösterdiği ve etkiden sorumlu bileşenlerin uçucu yağlar, flavonoitler ile saikosaponinlerin olduğu bildirilmiştir [63, 64].

Yapılan çalışmada, bitki ekstresinin ve saikosaponinlerin, NO, IL-1*β*, IL-6, TNF-*α*, ROS üretimini ve NF-κB aktivasyonunu inhibe ederek antiinflamatuvar etkiyi oluşturdukları kayıtlıdır [65]. *P. ginseng* (Araliaceae, Ginseng), Doğu Asya'da binlerce yıldır bitkisel ilaç olarak kullanılmaktadır [66]. Çin, Kore gibi uzak doğu ülkelerinde yetiştirilirken Türkiye'de yetişmediği belirtilmiştir [67]. Türün temel aktif bileşenleri, saponin yapısındaki ginsenozitlerdir [68]. Bitki köklerinin fiziksel veya psikomotor performansın iyileştirilmesi, antikanser, antiinflamatuvar ve immünomodülatör aktiviteleri bulunmaktadır [69]. Adaptojenik etkisinden dolayı bitkisel preparatların içeriğinde bulunduğu bildirilmiştir [70].

Etki mekanizması incelendiğinde ginsenozitlerin COX-2, NALp3 ve NF-κB aktivitesini inhibe ettiği, ayrıca IL-1*β* ile TNF-*α* üretimini azalttığı belirtilmiştir [71]. Ayrıca bitkinin polisakkarit bileşeni ginsanın NF-κB aktivitesini inhibe ederek immünomodülatör aktivite gösterdiği bildirilmiştir [72]. *P. ternata* (Araceae, karga kepçesi), Çin'de geleneksel olarak yaygın kullanılan tıbbi bitkilerdendir [73]. Bitkinin yumruları analiz edildiğinde çok sayıda alkaloit, yağ asitleri ve türevlerini içerdiği, farmakolojik çalışmalarda ise antifungal ve antiinflamatuvar aktivitelerinin olduğu bildirilmiştir [73, 74]. *S. baicalensis* (Lamiaceae, çin takkesi), biyolojik ve farmakolojik etkilerinden dolayı Çin'de uzun süredir kullanılmaktadır. İçeriğinde antibakteriyel aktiviteden sorumlu uçucu yağlar bulunmaktadır. Ayrıca antiviral, hepatoprotektif ve immünomodülatör etkilerinin bulunduğu gösterilmiştir [75].

Z. jujuba (Rhamnaceae, hünnap), Türkiye'de Batı ve Güney Anadolu'da kültürleri yapılmaktadır. Ancak, *Z. jujuba* hakkında ülkemizde yeterli sayıda farmakolojik çalışma

olmadığı için ilaç kaynağı olarak kullanılmadığı belirtilmiştir. Çin'de ise 7000 yıldır tıbbi amaçlı kullanılmak için yetiştirilmektedir [76]. Bitkinin her kısmında yaygın olarak betulinik asit bulunmakta olup anitinflamatuvar ve antibakteriyel aktiviteden sorumludur [77]. Meyvelerinde ise çok sayıda flavonoit bulunmaktadır [77, 78]. Meyvelerinin antiinflamatuvar, antimikrobiyal ve immünomodülatör aktivitelere sahip olduğu belirtilmiştir [76]. Yapılan çalışmada, bitkinin NF-κB aktivasyonunu inhibe ederek ve makrofajlarda inflamatuvar sitokin seviyelerini azaltarak antiinflamatuvar aktivite gösterdiği bildirilmiştir [79].

Z. officinale (Zingiberaceae, zencefil), Türkiye'de tıbbi amaçlı yaygın olarak kullanılsa da Türkiye florasında bulunmamaktadır. Bundan dolayı, bitki rizomlarının başka ülkelerden ithal edildiği belirtilmiştir. İthal edilen droglar analiz edilip Avrupa ve Türk farmakopesiyle karşılaştırıldığında drogların %60'ının tıbbi kullanıma uygun olmadığı belirtilmiştir [80].

Z. officinale, Çin ve Ayurvedik bitkisel ilaçlarında ise yaygın olarak kullanılan tıbbi bitkilerdendir. Zencefil, ana bileşen olarak zingiberen, kurkumen, zingiberol vb. içermektedir [81, 82]. İçeriğindeki etkin maddelerin analjezik ve antiinflamatuvar aktiviteleri vardır, artrit tedavisinde etkilidir [82]. *G. uralensis* (Fabaceae, Çin meyanı), en eski bitkisel ilaçlardan olup yaklaşık 3000 yıldır kullanıldığı gösterilmiştir [83]. Bitkiden izole edilmiş izoliquiritigeninin NO üretimini azalttığı ve NF-κB aktivasyonunu inhibe ederek IL-6 salgılanmasını azalttığı belirtilmiştir [84]. 7,5 g Sho-saiko-to numunesi 4,5 g Sho-saiko-to'nun kurutulmuş ekstresini (infüzyon yöntemi ile hazırlanmış) içerir. 7,5 g numunede bulunan bitkilerin miktarı ve etkin maddelerin yaklaşık konsantrasyonları Tablo 1'de gösterilmiştir [85].

Sho-Saiko-To'nun etki mekanizması henüz belirlenmemiştir, ancak inflamatuvar ve immün reaksiyonlarla ilgili farmakolojik etkilerinin olduğu bilinmektedir. Japonya'da kronik hepatit ve karaciğer sirozu olan hastalarda yaygın olarak kullanılmaktadır. Kronik viral hepatitli hastalarda IL-1β ve IL-6 üretimi düşükken Sho-Saiko-To verildikten sonra sitokin üretiminin arttığı bildirilmiştir [86]. Sho-Saiko-to ilacının AAA ataklarının profilaksisinde etkinliği araştırılmak üzere vaka çalışması yapılmıştır. Vaka 3 yaşından itibaren tekrarlayan ateş atakları olan 6 yaşında bir Japon erkek çocuğu ile ilgilidir. Ataklar sırasında CRP seviyesi artışıyla oluşan periyodik ateş ve lökositoz gözlenmiştir. Hastanın klinik bulguları değerlendirildiğinde AAA düşünülüp ailede MEFV geninin

mutasyon analizi yapılmıştır ve sonucunda hastaya AAA teşhisi konulmuştur [87].

Tablo 1 7,5 g Sho Saiko-To Bileşimi [85]

Table 1 Composition of 7.5 g Sho Saiko-To [85]

Bitkisel içerik	Miktar (g)	Etkin madde	Konsantrasyon
Radix Bupleuri (*Bupleurum falcatum)*	7,0	ıikosaponin b_1, b_2, c, d	100 mcg/mL; %0,2
Pinellia tuber (*Pinellia ternata)*	5,0	Efedrin	
Radix Scutellaria (*Scutellaria baicalensis)*	3,0	Baikalin	1,75 mg/mL; %3,5
		Baikalein	150 mcg/mL; %0,3
		Wogonin	20 mcg/mL; %0,04
		Viscidulin III	50 mcg/mL; %<0,1
Fructus Jujubae (*Zizyphus jujuba)*	3,0	Siklik AMP (cAMP)	
Radix Ginseng (*Panax ginseng)*	3,0	Ginsenozit Rb1 Ginsenozit Rg1	%0,2 %0,2
Liquiritiae radix (*Glycyrrhıza uralensis*)	2,0	Glisirizin Liquritin	500 mcg/mL; %1
Zingiberis rhizoma (*Zingiberis officinale*)	1,0	6-Gingerol 6-Shogaol Zingeron	

Hastaya 5 g/gün Sho-Saiko-to tedavisi 36 ay uygulanmıştır. Tedavi boyunca hastada AAA ataklarının gözlenmediği ve CRP ile lökosit düzeyinin azaldığı belirtilmiştir [86]. Sonuç olarak, Sho-Saiko-to'nun AAA ataklarının önlenmesinde etkili olabileceği ve tedavide rutin kullanımını belirlemek için daha fazla klinik çalışmanın gerekli olduğu bildirilmiştir [86, 87].

Tartışma

AAA gibi nadir otoinflamatuvar hastalıklarda karşılaşılan yüksek düzeydeki rekürrent ve sistemik enflamasyonun tedavisinde başlıca antiinflamatuvar ilaçlar ve immünsüpresif ajanlar kullanılmaktadır. Tıbbi bitkiler ve doğal ürünler kronik hastalıkların tedavisinde tarihin eski dönemlerinden beri kullanılmaktadır [88,89]. Geleneksel bitkisel tıbbın "çoklu hedef" özelliği, inflamatuvar hastalıkların tedavisinde özel bir avantaja sahiptir. Tıbbi bitkiler, NF-κB, TNF-α, indüklenebilir nitrik oksit sentaz (iNOS) ve COX-2'nin yanı sıra hücreler arası adhezyon molekülleri de dahil olmak üzere inflamasyon

yolaklarını ve bu hastalıkların patojenezinde kritik bir rol oynadığı düşünülen IL-1*β* gibi proinflamatuvar sitokinlerin aktivasyonunda yer alan NALp3 inflamazomunu inhibe ederek antiinflamatuvar etki gösterdikleri kayıtlıdır [79, 88].

Spesifik bitkisel ilaçların veya bitki sekonder metabolitlerinin inflamatuvar hastalıklarda kullanımında, atak semptomları ile patolojik hasarı azaltabilir ve konvansiyonel ilaç tedavileri ile kombinasyonunda sinerjik etkiler gözlenebileceği belirtilmiştir [90]. *Andrographis paniculata, Cannabis sativa, Panax ginseng ve Glycyrrhiza glabra* gibi tıbbi bitkilerin, AAA tedavisinde etkili-güvenli kabul edilen kolşisinin NALp3 inflamozomunu ve NF-κB aktivitesini inhibe eden etki mekanizmasına benzer mekanizmalar üzerinden antiinflamatuvar aktivite gösterdikleri kayıtlıdır [33, 43, 72].

Örnek olarak, *Andrographis paniculata*'dan elde edilen andrografolit, *Cannabis sativa*'dan elde edilen THC ve CBD ve *Panax ginseng*'den elde edilen ginsenozitler, NALp3 inflamozomunun aktivasyonunu inhibe ederek antiinflamatuvar etki gösterdiği belirtilmiştir [33, 43 72]. *Bupleurum falcatum'dan* elde edile saikosaponinler; *Eleutherococcus senticosus*'tan eloterozit E [54]; *Glycyrrhiza glabra'*dan likoflavononlar [91]; *Glycyrrhiza uralensis'*den izoliquiritigenin [84]; *Pinellia ternata'*dan lectin [92]; *Schisandra chinensis*'ten şizandrin [58, 59]*; Scutelleria baicalensis*'ten baikalin [93]; *Zingiber officinale*'den gingerol ve shogaol [94]; *Ziziphus jujubae*'dan flavonoitler, NF-κB aktivitesini inhibe ederek antiinflamatuvar etki gösterdiği belirtilmiştir [79].

Ek olarak, bazı gıdaların inflamasyon ve endotel disfonksiyonun artışı ile ilişkili olduğu, belirli makro ve mikro besinlerin eksikliği veya fazlalığının immün sistem fonksiyonlarını büyük ölçüde etkileyerek kronik inflamatuvar hastalıkların gelişme riskini artırdığı belirtilmekte, anti-inflamatuvar/pro-inflamatuvar oranı yüksek tıbbi bitkilerce zenginleştirilmiş kapsamlı bir beslenme programının da AAA akut ataklarının kontrolünde önemli bir rol oynayabileceği kaydedilmektedir. Ayrıca antiinflamatuar etkili takviyelerin ve antioksidanlar açısından zengin bir diyetin AAA semptomlarını azaltıp hastanın yaşam kalitesini artırdığı bildirilmektedir [95]. AAA'nın birinci basamak tedavisinde güncel olarak kullanılan kolşisin ciddi yan etkilere neden olmaktadır, terapötik aralığı düşüktür ve bazı vakalarda tedaviye direnç ve intolerans gözlenmektedir [10, 16].

Bu çalışmada AAA'da kullanılan potansiyel tıbbi bitkiler veya kombinasyonları değerlendirilmiş, *Cannabis sativa* L., *Andrographis paniculata* Nees. ve diğer bitki

kombinasyonlarının sınırlı bir hasta grubunda yapılan çalışmalarında; antiinflamatuvar ve immünomodülatör etkilerinden dolayı atak şiddetini azaltmada etkili olduğu gözlenmiştir [35, 36, 45]. Tıbbi bitkilerin otoinflamatuvar hastalıklardaki etkinliğini anlamak için daha fazla klinik çalışmaya ihtiyaç vardır. Diğer bitkilerin veya bitki kombinasyonlarının AAA tedavisinde terapötik olarak kullanımı için daha fazla klinik çalışma gerçekleştirilmelidir.

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Data Availability statement / Veri Kullanılabilirliği bildirimi
The authors confirm that data supporting the findings of this study are available in the article.
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Compliance with ethical standards / Etik standartlara uyum
Conflict of interest / Çıkar çatışması
The authors declare no conflict of interest.
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Ethical standards / Etik standartlar
The study is proper with ethical standards.
Çalışma etik standartlara uygundur.
Authors' contributions / Yazar katkıları
All authors conceived and designed the work. All authors reviewed the literature, collected data and contributed to the preparation of the manuscript.
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DECLARATION: This work is part of Gülfidan KUYUMCU's MA thesis. Articles on 27-29 September 2017 held in Bayburt in Turkey was presented as a Oral Presentation I. International Organic Agriculture and Biodiversity Symposium

Genetic Analysis Related To Organized Genetic Changes in Potato And Processed Potatoes

Gulfidan Kuyumcu[1*], Muhammed Majed Abed[2]

Author Addresses: [1] Samsun Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, Samsun / Turkey
[2] Samsun Ondokuz Mayıs University, Faculty of Agriculture, Department of Agricultural Biotechnology, Samsun / Turkey

*Corresponding Autor: Gulfidan Kuyumcu, e-mail: ijlsb@intsa.org

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