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Effect of Additional Prebiotics on Glycemia and Lipid Profile in Individuals with Type 2 Diabetes

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Abstract

This study was conducted to determine the effect of prebiotic fiber intake on glycemia and lipemia in individuals with Type 2 Diabetes Mellitus (Type 2 DM). Over a 8-week period, 14 individuals with Type 2 DM between the ages of 20-50 with a Body Mass Index (BMI) above 25 kg/m² were randomly assigned to either an intervention group, in which participants were given additional prebiotic fiber with diabetic diet (n = 8, consuming 10 g/d of oligofructose-enriched inulin to diabetic diet) or to a control group, in which participants were given only with a diabetic diet (n = 6, consuming only diabetic diet). Fasting Plasma Glucose (FPG), fasting insulin, insulin resistance (HOMA-IR), glycosylated hemoglobin (HbA1c), Triglyceride (TG), Total Cholesterol (Total-C), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C) and Very Low-Density Lipoprotein Cholesterol (VLDL-C) values were measured at the beginning and end of the study. There was no significant difference between the initial and last measurements in HbA1c, fasting insulin, HOMA-IR, and HDL-C values only in the diabetic diet group ($p>0.05$); whereas FPG, TG, Total-C, LDL-C, and VLDL-C levels decreased ($p<0.05$). In the group that received additional prebiotics in the diet, the FPG, HbA1c, TG, Total-C, VLDL-C, and LDL-C levels decreased significantly ($p<0.05$) at the end of the study compared to the beginning; HDL-C ($p<0.05$) was elevated. Prebiotics can be used as a new option in the treatment of many metabolic diseases, especially diabetes. Prebiotics will make a positive contribution to metabolic markers by changing the composition of the microbiota.

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1. Introduction

Diabetes has become a serious worldwide health problem that arises with elevated blood sugar levels (Wang et al., 2021). According to the data from the International Diabetes Federation (2021), there are 536.6 million adults with diabetes between the ages of 20-79 in the world, and this number is expected to reach 642.7 million in 2030. In recent years, besides the risk factors such as diet, environmental and genetic factors that affect the development of chronic diseases such as DM, obesity, metabolic syndrome, and cardiovascular disease (CVD) the role of changes in the intestinal microbiota has also been studied (Gholizadeh et al., 2019; Gurung et al., 2020). In the intestinal microbiota of individuals with Type 2 DM, while there is a reduce in functional bacteria number such as *Bifidobacteria* and *Bacteroidetes/Firmicutes* ratio; an raise is observed in both opportunistic pathogenic bacteria and some gram-negative bacteria that produce endotoxin (Larsen et al., 2010; Woldeamlak et al., 2019).

The contribution of the microbiota to the formation of insulin resistance and Type 2 DM is explained by many molecular mechanisms. The intestinal microbiota has an important role in the development of Type 2 DM by influencing metabolic pathways through different potential mechanisms, including intestinal permeability, modulation of inflammation, and glucose and lipid metabolism (Gurung et al., 2020). Therefore, regulation of the microbiome may be a beneficial strategy in the treatment of Type 2 DM (Wang et al., 2021).

Prebiotic fibers are classified as Inulin Type Fructans (ITF) (oligofructose, inulin, fructo-oligosaccharides), galactooligosaccharides, oligosaccharides from starch and glucose (resistant starch), non-carbohydrate oligosaccharides (such as flavanol), and other oligosaccharides (pectic oligosaccharides). Prebiotics are non-digestible nutritional components in the structure of oligosaccharides that enhance host health by selectively stimulating the activity and/or growth of one or more bacteria in the colon (Davani-Davari et al., 2019).

With the understanding of the role of intestinal microbiota in the pathophysiology of metabolic diseases, it was thought that prebiotics could be used as a new option in the treatment of diabetes (Davani-Davari et al., 2019; Wang et al., 2021). It has been stated that prebiotics have a positive contribution to metabolic markers by transforming the structure of the microbiota. It has been shown that prebiotic treatment improves intestinal microbiota fermentation, reduces hunger, and improves postprandial glucose response (Cani et al., 2009; Parnell & Reimer, 2012).

Prebiotics produce short-chain fatty acids (SCFA) by fermentation in the colon, which can slow down gastric discharge by regulating stomach movements. In studies conducted on people with Type 2 Diabetes, it has been shown that ITF have an improving impact on serum glucose levels by delaying gastric emptying, reducing postprandial glucose levels (Dehghan et al., 2014; Wang et al., 2019), and significantly decreasing FPG, HbA1c (Dehghan et al., 2016; Dehghan et al., 2014) TG, Total-C and LDL-C, while increasing HDL-C (Dehghan et al., 2016; Roshanravan et al., 2017). In order to show these effects, it is recommended that ITF supplementation be 10 g daily

for 6 weeks or more (Dehghan et al., 2016; Dehghan et al., 2014; Roshanravan et al., 2017; Wang et al., 2019).

Therefore, dietary interferences can be recommended as a beneficial strategy to prevent, delay or treat Type 2 DM by causing positive variations in the gut microbiota. It has been proposed that additional prebiotics to diet may be an effective method to change the composition of the intestinal microbiota in individuals with pre-diabetes (Larsen et al., 2010; Wang et al., 2021).

In our study we planned in light of this information, it was aimed to evaluate the effect of prebiotic fiber intake on glycemia and lipemia in individuals with Type 2 DM. Certain limitations existed in the present study. The sample size of our study was small. Additionally, the duration of the intervention was 2 months. The intervention period is insufficient in terms of evaluation of biochemical markers such as HbA1c. Therefore, randomized controlled trials with larger samples are needed to determine the short and long-term effects of prebiotics on microbiota.

2. Materials and Methods

2.1. Individuals

This study was conducted with 14 individuals with Type 2 DM who were diagnosed and treated by the Department of Endocrinology at Selçuk University Hospital, between the ages of 20-50 y, with a BMI above 25 kg/m². Inclusion criteria for the study: diagnosed with Type 2 DM for more than 6 months, having a regular diabetic diet, and currently using oral antidiabetic drug. A history of any diagnosed pancreatic, gastrointestinal, thyroid, cardiovascular, kidney and liver disease, using insulin, regular use of probiotics, prebiotics, antibiotics, antacids, alcohol, antidiaretic and anti-inflammatory medications, being pregnant or breastfeeding, daily fiber intake of 30 g and above, and changing antidiabetic medication during the study are the exclusion criteria of the study.

2.2. Study Design

The intervention group received 10 g of prebiotic fiber (inulin+fructooligosaccharide) daily in addition to the diabetic diet (n=8), and the control group received a diabetic diet (n=6) for 8 weeks. The prebiotic supplement given to the volunters has been met by the researchers. The diabetic diet programs previously applied by the individuals in the study and control groups were rearranged by the researchers according to current body weight and height. The 'Mifflin-St. Jeor Equation' based on current weight was used to calculate the Basal Metabolic Rate (BMR) of individuals (Mifflin et al., 1990). The macronutrient pattern of the diabetic diet is organized so that 45-60% of energy comes from carbohydrates, 15-20% from protein and 25-30% from fat (TEMD, 2022). The volunteers were randomly assigned to two groups. At the beginning and end of the study, all participants' FPG, fasting insulin, HOMA-IR, HbA1c, TG, Total-C, LDL-C, and VLDL-C values were measured. The study was initiated after determining the sample size within the population with 95% confidence interval and 80% power and a total sample size of at least 14. The study has been approved by the ethics committee of Gazi University (Decision No: 14.06.2021 / 578).

2.3. Statistical Analysis

Data were analyzed using Statistical Package for the Social Science (SPSS) version 23.0 software. Non-

parametric methods were used for the measurement values that did not conform to the normal distribution. In accordance with non-parametric methods, the "Mann-Whitney U" test method was used to compare the measurement values of two independent groups. The "Wilcoxon" test method was used to compare the double-repeated measurements of two dependent groups.

3. Results

There was no statistically significant difference between the groups in terms of gender, educational status, and occupation (p>0.05) (Table-1).

There was no statistically significant difference between the groups in terms of age and diabetes duration (p>0.05). There was no statistically significant difference between the groups in the FPG, HbA1c, fasting insulin, HOMA-IR, TG, HDL-C, and VLDL-C values measured at the beginning and end of the study (p>0.05). At the beginning of the trial, Total-C and LDL-C values were lower in the diet group than in the prebiotic fiber group (p<0.05). In the diet group, FPG, TG, Total-C, LDL-C, and VLDL-C values were significantly lower compared to the baseline values at the end of the study (p<0.05); while there was no significant difference between the first and last measurement in HbA1c, fasting insulin, HOMA-IR and HDL-C values (p>0.05).

Fasting plasma glucose, HbA1c, fasting insulin, HOMA-IR, TG, Total-C, LDL-C, and VLDL-C values decreased significantly at the end of the study compared to baseline in the group receiving additional prebiotics in the diet; whereas HDL-C increased (p<0.05) (Table- 2).

Table 1. Distribution of Individuals by General Characteristics

Variables	**Diet group [n=6]**	**Diet+prebiotic group [n=8]**	**p**
	n (%)	n (%)	
Gender			
Female	4 (%66.7)	2 (%25.0)	0.119
Male	2 (%33.3)	6 (%75.0)	
Educational status			
Middle school	3 (%50.0)	-	
High school	2 (%33.3)	3 (%37.5)	0.058
University	1 (%16.7)	5 (%62.5)	
Occupation			
Officer	-	3 (%37.5)	
Employee	2 (%33.3)	3 (%37.5)	0.171
Self-employment	2 (%33.3)	2 (%25.0)	
Housewife	2 (%33.3)	-	

Data are presented as number (percent)
p values for chi-square test

4. Discussion

Medical Nutrition Therapy (MNT), together with exercise and drug therapy, forms the basis of the medical treatment of diabetes patients. In order to provide effective glycemic and metabolic control in diabetes and to delay or prevent complications of diabetes, MNT should continue to be a part of the treatment throughout life (ElSayed et al., 2023). Regular and adequate intake of soluble fiber is one of the important dietary factors that play a role in the clinical management of diabetes by lowering the postprandial blood glucose level and reducing insulin resistance (Xie et al., 2021).

The presence of Type 2 Diabetes is related with the deterioration of the balance in the intestinal microbiota. Dysbiosis in the microbiota causes inflammation and affects glucose metabolism, insulin sensitivity, and tissue fatty acid composition. Dietary interventions such as increasing prebiotic fiber consumption have a dominant therapeutic effect in the prevention and management of Type 2 DM by changing the intestinal microbiota (Birkeland et al., 2021; Liu et al., 2017; Pourghassem Gargari et al., 2013).

It has been shown in many studies that soluble dietary fiber added to the diet plays an important role in glucose homeostasis with its positive effects on HbA1c, postprandial blood sugar, insulin resistance (Liu et al., 2017; Xie et al., 2021), and FPG (Liu et al., 2017; Silva et al., 2013) in individuals with Type 2 DM. In a meta-analysis study, 33 randomized clinical studies were evaluated, and it was shown that ITF, which is 10 g of soluble dietary fiber added to the diet for 6 weeks and longer, is effective in improving glycemic control (Wang et al., 2019).

Another meta-analysis study managed by Paul et al. (2022) reported a decrease of 0.45% in HbA1c, 13.98 mg/dl in FPG, 0.75 μU/mL in fasting insulin, and 0.88 units in HOMA-IR with the addition of prebiotics in individuals with type 2 DM. In this study, it was also observed that ITF supplementation used in addition to the diet for 8 weeks significantly reduced the glycemic indicators; FPG, HbA1c, HOMA-IR, and fasting insulin ($p<0.05$). While the change in HbA1c, fasting insulin, and HOMA-IR values was not significant in the diet intervention group ($p>0.05$); the decrease in fasting blood sugar was significant ($p<0.05$). These results suggest that prebiotics may contribute to glucose metabolism by positively changing the intestinal microbiota. Inulin-type fructans can improve serum glucose levels by delaying gastric discharge, reducing postprandial serum glucose, and slowing the entry of glucose into the bloodstream. In addition, prebiotics may indirectly affect hepatic glucose metabolism by decreasing the concentration of plasma fatty acids. In addition, it has been shown that oligofructose can improve blood glucose metabolism by increasing the levels of GLP-1 and Glucagon-Like Peptide 2 (GLP-2) (Wang et al., 2019). Prebiotics increase the production of GLP-2, which decreases intestinal permeability, thereby reducing endotoxemia and reducing insulin resistance (Ho et al., 2016).

Table 2. Comparison of the Groups by Demographic Characteristics and Pre and Post-Treatment Biochemical Results

	Diet group [n=6]	**Diet+prebiotic group [n=8]**	**p[1]**
	Median (I-III QR)	**Median (I-III QR)**	
Age (years)	41.5 (31.2-50.0)	42.0 (37.0-46.7)	0.897
Diabetes duration (years)	1.0 (0.87-8.75)	6.0 (5.0-9.7)	0.268
FPG (mg/dl)			
Initial	130.5 (95.0-196.0)	132.0 (107.5-172.0)	0.561
End	98.0 (91.2-122.5)	117.5 (94.5-147.5)	0.517
p[2]	0.046	0.012	
HbA1c			
Initial	6.6 (6.2-8.6)	8.1 (6.6-8.5)	0.243
End	6.1 (5.9-6.6)	6.6 (5.8-7.5)	0.603
p[2]	0.093	0.012	
Fasting Insulin			
Initial	17.4 (9.2-29.4)	10.2 (5.7-15.9)	0.245
End	9.3 (7.4-12.0)	8.3 (7.0-11.5)	0.519
p[2]	0.116	0.050	
HOMA-IR			
Initial	3.7 (2.4-10.6)	4.3 (1.9-4.9)	0.698
End	2.7 (2.4-3.9)	2.7 (1.8-3.3)	0.796
p[2]	0.173	0.036	
TG(mg/dl)			
Initial	201.5 (142.7-221.0)	215.0 (151.5-299.0)	0.366
End	142.0 (116.0-193.2)	165.5 (121.0-195.0)	0.699
p[2]	0.028	0.012	
Total-C (mg/dl)			
Initial	211.0 (201.5-218.2)	253.0 (235.5-279.2)	0.002
End	184.5 (168.2-200.2)	218,5 (195.2-231.5)	0.014
p[2]	0.028	0.012	
LDL-C (mg/dl)			
Initial	129.5 (107.5-136.5)	160.5 (152.7-181.7)	0.002
End	114.5 (102.0-119.2)	134.5 (125.7-165.5)	0.004
p[2]	0.028	0.012	
HDL-C (mg/dl)			
Initial	43.0 (40.2-50.7)	42.0 (38.0-45.0)	0.361
End	45.5 (42.5-53.0)	52.0 (44.5-54.0)	0.476
p[2]	0.279	0.017	
VLDL-C (mg/dl)			
Initial	40.3 (28.5-44.2)	43.1 (30.3-59.8)	0.366
End	28.4 (23.2-38.6)	33.1 (24.2-39.0)	0.699
p[2]	0.028	0.012	

Values are given as median.
FPG, fasting plasma glucose; HbA1c, glycosylated hemoglobin; HOMA index, insulin resistance; TG, triglyceride; Total-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.
p[1]<0.05, "Mann-Whitney U" test for comparison of measurement values of two independent groups
p[2]<0.05, "Wilcoxon" test statistics were used to compare the difference between baseline values and post-treatment measurements.

Studies have shown that ITF consumption enhances the serum lipid profile and thus helps to reduce the risk of CVD (Brighenti, 2007; Reis et al., 2014). Inulin is a viscous and soluble compound that inhibits cholesterol absorption. As a result of colonic fermentation of prebiotics, short-chain fatty acids butyric acid, propionic acid, and acetic acid are produced. These acids, in addition to providing energy to colon epithelial cells, are involved in supporting the survival of beneficial bacteria by lowering the ambient pH, regulating blood sugar, and secreting satiety intestinal hormones. It also plays an important role in improving the profile of lipids, including Total-C, HDL-C, LDL-C, and VLDL-C. Inulin-type fructans decrease the expression of genes encrypting liver enzymes required for de novo synthesis of lipids, increase muscle lipoprotein lipase enzyme mRNA transcript and activity, increase the production of SCFAs, excretion of fecal bile salt and cholesterol, and increase *Bifidobacterium spp* growth (Liu et al., 2017; Tawfick et al., 2022).

Uncontrolled diabetes patients typically have low HDL-C and high TG level. High triglyceride levels affect LDL cholesterol metabolism and lead to the formation of smaller and denser LDL particles, which are atherogenic (Mach et al., 2020). Some randomized placebo-controlled studies have reported that ITF intake improves the lipid profile therefore has a possible influence on reducing the risk of CVD (Dehghan et al., 2016; Dehghan et al., 2014; Reis et al., 2014; Roshanravan et al., 2017). Dehghan et al. (2014) announced that intake of 10 g/d inulin over 8 weeks had significant impacts on lowering FPG, fasting insulin, Total-C, LDL-C, and TG, increasing HDL-C. In this study, ITF supplement used in addition to the diet significantly decreased TG, Total-C, LDL-C, and VLDL-C values at the end of the study compared to the beginning ($p<0.05$); It was found to increase HDL-C ($p<0.05$). The change in HDL-C values was not significant ($p>0.05$); The decrease in TG, Total-C, LDL-C, and VLDL-C values was significant ($p<0.05$) in the diet group only. The recommended LDL-C value for individuals with diabetes at moderate cardiovascular risk is <100 mg/dL. In the 2019 EAS/ESC guideline, lowering the LDL-C level is recommended as the primary treatment to reduce the risk of CVD (Mach et al., 2020). With the addition of ITF, this reduction in LDL-C constitutes 2.7% of the recommended value (Liu et al., 2017). It has been shown that each 40 mg/dL decrease in LDL-C provides a 23% reduction in cardiovascular conditions (Baigent et al., 2005). Therefore, ITF supplementation with diet may be a preventive measure for CVD mainly through the reduction in LDL-C concentration and other positive effects on lipid homeostasis.

5. Conclusion

According to the results of this study, it has been shown that a daily intake of 10 g oligofructose-enriched inulin in addition to the diabetic diet for approximately 8 weeks improves the glycemic status and lipid profile in Type 2 DM. It suggests that the use of a prebiotic (ITF) that supports the positive effects of a diabetic diet may be an effective way to change gut microbiota and/or function. All these findings show that ITF can be used as adjunctive treatment for glycemic and lipemic control, particularly for patients with Type 2 DM in clinical practice.

Ethical Statement

Ethical approval for this study was obtained from Ethics Committee of Gazi University on 14.06.2021 with approval number 578.

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This study did not receive any financial support.

Presentation Information

The data from this study were presented as an oral presentation at I. International Congress on Medical Sciences and Multidisciplinary Approaches. The presentation date was February 20th, 2021.

Conflicts of Interest

The authors declare no conflicts of interest regarding this study. Any institution or organization providing funding for this research did not have any role in the design, data collection, analysis, interpretation, or publication to influence or distort the findings.

Author Contributions

The contributions of the authors are as follows: Ayşe Ayda Demirtaş participated in data collection and analysis, prepared the draft of the paper; Nilüfer Acar Tek conducted the supervision and final revision of the manuscript.

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Review

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The Effect of Forced Migration on Mental Health in Elderly Individuals: A Struggle in the Shadow of Disasters

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Abstract

Natural disasters are social crises that affect people together with their environment, which are unpredictable where and when they will occur worldwide. Natural disasters are a process that deeply affects people's lives, often necessitating relocation. Natural disasters affecting individuals from all age groups significantly affect elderly individuals, who are a vulnerable and fragile group due to physical limitations, chronic diseases and other age-related factors. Uncertainty and anxiety together with difficult experiences and change of location after the disaster is a traumatic process that especially affects elderly individuals. The fact that elderly individuals constitute the majority of the increasing population on a global scale and the increasing number of natural disasters today makes it a priority to address the mental health of elderly individuals and to carry out more studies on survival strategies. From this point of view, this review study aims to provide strategies for the protection, development and rehabilitation of the mental health of elderly individuals by focusing on the mental health problems and coping methods of elderly individuals who try to establish a new life by having to relocate due to natural disasters and natural disasters. Pre-disaster preparation and post-disaster intervention programs should be prepared specifically for elderly individuals and should be structured taking into account cultural/geographical differences.

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1. Introduction

Natural disasters are a worldwide reality and are important events that deeply affect people's lives, societies and the environment (Bayraktar & Dal Yılmaz, 2018). These disasters can cause people to leave their homes, communities, and familiar living spaces by forcing displacement (Cofini et al., 2015; Şengül & Turan 2013; Yıldız et al., 2018). Due to the nature of natural disasters, the unpredictability of when and where they may occur further increases their destructiveness. It is a fact that natural disasters always pose a threat worldwide, and the simultaneous fires, floods and the recent earthquake in Turkey in recent years have once again revealed this fact (T.C. İçişleri Bakanlığı Afet ve Acil Durum Yönetimi Başkanlığı, 2023). According to the data of the Disaster and Emergency Management Presidency of the Ministry of Interior of the Republic of Turkey (T.C. İçişleri Bakanlığı Afet ve Acil Durum Yönetimi Başkanlığı), the impact area of the 6 February 2023 earthquake was quite large and spread over an area of 108,812 square kilometers covering 11 provinces, and the settlements and rural areas within this area were largely affected, and according to the June report, 50,783 people lost their lives, 115,353 people were injured and 37,984 buildings were destroyed due to the earthquake. Furthermore, according to the Turkish Statistical Institute (Türkiye İstatistik Kurumu) (2022), an estimated 14.013.196 people were affected as a result of this earthquake, which revealed a serious housing problem. According to the report published by the International Organization for Migration, an estimated 2.7 million people, including migrants, needed to relocate due to the need for forced shelter (IOM, 2023).

Natural disasters that cause forced displacement cause serious physical, emotional and spiritual traumas to elderly individuals, as in all age groups. Although these traumas sometimes have different effects on individuals, older individuals are more sensitive to traumatic experiences than young people and their reactions to trauma may also differ (Şevik, 2023). However, there are not enough studies to reveal these differences, especially in elderly individuals who have had to relocate or migrate. For this reason, studies on this subject are needed to guide future planning and research.

2. Compulsory Relocation After Natural Disasters and Its Impact on Elderly Individuals

2.1. Compulsory Relocatioin After Natural Disaster

Natural disasters are events that deeply affect people's lives and social structures (Mayer et al., 2020). The massive destruction caused by these disasters can cause people to lose their living spaces, homes and communities they belong to, leading to forced displacement (Cofini et al., 2015; Şengül & Turan, 2013; Yıldız et al., 2018). Such displacement can be temporary, long-term or permanent and can take place both in the same geography and in different geographies. This situation can seriously affect the social structure and solidarity of local communities and result in economic-psychological difficulties.

2.2. The Effect of Forced Relocation on Elderly Individuals

Natural disasters cause many people to lose their lives, but they can also cause forced displacement by destroying people's living spaces, homes and communities they belong to. This displacement may be temporary in terms of duration, long-term or permanent, or it may be in the same geography or another geography (Cofini et al., 2015; Şengül & Turan 2013; Yıldız et al., 2018). Disasters cause individuals of all age groups to leave their homes and the environment they live in due to the chaos, uncertainty and anxiety environment it causes, and have a deep impact on their lives as a result of leaving their roots and leaving their familiar surroundings behind. Especially elderly individuals, who are among the vulnerable groups against the negative effects of natural disasters, are negatively affected by the experience of forced displacement (Bayraktar & Dal Yılmaz 2018; Donatelli & Somes 2012; Claver et al.,2013).In addition to the biological progression of old age, physical limitations in the aging process and diseases, changes in cognitive functions, perception, decreased attention and concentration, memory weakness, difficulty in adapting to the new, withdrawal from life due to loss of role, status and income, loneliness, fragility, inability to use technology and the fear of novelty caused by all these reasons cause them not to want to move away from the environment they are accustomed to and to show avoidance behavior to avoid relocation under any circumstances (Kalınkaya, 2021; Kart & Kinney 2001). Chronic diseases of elderly individuals, which increase with age, further increase their vulnerability and fragility. The difficulties experienced by elderly people who have to relocate due to natural disasters due to chronic diseases are very important and this issue should be taken into consideration for the planning of disaster management health services. Access to medication, access to medical care and difficulties in controlling chronic diseases due to stress and anxiety are challenging for elderly people with chronic diseases. In addition, it should not be ignored that the caregiving conditions of elderly individuals who need continuous care due to their chronic diseases change and become more difficult for their caregivers. Approximately 80% of the elderly affected by disasters have at least one chronic disease and 50% have at least two chronic diseases (Centers for Disease Control and Prevention, 2011). It has been reported that the majority of deaths during and after the earthquake, tsunami and nuclear disaster that occurred in Japan in 2011 were elderly individuals (Help Age, 2013).Studies have emphasized the vulnerability and vulnerability of elderly individuals with chronic diseases. (Yerli, 2017; Küçük & Karadeniz, 2021) In addition to all these, the increase in studies on elderly individuals who need continuous care and their caregivers will provide a different perspective in disaster management. All these factors show the impact of natural disasters on older persons and the difficulties of forced displacement on older persons.

3. Post Traumatic Stress And Recovery Process in Elderly People After Natural Disasters

Individuals affected by disasters may have negative feelings about their lives in addition to the pathological problems they experience, have difficulty in fulfilling their roles and responsibilities in daily life in the changing environment after disasters

and experience coping difficulties. Although it is accepted as a social reality that survivors recover after natural disasters such as earthquakes and recover after a while, it should be noted that elderly individuals, who are considered as a vulnerable group in natural disasters, may be exposed to longer-term psychological disorders following the end of the acute period after a disaster (Şevik, 2023). Mental health problems are observed in individuals who have experienced losses and witnessed these losses (Kim et al., 2022). As a traumatic life event, natural disasters are potentially serious sources of vital, existential stress. Especially post-traumatic stress disorder emerges after disasters as a common mental health problem (Adhikari Baral & KC., 2019). In DSM-IV-TR, Posttraumatic Stress Disorder (PTSD) is defined as a psychiatric disorder lasting more than one month after the event, characterized by specific symptoms such as hyperarousal, re-experiencing, avoidance, decreased interest in the outside world, slowed reactions and alienation, causing clinically significant distress and impairment in social, occupational or other areas of functioning and other areas (Battal & Özmenler, 1997). Many studies have been conducted to investigate mental disorders that may occur after natural disasters, which are a source of great existential stress. In a study conducted after the earthquake in Nepal in 2015, it was emphasized that PTSD was significantly related to the age of adult survivors of the earthquake, and PTSD was detected in more than half (62.7%) of the elderly survivors (Adhikari Baral & KC., 2019). Some studies conducted in China and Italy also revealed similar findings (Jia et al., 2010). As a result of the studies, the increased risk of PTSD with age has been attributed to vulnerability, physical frailty in older individuals, chronic diseases, low coping levels and decreased adaptation to stress (Blevins et al., 2015). After natural disasters, it is accepted as a social expectation that individuals exposed to the disaster will recover and hold on to life after a short period of time. However, variables such as the magnitude of the disaster, losses experienced, individual differences, age, gender, social roles, etc. should be taken into consideration in the aftermath of the acute period and mental health disorders that may occur in the long term (Şevik, 2023). Although definitions such as getting used to the new normal, continuation of life or the effort to hold on to life are created, the loss and deaths of families, close relatives who have witnessed the destructiveness of natural disasters, destroyed houses and neighborhoods, and on top of all these, having to migrate to other places from the living spaces they know where they live is an experience that challenges all individuals, even if it changes in proportion to the losses experienced. Especially for elderly individuals with high vulnerability and fragility in natural disasters, coping methods for holding on to life and getting used to the new may vary individually and socially. In a cross-sectional descriptive study on PTSD and coping strategies, it was found that individuals with PTSD affected by the earthquake had significantly higher scores of religious coping, passive coping, self-distraction coping and substance use coping, while those without PTSD had significantly higher scores of active coping (Adhikari Baral & KC., 2019). In addition, studies have also shown that they resort to religious coping methods such as praying, worshiping, believing in the help of the creator, and doing charity work as coping strategies (Adhikari Baral & KC., 2019); Ali et al.,2012; Hollifield,2008; Feder et al.,2013). In a

similar result, it was determined that 76% of individuals used religious coping methods during the 1999 Kocaeli and Düzce earthquakes (Kula, 2002). In another study, emphasizing the importance of cultural context in the recovery process after disasters in Myanmar and Taiwan, it was concluded that disaster victims with strong social networks and a good social life were more resilient (James & Paton,2015). In a study conducted after the Italian earthquake, it was determined that increasing age did not have a linear relationship with coping scores, but acceptance affected coping mechanisms, especially religious coping and behavioral distancing increased with age (Cofini et al., 2015).

4. Conclusion and Recommendations

This review study aimed to provide strategies for the protection, promotion and rehabilitation of mental health of older adults by focusing on the effects of natural disasters on older adults and the mental health problems and coping methods of forced displacement. As a result, natural disasters cause serious traumas on elderly individuals both physically and emotionally. Especially when elderly individuals have to relocate after a disaster, they face physical limitations, social isolation and emotional difficulties brought about by this process. In addition, elderly individuals have a high risk of PTSD and this risk increases with age. It is thought that the evaluation of psychological problems that may arise after natural disasters and holding on to life in the case of elderly individuals will contribute to the literature, especially the studies examining religious coping methods in the spiritual dimension, which enables to see cultural, social and individual differences in a wider range. Considering that elderly individuals are more sensitive to traumatic experiences and react differently to these experiences, post-disaster support and rehabilitation programs should specifically address the elderly population. In addition, it should not be forgotten that cultural and social factors also play an important role in this process. This study aims to contribute to the development of further research and interventions to help older adults better prepare for the effects of natural disasters.

Ethical Statement

There is no need to obtain ethics committee permission for this study due to the use of open access sources. However, the study was conducted in accordance with ethical principles.

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The findings of this study have not been presented at any conference or journal.

Conflicts of Interest

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Author Contributions

The entirety of the writing process of the article belongs to the author.

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Research Article

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Investigation of the Resistance of Some Disinfectant Active Substances in ESBL-Producing *Enterobacteriaceae*

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Abstract

In this study, a total of 200 samples, including 100 neck skin and 100 cecum samples, were collected and analyzed from various poultry slaughterhouses on different sampling days. ESBL-producing *Enterobacteriaceae* were isolated and ESBL production was confirmed phenotypically by combined disk diffusion and E-test gradient strips. While ESBL production was confirmed in 10 (10%) of 100 neck skin samples, no significant ESBL production could be confirmed in 100 cecal samples. The broth microdilution method of Clinical and Laboratory Standards Institute (CLSI) was used to determine the resistance profiles against benzalkonium chloride (BC), cetylpyridinium chloride (CPC), N-alkyl dimethyl benzyl ammonium chloride (ADBAC) and potassium peroxymonosulfate (PPMS) disinfectants in 10 neck skin isolates with confirmed ESBL production. In the study, it has been determined that MIC_{50} and MIC_{90} values were respectively ADBAC (8 and 16 mg/L), BC (16 and 32 mg/L), CPC (16, and 32 mg/L), PPMS (≥ 1024 mg/L). The impacts of *Enterobacteriaceae* strains on food safety and public health are significant; Disinfectant resistance can lead to increased transmission of antibiotic-resistant bacteria, leading to serious infections in humans that are difficult to treat. For that reason, it is of great importance to develop effective control methods, including appropriate disinfectant use, hand hygiene and appropriate isolation measures, to prevent the spread of disinfectant resistant *Enterobacteriaceae* strains in food production systems.

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1. Introduction

Until the late 20th century, the safe use of empiric cephalosporins and fluoroquinolones by the clinician against major infections caused by *Enterobacteriaceae* strains has now been compromised by resistance conferred by extended-spectrum beta-lactamases (ESBLs) and AmpC enzymes. ESBLs are enzymes that can hydrolyze a wide variety of substrates, including most penicillins, cephalosporins (except cephamycins), and monobactams. ESBLs came to prominence in the early 1980s, first in Germany and shortly thereafter in France. These early ESBLs were reported to be structural mutants of TEM-1, TEM-2, and SHV-1, penicillinases common among *Escherichia coli* and *Klebsiella pneumoniae* (Denton, 2007).

Subsequently, in the 1990s, an increasing number of reports of TEM and SHV-induced ESBLs were published from many parts of the world, including the United Kingdom, particularly in association with nosocomial outbreaks. ESBL-producing *E. coli* (Palucha et al., 1999), *Salmonella enterica* serovar Typhimurium (Vahaboglu et al., 1996), *Citrobacter diversus* and *Klebsiella oxytoca* (El Harrif-Heraud et al., 1997) and *K. pneumoniae* have caused epidemics in various units of healthcare institutions (Venezia et al., 1995).

Although many epidemics are caused by the spread of microorganisms carrying certain resistance genes from patient to patient, Hibbert-Rogers et al. (1995) reported that the same microorganism carrying different resistance genes was transferred to different microorganisms through plasmid transfer between microorganisms.

Some studies have reported a significant increase in ESBL-producing *Enterobacteriaceae* from both community and patient origin. This increases the possibility that animals and food may play an important role in the epidemiology of ESBLs in community-acquired *Enterobacteriaceae* (Valverde et al., 2004; Liebana et al., 2006; Bertrand et al., 2006; Hasman et al., 2005; Riano et al., 2006).

Disinfectant resistance in ESBL-producing *Enterobacteriaceae* strains has become an issue of concern in the healthcare industry. Disinfectant resistance refers to the ability of bacteria to withstand the effects of disinfectants used to eliminate pathogen microorganisms from surfaces and equipment (Tong et al., 2021). ESBL-producing *Enterobacteriaceae* strains are a group of bacteria that produce extended-spectrum β-lactamases (ESBLs), enzymes that can break down certain types of antibiotics, making them resistant to treatment (Shaikh et al., 2015). These strains are often responsible for healthcare-associated infections and are a significant cause of morbidity and mortality worldwide (Navon-Venezia et al., 2017). Understanding the mechanisms of disinfectant resistance in these strains is crucial for developing effective infection control strategies and maintaining the effectiveness of disinfectants (Centers for Disease Control and Prevention [CDC], 2019).

Some different disinfectant resistance mechanisms have been reported in ESBL-producing *Enterobacteriaceae*. Efflux pumps are one of the main mechanisms of disinfectant resistance in ESBL-producing *Enterobacteriaceae* strains. Efflux pumps can facilitate the extrusion of a wide range of

substrates, conferring resistance to multiple antimicrobials (Anes et al., 2015). Many studies have reported efflux pumps as the primary mechanism of resistance to quaternary ammonium compounds (QACs), a common disinfectant used in healthcare settings (Nguyen et al., 2023; Hrovat et al., 2023). Efflux pumps expel the disinfectant from the bacterial cell, reducing its effectiveness and increasing the likelihood of bacterial survival (Tong et al., 2021).

Changes in cell membrane permeability may also contribute to disinfectant resistance in ESBL-producing *Enterobacteriaceae* strains. Resistance to disinfectants may result from changes in bacterial cell wall structure and function, which may reduce the ability of disinfectants to penetrate the bacterial cell (Boyce, 2023). Chlorine compounds commonly used as disinfectants can cause changes in membrane permeability, leading to resistance in bacterial strains (Rolbiecki et al., 2022). These changes may lead to reduced effectiveness of disinfectants and increased survival of bacteria, contributing to the spread of multidrug- resistant strains (Breijyeh et al., 2020; Chapuis et al., 2016).

Biofilm formation is another mechanism of disinfectant resistance in ESBL-producing *Enterobacteriaceae* strains. Biofilms are communities of bacteria that adhere to surfaces and can be difficult to eliminate with disinfectants (Dumaru et al., 2019). ESBL-producing *Enterobacteriaceae* strains have been shown to form biofilms that can protect themselves from disinfectants and antibiotics (Damiano et al., 2021; Laconi et al., 2023; Yılmaz & Güvensen, 2016). Murugesan et al. (2022) reported that ESBL-producing *E. coli* isolates had higher biofilm formation rates compared to non-ESBL-producing *E. coli* isolates (Murugesan et al., 2022). Biofilm formation may contribute to the persistence of antibiotic-resistant strains in healthcare settings, making them difficult to eradicate and increasing the risk of transmission to vulnerable patients.

The aim of the study was to determine the disinfectant resistance in phenotypically confirmed ESBL-producing *Enterobacteriaceae* isolates.

2. Materials and Methods

2.1. Isolation and Identification of ESBL-Producing *Enterobacteriaceae*:

Within the scope of this study, a total of 200 samples, including 100 neck skin and 100 cecum samples were collected from different poultry slaughterhouses in different time periods. Isolation of *Enterobacteriaceae* was done by classical culture technique using chromogenic ESBL (Extended spectrum beta-lactamase) medium (Himedia, M1829-500G). ESBL production in the obtained isolates was determined phenotypically by the combined disk diffusion method (Himedia, SD238-1KT) and E-test gradient strips (Himedia, EM132-30ST). Finally, the MIC values of the isolates that were found to produce ESBL phenotypically were determined with E-test gradient strips (Himedia, EM132-30ST) (The European Committee on Antimicrobial Susceptibility Testing [EUCAST], 2017).

2.2. Detection of Disinfectant Resistance in Isolates:

The broth microdilution method recommended by CLSI was used to determine the resistance against BC (benzalkonium chloride, Sigma Aldrich 12060), CPC (cetylpyridinium chloride, Himedia GRM1526), ADBAC (alkyl (C12-16) dimethylbenzyl ammonium chloride, Sigma Aldrich) and PPMS (potassium peroxymonosulfate, Himedia RM2406) disinfectants (Clinical and Laboratory Standards Institute [CLSI], 2018) The disinfectant concentrations used to determine the disinfectant MIC values of the isolates were kept between 0.125- 1024 mg/L (Wu et al., 2015).

Enterobacteriaceae isolates, previously kept at -85 °C, were revived by incubating in Tryptic soy broth (Millipore, 43592) at 37 °C for 24 hours. The next day, they were planted on Tryptic soy agar (Millipore, 22091) and incubated at 37 °C for 24 hours. Colonies were taken from the agars and suspended in tubes containing 0,9 % sterile saline. The turbidity standard was verified using NanoDrop Spectrophotometer (NanoDrop ND-100, Delaware, USA). The value at which the culture in the broth was fixed at a wavelength of 0.08- 0.13 at 625 nm was considered optimum, and it was diluted at a ratio of 1:100 and inoculated into Mueller-Hinton broth (Millipore, 70192) (Wu et al., 2015). 96-well microtiter test plates containing double concentrations of disinfectant solution and MHB were added with 50 µl of suspended culture at 10^5 cfu/ ml bacteria per well. The plates were incubated at 37 °C for 24 hours. MICs were recorded as the lowest disinfectant concentrations that prevented visible growth of microorganisms (absence of turbidity) in the wells of microtiter plates (Wu et al., 2015). *Escherichia coli* ATCC 25922 and *Salmonella* Typhimurium ATCC 14028 were used as controls for disinfectant susceptibility tests.

2.3. Statistical Analysis

The data were evaluated using the Microsoft Excel application. Table 1 and Figure 1 were prepared with Microsoft Excel application.

3. Results

In the combined disk method applied for the phenotypic determination of ESBL production, ESBL production was detected in 66 (66%) of 100 neck skin isolates. In addition, ESBL production was detected in 50 (52.6%) of 95 cecum isolates. ESBL production was detected in 50 of 95 cecum isolates by the combined disk diffusion (Himedia, SD238-1KT) method. However, ESBL production could not be detected in the E-Test gradient strip (Himedia, EM132-30ST) application performed to confirm ESBL production and ESBL production was evaluated as negative. In the E-test gradient strip application performed to verify ESBL production and ESBL production was confirmed in 10 (10%) of the neck skin isolates.

E-test strips containing different cephalosporin group antibiotics were applied to these 10 confirmed isolates, based on the guideline determined by EUCAST (2017), to determine the MIC values.

Table 1. MIC values of ESBL-positive *Enterobacteriaceae* strains, respectively, against specific disinfectants.

Enterobacteriaceae **Isolates Number**	**Disinfectants and MIC's (mg/L)**			
	ADBAC*	**BC***	**CPC***	**PPMS***
1	8	16	16	>1024
2	16	16	16	>1024
3	2	8	16	>1024
4	8	16	16	>1024
5	4	8	8	>1024
6	8	4	16	>1024
7	4	8	16	>1024
8	32	64	32	>1024
9	16	32	32	1024
10	8	16	16	>1024

*ADBAC: N- alkyl dimethyl benzyl ammonium chloride
*BC: Benzalkonium chloride
*PPMS: Potassium peroxymonosulfate
*CPC: Cetylpyridinium chloride

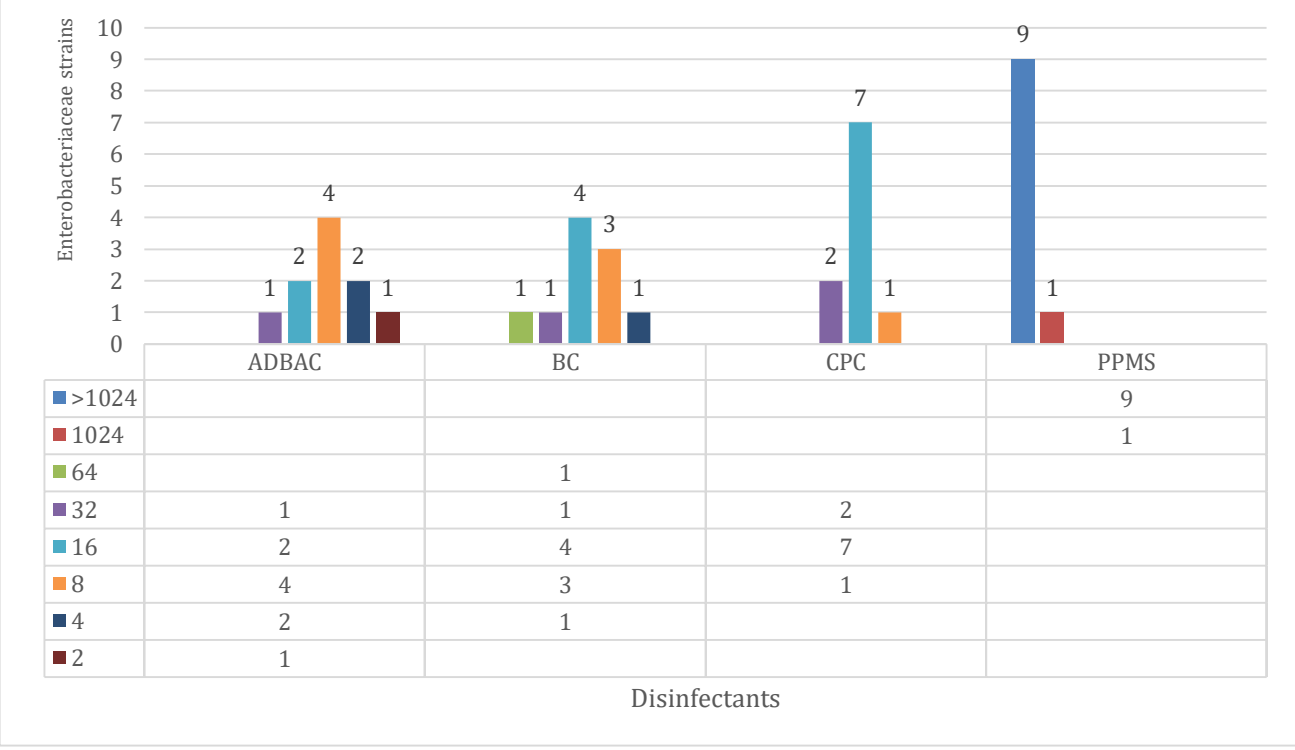

Figure 1. MIC values of ESBL-positive *Enterobacteriaceae* strains according to the microbroth dilution ratios developed against a certain disinfectant. (The numbers show how many strains in total have developed resistance to the relevant disinfectant at the relevant dilution rate, and the colors used symbolize the micro dilution rates.

As a result of the application, 6 (60%) of 10 chicken neck skin isolates were found to be highly resistant to ceftazidime and the other 4 (40%) were moderately resistant. Again, all 10 chicken neck skin isolates (100%) were found to be highly resistant to cefotaxime. Finally, 4 (40%) of 10 chicken neck skin isolates were found to be highly resistant to cefepime, the other 5 (50%) were moderately resistant and 1 (10%) was sensitive.

MICs of analyzed disinfectants were determined by CLSI's Broth microdilution method (2018) The highest MIC values were determined as >1024 mg/L (90%, n: 9) and 1024 mg/L (10%, n: 1) in PPMS. The other MIC values found 32 mg/L (10%, n: 1), 16 mg/L (20%, n: 2), 8 mg/L (40%, n: 4), 4 mg/L (20%, n: 2) and 2 mg/L (10%, n: 1) in ADBAC; 32 mg/L (20%, n: 2), 16 mg/L (70%, n: 7) and 8 mg/L (10%, n: 1) in CPC; 64 mg/L (10%, n: 1), 32 mg/L (10%, n: 1), 16 mg/L (40%, n: 4), 8 mg/L (30%, n: 3) and 4 mg/L (10%, n: 1) in BC.

In the study, it was determined that the samples had an ADBAC average of 10,6 mg/L and that it was the most sensitive active ingredient among the disinfectant active ingredients. It was found to have MIC values CPC 18,4 mg/L, BC 18,8 mg/L and PPMS >1024 mg/L, respectively.

It was found that MIC values varied between 32-2 mg/L for ADBAC, 64-4 mg/L for BC, 32-8 mg/L for CPC, and 1024 and above for PPMS. In the study, it has been determined MIC50 and MIC90 values were respectively ADBAC (8 and 16 mg/L), BC (16 and 32 mg/L), CPC (16 and 32 mg/L), PPMS (> 1024 mg/L).

4. Discussion

Studies have generally been carried out on the investigation of species and genus-specific disinfectant resistance in *Enterobacteriaceae*. For example, Boutarfi et al. (2019) conducted a study in Algeria with 77 *Enterobacter* spp. isolates, using hexachlorophene (CF) and benzalkonium chloride (BC) active ingredients. They found that their isolates tolerated disinfectants containing hexachlorophene (CF) and benzalkonium chloride (BC) at high rates such as 128 mg/L and 64 mg/L- 128 mg/L respectively. In another comprehensive study conducted by Morrissey et al. (2014), they collected 901 *Salmonella* spp., 368 *E. coli*, 60 *Klebsiella pneumonia*, 53 *Enterococcus faecium*, 56 *Enterococcus faecalis* and 54 *Enterobacter* spp. isolates obtained from certain university hospitals in Spain, the United Kingdom and Türkiye to determine the tolerance of benzalkonium chloride (BC). They found MIC_{50}- MIC_{90} rates 16- 16mg/L in *Salmonella* spp. isolates, 16- 32 mg/L in *E. coli* isolates, 8- 16 mg/L in *Klebsiella pneumonia* isolates, 16- 32 mg/L in *Enterobacter* spp isolates, 4- 8 mg/L in *Enterococcus faecium* isolates and 2 - 4 mg/L in *Enterococcus faecalis* isolates. In another comprehensive study conducted by Wu et al. (2015) collected 53 *Salmonella* spp. isolates, 33 *E. coli* isolates, 22 *Klebsiella pneumonia* isolates from retail raw meats, they found *Salmonella* MIC_{50}- MIC_{90} 128 mg/L in BC, 256- 256mg/L in CPC, 32-32 mg/L in DDAC (Didecyldimethylammonium chloride); for *E. coli* MIC_{50}- MIC_{90} 128- 128 mg/L in BC, 128- 128 mg/L in CPC, 16-32 mg/L in DDAC; for *Klebisella pneumonia* MIC_{50}- MIC_{90} 128- 128 mg/L in BC, 256- 256 mg/L in CPC, 32-32 mg/L in DDAC. Chapuis et

al. (2016) found that ADBAC and DDAC levels were between 64 and 512 mg/L in 43 environmental and clinical ESBL-positive *E. cloacae* isolates with microbroth dilution method in France. Zhang et al. (2016) found The MIC's of BC, CPC and DDAC in the 255 *E. coli* strains, from retail meat samples, were 16- 1024 mg/L, 8-512 mg/L, 4-1024 mg/L respectively. In another study conducted by Deus et al. (2017) in Germany, in 174 *E. coli* isolates were found to be ESBL-positive and the MIC_{50} - MIC_{90} values against BC were found to be 8 and 16 mg/L, respectively, according to the microbroth dilution method.

However, in this study, all strains belonging to the *Enterobacteriaceae* family, regardless of species and genus, were isolated from neck skin and cecum samples collected from poultry slaughterhouses, and the resistance of some disinfectant active substances from culture samples in this isolates was investigated. Since it is assumed that resistance transfer is easier among bacteria belonging to the *Enterobacteriaceae* family, studying disinfectant resistance as a whole with this bacterial family has made this study of high practical importance regarding the spread of antibiotic and disinfectant resistance in bacteria. Since this study contains important data for public health, it is thought to be a guide for future studies to be used in the food industry. In this study, although ADBAC and BC MIC values varied more among strains, these values were found to be more stable for CPC and PPMS. This suggests that resistant strains have emerged as a result of more frequent use of disinfectants containing BC and PPMS active ingredients in the field. Based on this, in order to inhibit all strains, PPMS (1024 mg/L <) should be used at the highest concentration and BC (64 mg/L) should be used at higher concentrations than ADBAC and CPC (32 mg/L).

Some studies have investigated whether there is a correlation between disinfectant resistance and biofilm formation. In this context. Sun et al. (2019) observed the susceptibility of 510 *E. coli* isolates isolated from retail poultry meat to 5 disinfectants and whether they formed biofilms. 194 isolates showed biofilm formation. Then, it was examined whether there was a correlation between disinfectant resistance and biofilm formation capacity of these isolates. They reported that while the biofilm formation capacity was directly correlated with BC resistance, no correlation was found with other disinfectants. In another study, Cai et al. (2018) investigated the response of biofilms formed by *Enterobacter cloacae*, *Klebsiella oxytoca* and *Citrobacter freundii* isolates, members of the *Enterobacteriaceae* family, to chloride-based disinfectants. For this purpose, chlorite-based SH (sodium hypochlorite), CD (chlorine dioxide), StAEW (strongly acidic electrolyzed water) and NEW (neutral electrolyzed water) were used. Biofilms formed by *E. cloacae* were more resistant to disinfectants than biofilms of the other two strains and SH (200 mg/L) was the most effective in the reduction of cell number in the biofilms of all strains.

Since biofilm formation varies depending on the surface, nutrient, pH, temperature, and other environmental conditions, conducting such studies by taking field conditions into account will significantly increase the applicability of the results to the field. (Chmielewski & Frank, 2003).

With the 2019 COVID epidemic, awareness of microorganisms in society increased and the use of

disinfectants increased approximately 3 times (Akyüz & Aytekin, 2022). There are 213 disinfectant and biocidal products licensed by the General Directorate of Public Health (Halk Sağlığı Genel Müdürlüğü, 2023) in Türkiye and allowed for use in personal areas, and the data obtained is expected to guide the use of disinfectants in the food and health sectors.

5. Conclusion

It is obvious that disinfectants will continue to exist in our lives as an indispensable element in the control of infectious diseases in abiotic environments by preventing or destroying the development of many pathogens, including bacteria and viruses. Understanding disinfectant resistance mechanisms in ESBL-producing *Enterobacteriaceae* strains; Using appropriate disinfection methods, selecting an effective disinfectant and using it in appropriate doses are of great importance in infection control and improving public health.

HACCP systems used in food processing plants do not directly mention bacterial biofilms, which can limit the effectiveness of disinfectants. Therefore, the creation of an updated HACCP system that foresees the assessment of biofilms in food environments and establishes an appropriate sanitisation plan, thus providing a much clearer contamination information and studies should be carried out to facilitate food production in biofilm-free processing systems of the food industry.

Among the disinfectant active ingredients used in this study, the most effective was found to be ADBAC, followed by CPC, BC and PPMS, respectively.

Frequent and high doses of the same disinfectant can cause damage to abiotic environments, chemical contamination of the environment, and the development of resistance. In order to prevent the use of high doses of disinfectants and to use appropriate disinfectants in effective doses, sero survey studies should be carried out to allow the development of resistance to be investigated at certain periods.

Future research directions should focus on identifying new disinfectants that can effectively control disinfectant-resistant bacteria. Additionally, research should adopt alternative and green approaches such as bacteriophages or probiotics to control the spread of disinfectant-resistant bacteria. In addition, more comprehensive studies should be conducted to understand the genetic basis of disinfectant resistance in ESBL-producing *Enterobacteriaceae* strains and to obtain information about the evolution and spread of these resistance mechanisms.

Ethical Statement

There is no need to obtain ethics committee permission for this study due to his article does not contain any studies with human or animal subjects. However, the study was conducted in accordance with ethical principles.

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Presentation Information

The findings of this study have not been presented at any conference or journal.

Conflicts of Interest

The authors declare no conflicts of interest regarding this study. Any institution or organization providing funding for this research did not have any role in the design, data collection, analysis, interpretation, or publication to influence or distort the findings.

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Holmes Tremor in A Case of AIDS with Toxoplasma Abscess: Is A Common Result of Different Pathogenetic Mechanisms?

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Abstract

Holmes tremor is a low-frequency tremor clinic that predominantly affects proximal extremities during rest, posture, and action. It is generally associated with lesions located in the mesencephalon or thalamus. It is thought that the clinic is formed in similar phenomenology but as a result of different physiopathologic events. It is known that weeks, months, or even years pass from the underlying cause for the duration of symptoms. In this article, it is aimed to present a case of Holmes tremor, developing approximately nine months after HIV-related toxoplasmic abscess located in the mesencephalon and the thalamus and to discuss these rare associations with the literature.

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1. Introduction

Holmes tremor is a rare, unilateral, low-frequency tremor that dominates the proximal extremity and is observed during rest, posture and action. The amplitude of tremor may be low at rest, but it increases during posture and reaches its highest level during movement (Holmes, 1904). The clinical picture is be due to various etiologies such as ischemia, vascular malformation, tumor or demyelinating disease located in mesencephalon or thalamus. The clinic is mostly unilateral and prominent in upper extremity, and sometimes accompanied by ipsilateral dystonia (Deuschl et al., 1996; Deuschl et al., 1998). Clinical signs appear weeks, months or even years after the primary lesion. Cerebellothalamic and nigrostriatal system degeneration or structural and functional impairment due to interruption of pallidothalamic pathways is involved in the pathogenesis (Kim et al.,2009; Deuschl & Bergman, 2002). The primary goal of treatment is to eliminate the underlying cause. If additional treatment is required, levodopa, dopamine agonists, clonazepam, propranolol, amantadine, valproate or levetiracetam can be preferred (Raina et al., 2016, ; SchreudFerlazzo et al., 2008). Surgery is recommended in resistant cases who do not respond to medical treatment. It has been reported that stereotaxic thalamotomy and deep brain stimulation to the thalamic ventral intermediolateral (VIM) nucleus are beneficial in terms of dystonia in addition to tremor (Yuk et al., 2019). Holmes tremor cases with brain abscess due to HIV-related opportunistic toxoplasma infection are extremely rare in the literature (Lekoubou et al., 2010).

In this article, it is aimed to discuss the clinical features, etiologies and treatment methods in the light of the literature in a patient who was diagnosed with Holmes tremor nine months after the diagnosis of brainstem abscess with AIDS.

2. Case Report

41-year-old female patient was admitted to the Neurology department with weakness in the right half of the body and facial asymmetry nine months ago. In the first examination with magnetic resonans imaging (MRI), there were two abcesses; first one is a 12x15x18 mm lesion which appears to be central hypointense in T2-weighted and FLAIR sequences and whose periphery appears to be hyperintense in T1-weighted sequences at the left thalamus, internal capsule and also the mesencephalon tegmentum. The second one was smaller than 1 cm and located at adjacent to the anterior horn of the lateral ventricle on the right (Figure 1).

There were HIV and toxoplasma Ig G positivity in laboratory tests. She was diagnosed with opportunistic toxoplasma-induced brainstem abscess secondary to human immunodeficiency virus (HIV) infection. After the antiparasitic (Trimethoprim-sulfamethaxazole, azithromycin) and antiviral treatment (dolutegravir, tenofovir disoproxil/emtricitabine), she was discharged with a partial neurological recovery. After 3 months, he went to the ophthalmologist because of poor vision. She was diagnosed with cytomegalovirus retinitis and valganciclovir was added to the treatment. At the end of the fifth month, she presented with gradually increased involuntary movements in the right arm.

In neurological examination, there was dystonic posture in the right hand with low-frequency tremor observed during rest and posture in the right upper extremity, with additional sequelaes as right hemiparesis, central facial paralysis and 0.3 / 0.6 vision. Neuroimaging showed the same lesions with the difference of markedly reduced edema effect (Figure 2).

With the diagnosis of Holmes tremor, levetiresetam was started by dose titration in addition to the current antiviral treatment in order to avoid of aggravation of liver dysfunction. Significant improvement in tremor was observed in the third month of clinical follow-up. However, Clonazepam was added to the treatment by planning dose titration due to the continuation of dystonia and it had to be discontinued due to intolerance. Thereupon, she was directed to a center aiming to completely eliminate the functional loss caused by tremor and dystonia to apply deep brain stimulation in addition to other medical treatment options. During the period, she was diagnosed with inflammatory breast cancer with axillary lymph node and mandibular bone metastasis after a palpable mass

in the left breast and decided to give priority to oncological treatment. In the sixth month of the neurological follow-up, a significant regression was observed in the abscess lesion in the mesencephalon and thalamus in neuroimaging performed for oncological treatment (Figure 3). She has been following up in our neurology, infectious diseases and oncology outpatient clinics.

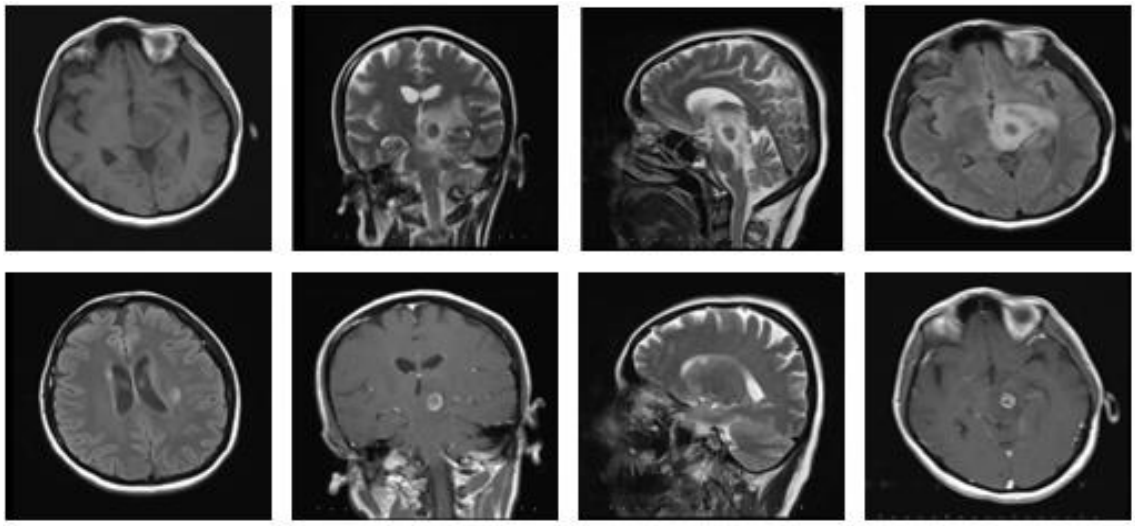

Figure 1. T1A iso-hypointense, central hypointense and peripherally hyperintense in T2A and FLAIR sequences, peripheral enhancing abscess lesion at left thalamus, capsule interna and mesencephalon causing mass effect with intense edema and a second abscess lesion at adjacent to the anterior horn of the lateral ventricle on the right.

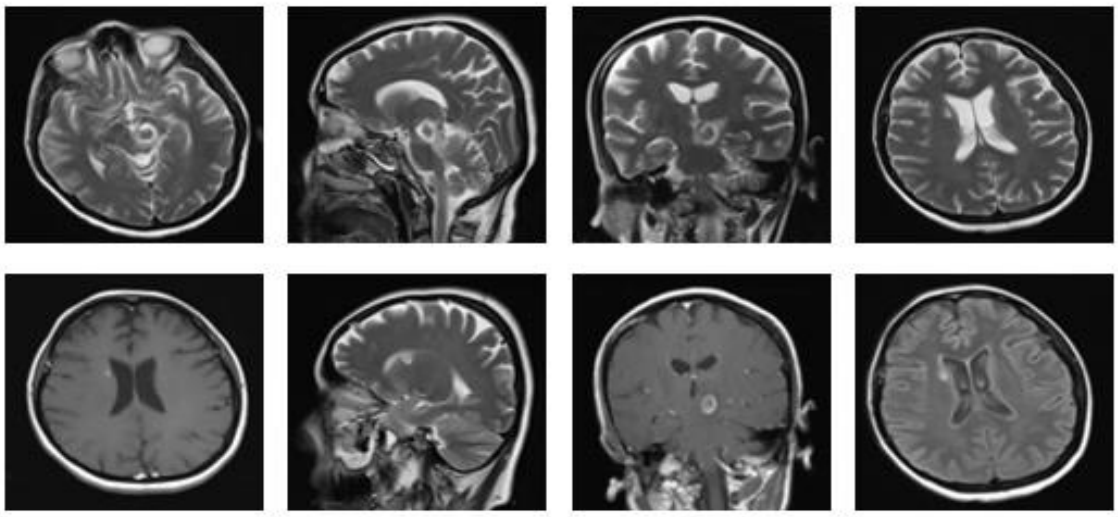

Figure 2. Abscess lesion preserving its size but with markedly reduced edema effect.

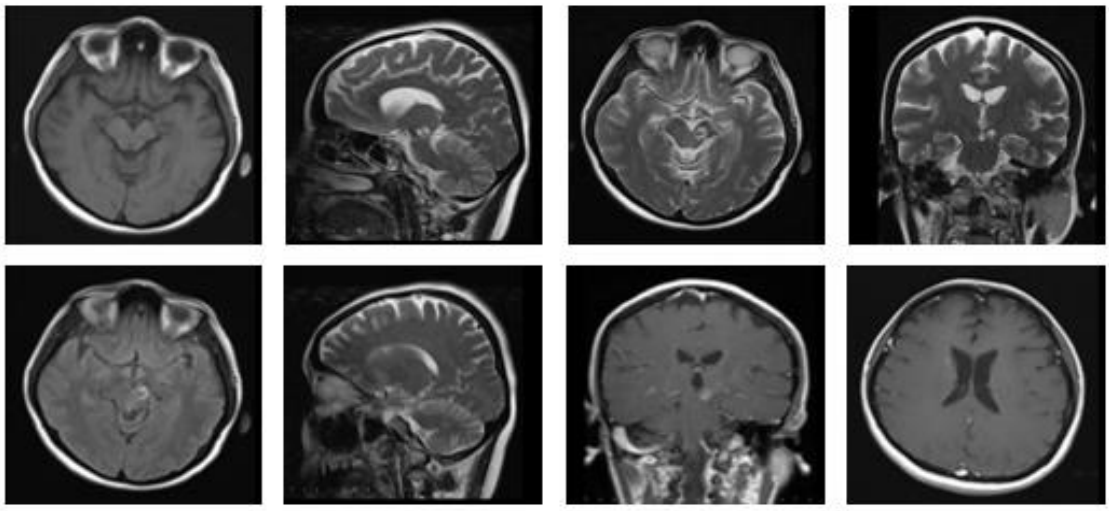

Figure 3. Reduction in size of both abscess lesions and marked reduction in peripheral edema and partial enhancement.

3. Discussion

Gordon Holmes first described this tremor in 1904 and named the cases by associating with the location of the lesion such as mesencephalon tremor, thalamic tremor or rubral tremor (Holmes, 1904). In 1997, it was decided to use the name 'Holmes tremor' in the diagnosis with the criteria defined at the Tremor Symposium held in Germany.

According to the criteria, Holmes tremor has a clinic that occurs at rest and during action, with a low frequency (usually below 4.5 Hz), after a latent period of four weeks to two years from the causative lesion (Deuschl et al., 1996; Deuschl et al., 1998).

Cerebellothalamic and nigrostriatal system dysfunction is held responsible for the physiopathology of the combination of resting and kinetic tremor (Kim et al.,2009). Mesencephalon tegmentum and thalamus are affected together as a rare form of involvement in presented case.

It is known that occurence of tremor needs time, at least four weeks, after the hemiparesis caused by the involvement of the corticospinal tract. As stated in the diagnostic criteria, the latent period between four weeks and two years suggests that tremor may be the result of a secondary degeneration process. The presence of cases with similarly located lesions but no tremor suggests that this secondary degeneration does not always develop or regenerates before clinical manifestation (Lekoubou et al., 2010; Ling et al., 2022).

Although various causes have been reported in the etiology, cerebrovascular disease, trauma, vascular malformation and multiple sclerosis play the most common role (Deuschl et al., 2002). Focal lesions caused by HIV-associated opportunistic toxoplasma infection are a rare cause of Holmes tremor. The first disease to be considered in the presence of central system involvement in HIV-positive cases is toxoplasma encephalitis. The most important risk factors include female gender, highly immunocompromised (CD4 + T lymphocyte count <100 / mm^3) and no primary prophylaxis. It is associated with a poor prognosis, with 40% having worsening AIDS status and 23% mortality after one year (San-Andrés et al., 2003). Typically, similar to presented case, it is characterized by multiple lesions with peripheral enhancement and edema effect.

Differential diagnoses include primary central nervous system lymphoma, cryptococcal meningitis, progressive multifocal leukoencephalopathy (PML), and cytomagalovirus (CMV) infection (Siddiqi & Koralnik, 2015). There are a very limited number of HIV-toxoplasma related Holmes tremor cases in the literature. Deep white matter localized lesions have been described in the left upper brain stem and frontal lobe for the first case (Koppel & Daras, 1990). Subsequently, five more HIV-associated toxoplasma abscess cases with similar localization, mostly unilaterally involved, were reported. These six cases were compiled and discussed from various sides; one of the striking features of this article is the emphasis that the main source of Holmes tremor is HIV-related primary neuronal damage, rather than toxoplasma-induced lesion (Lekoubou et al., 2010). The hypothesis put forward by DaTSCAN SPECT studies in the literature is supported by the presence of a single HIV-toxoplasma-related Holmes tremor case in which presynaptic dopaminergic nigrostriatal system involvement was shown, and there was no significant involvement difference in other cases (Yuk et al., 2019).

The first goal of Holmes tremor treatment is to eliminate the underlying cause. However, there may be cases that do not improve despite the appropriate treatment of the etiological factor. It is possible to encounter cases where surgical treatment is not possible due to the location and characteristics of cavernoma or mass lesions, which are among the common causes. In the light of the pathophysiology mentioned above, different agents such as levodopa, dopamine agonists, amantadine have been tried and responded in some cases.

However, the efficacy of carbamazepine, valproic acid, clonazepam and levetiracetam has been demonstrated and put into use in cases where no response was obtained (Raina et al., 2016,; SchreudFerlazzo et al., 2008).

Among the medical treatment options, levetiracetam has an important place due to its good tolerability characteristics such as low side effect profile, no difficulty in titration and renal elimination (SchreudFerlazzo et al., 2008).

Considering the continuity of multiple drug use and the possibility of hepatotoxicity in our case, levetiracetam, an agent that undergoes renal elimination, was preferred. The improvement observed in the follow-up of the patient from the first month onwards reached a level where she was able to fulfill her activities of daily living and self-care at the end of the third month. However, complete recovery was never achieved despite dose titration. In cases where there is still no response despite symptomatic medical treatment, invasive treatment options such as deep brain stimulation can be applied in addition to medical treatment (Garos et al., 2010). Thereupon, any other options could be used because of hepatic disfunctions releated to HIV infection therapies and deep brain stimulation had been recommended to the patient but this option had to be postponed due to the intervening breast cancer treatment process and the effect of deep brain stimulation could not be observed.

4. Conclusion

Holmes tremor; It still maintains its place as a very interestig subject with its etiological diversity of the same phenomenology and the areas waiting to be clarified in its physiopathology. Our case; It has been found worth sharing with its extremely rare etiology, rich imaging findings in emergency department, and aspects contributing to the primary neuronal injury hypothesis proposed for the Holmes tremor pathophysiology.

Ethical Statement

Since this is a case report, ethical approval was not obtained. However, signed informed consent was obtained from the patient.

Financial Support for the Study

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Presentation Information

This study was presented as a poster presentation at 59th National Neurology Congress in 2023.

Conflicts of Interest

The author declare no conflicts of interest regarding this study. Any institution or organization providing funding for this research did not have any role in the design, data collection, analysis, interpretation, or publication to influence or distort the findings.

Author Contributions

The entirety of the all process of the article belongs to the author.

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Review

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Most Known *Citrus* L. Species and Breast Cancer Relationship

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flavonoid
rutaceae

Abstract

The genus *Citrus* L. belongs to the Rutaceae family of flowering plants and shrubs. *Citrus* genus has valuable edible fruits such as oranges, lemons, grapefruits, pomelos, and limes. Australia, Melanesia, Southeast Asia, East Asia, and South Asia are the native habitats of the genus *Citrus.* Indigenous societies in these regions have long utilized and tamed various *Citrus* species. *Citrus* fruits and their secondary metabolites have been reported to be useful agents in numerous studies to possess anti-inflammatory, antioxidant, and anticancer properties. This review gives an overview of *Citrus* species and background information on the potential anticancer properties of the compounds identified, along with the related *in vitro* and *in vivo* research. Studies from the past have revealed a variety of biological functions that *Citrus* compounds can regulate, such as angiogenesis, apoptosis, metastasis, cell cycle regulation, and cell proliferation. These promising data call for more investigation into the chemopreventative activity of *Citrus* and phytoconstituents.

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1. Introduction

Citrus L. fruits offer a treasure trove of bioactive compounds beyond their refreshing taste, with potential for disease prevention and treatment. Studies consistently link high fruit and vegetable intake, including *Citrus* varieties like mandarin, pomelo, orange, and grapefruit, to reduced cancer risk by at least 20%, particularly for breast cancer (Cirmi et al., 2016; Wang et al., 2021). This aligns with the observed protective effect of the Mediterranean diet, which is rich in citrus, likely due to its abundance of fiber, antioxidants, and polyphenols (Koolaji et al., 2020).

Intriguingly, even citrus peels, traditionally used medicinally, contain bioactive compounds with potential for cancer treatment, particularly breast cancer. These peels are rich in flavonoids and other polyphenols, supported by preclinical and epidemiological evidence (Gómez-Mejía et al., 2019). This review delves into the diverse secondary metabolites of *Citrus* species, focusing on their potential connection to breast cancer prevention and treatment.

Beyond their delightful presence, citrus fruits boast a captivating history, originating around 2200 BC in Southeast Asia (Cebadera-Miranda et al., 2020). Traversing continents via trade and exploration, they held religious significance in India and graced the Mediterranean during Alexander the Great's era (Rouseff et al., 2009). However, this rich past presents a tangled taxonomic web. Prolific crossbreeding within and beyond *Citrus*, coupled with ancient cultivation and ambiguous boundaries, obfuscates classification (Agouillal et al., 2017).

The estimated range of 16 to 156 suggested species emphasizes the continuous difficulty with taxonomy (Ollitrault et al., 2020).

Amidst this labyrinth, four "basic taxa" – pomelo, citron, mandarin, and kaffir lime – stand as foundational pillars. Unravelling their role in hybridization and recombination is key to comprehending *Citrus* evolution and securing the future of these treasured fruits (de Araújo et al., 2003).

2. Chemical Composition of *Citrus* species

2.1. Coumarins

Beyond alluring scents, citrus coumarins (Figure 1) unveil an arsenal of potent anti-breast cancer activities. Their versatile structure and diverse substituents enable potent pharmacological effects and the development of active derivatives with enhanced efficacy (Wu et al., 2009)

Citrus coumarins (auraptene, imperatorin, phellopterin, scoparone, myrsellin, triphasiol, umbelliferone, citropten) offer a compelling multi-pronged attack (Kerekes et al., 2022; Prince et al., 2009).

Growth pathway disruption:

They target the vital PI3K/Akt/mTOR pathway, a regulator of cell growth and survival, ultimately inducing cancer cell death (Musa et al., 2008).

Hormone signaling modulation:

By inhibiting key enzymes like sulfatase and aromatase, coumarins decrease estrogen levels, depriving breast cancer cells of a crucial fuel source (Wu et al., 2020).

Resistance mechanism bypass:

Certain coumarin derivatives can bypass multidrug resistance, rendering them effective against established cancer therapies (Musa et al., 2008).

This multifaceted assault positions citrus coumarins as promising candidates for novel breast cancer treatment strategies.

2.2. Flavonoids

Beyond vibrant colors, citrus fruits unveil a treasure trove of flavonoids, and diverse chemical structures (Figure 2) with potent bioactivities (Chen et al., 2023). Their arsenal extends beyond aesthetics, wielding potent antioxidant, anti-inflammatory, and anti-mutagenic properties against chronic diseases like cancer (Panche et al., 2016).

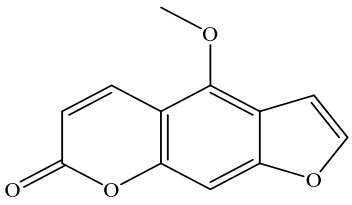
In the fight against cancer, citrus flavonoids (nobiletin, tangeretin, hesperetin, naringenin, eriodictyol, diosmin) become versatile warriors, employing a multifaceted attack (Qiu et al., 2023):

Suppressing cell proliferation:

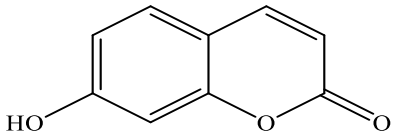
They hinder uncontrolled cell division, slowing tumor growth.

Inducing apoptosis:

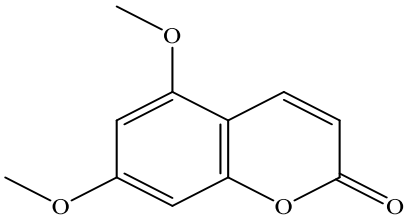
They trigger programmed cell death within cancer cells, leading to their elimination.



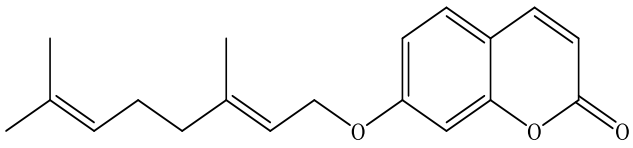
Bergapten



Umbelliferone



Citropten



Auraptene

Figure 1. Chemical structures of some citrus coumarins

Hinder metastasis:

They prevent cancer spread by interfering with cell movement and adhesion.

Notably, their ability to trigger apoptosis through both intrinsic and extrinsic pathways underscores their significant therapeutic potential (Park et al., 2022).

2.3. Terpenes

Beyond captivating fragrances, citrus terpenoids (mainly limonene) unleash a potent anticancer arsenal against this formidable foe (Kłos & Chlubek, 2022). Their diverse and flexible structures (Figure 3) enable a multi-pronged attack on tumor development, encompassing:

Thwarting cell proliferation and angiogenesis:

They curb uncontrolled cell growth and restrict blood vessel formation, which is vital for tumor nourishment (Luo et al., 2019).

Disrupting metastasis:

By interfering with critical cell signaling pathways, these terpenes hinder cancer cell migration and dissemination (Luo et al., 2019).

Targeting key signaling pathways:

They disrupt crucial cascades like NF-kB and JAK-STAT, impacting cell survival and proliferation (Olson, 1999; Saini et al., 2020).

Inhibiting critical enzymes:

By targeting enzymes like DNA topoisomerases and proteinases, they disrupt DNA replication and protein function, hindering tumor growth (Ghantous et al., 2010).

While programmed cell death (apoptosis) is the primary outcome, evidence suggests citrus terpenoids may trigger additional pathways like autophagy, further amplifying their antitumor potential (Kuttan et al., 2011).

2.3.1. Carotenoids

Beyond captivating hues, citrus fruits harbor a wealth of bioactive carotenoids categorized as vitamin A precursors and non-precursors (Young & Lowe, 2018). Key dietary members like α-carotene, β-carotene, and lycopene play significant roles in human health (Alquézar et al., 2008).

Carotenoids excel as antioxidants, shielding cells from oxidative damage (Rowles III & Erdman Jr, 2020). They further influence diverse biological functions, potentially mitigating chronic diseases, bolstering immunity, and exhibiting anti-inflammatory and anti-obesity properties (Rao & Rao, 2007). They demonstrate anticancer potential through mechanisms like free radical scavenging, cell proliferation modulation, and enhanced immune response (Olson, 1999; Saini et al., 2020) . Understanding the specific carotenoid profiles within different citrus varieties is crucial for maximizing their health benefits, as depicted in Figure 4 (Agócs et al., 2007).

The chemical composition of *Citrus* species and the major components of their essential oils were presented in Tables 1 and 2, respectively.

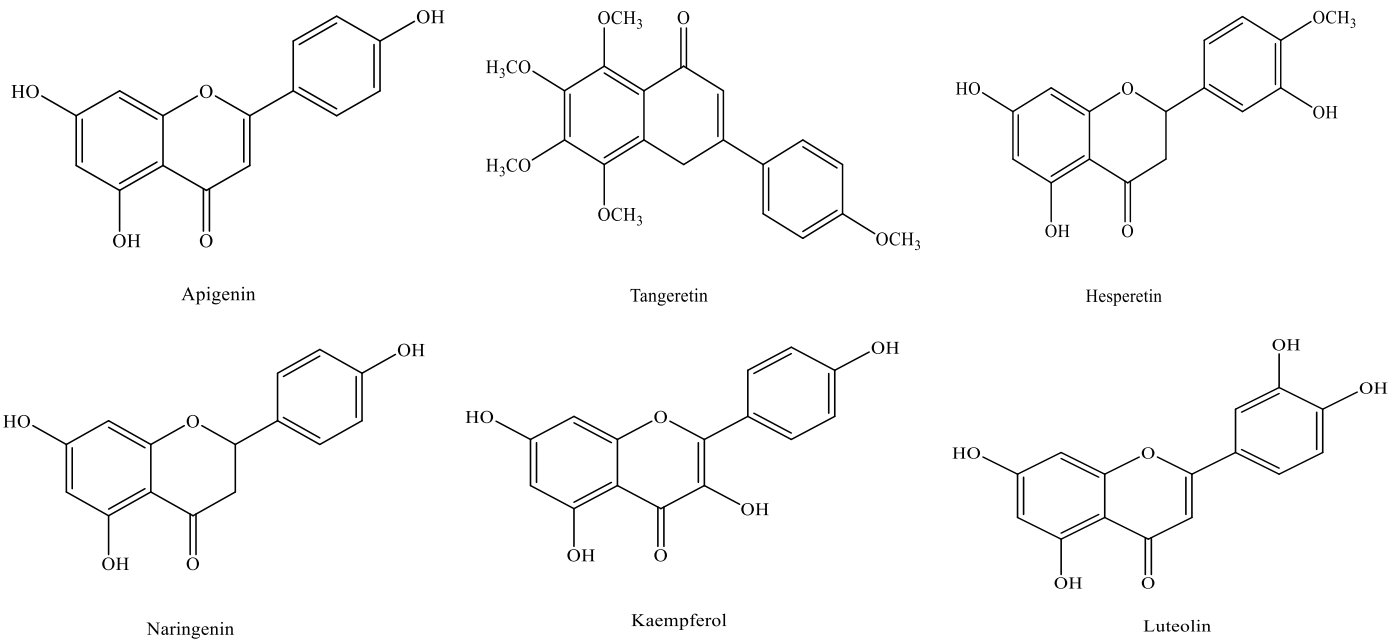


Figure 2. Chemical structures of some citrus flavonoids

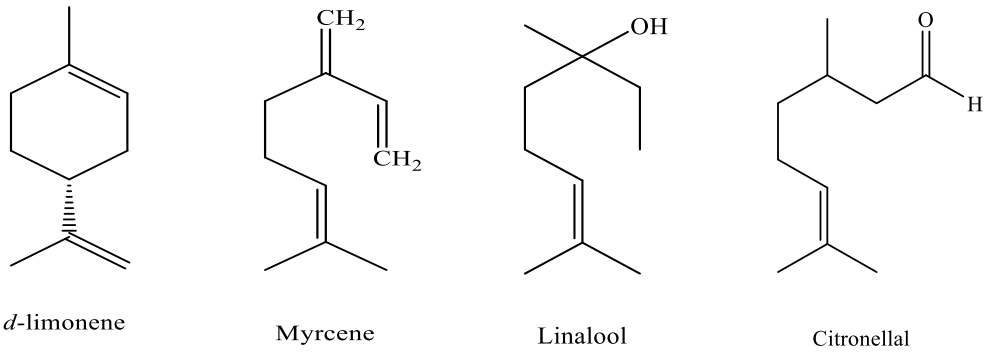


Figure 3. Chemical structures of some citrus terpenoids

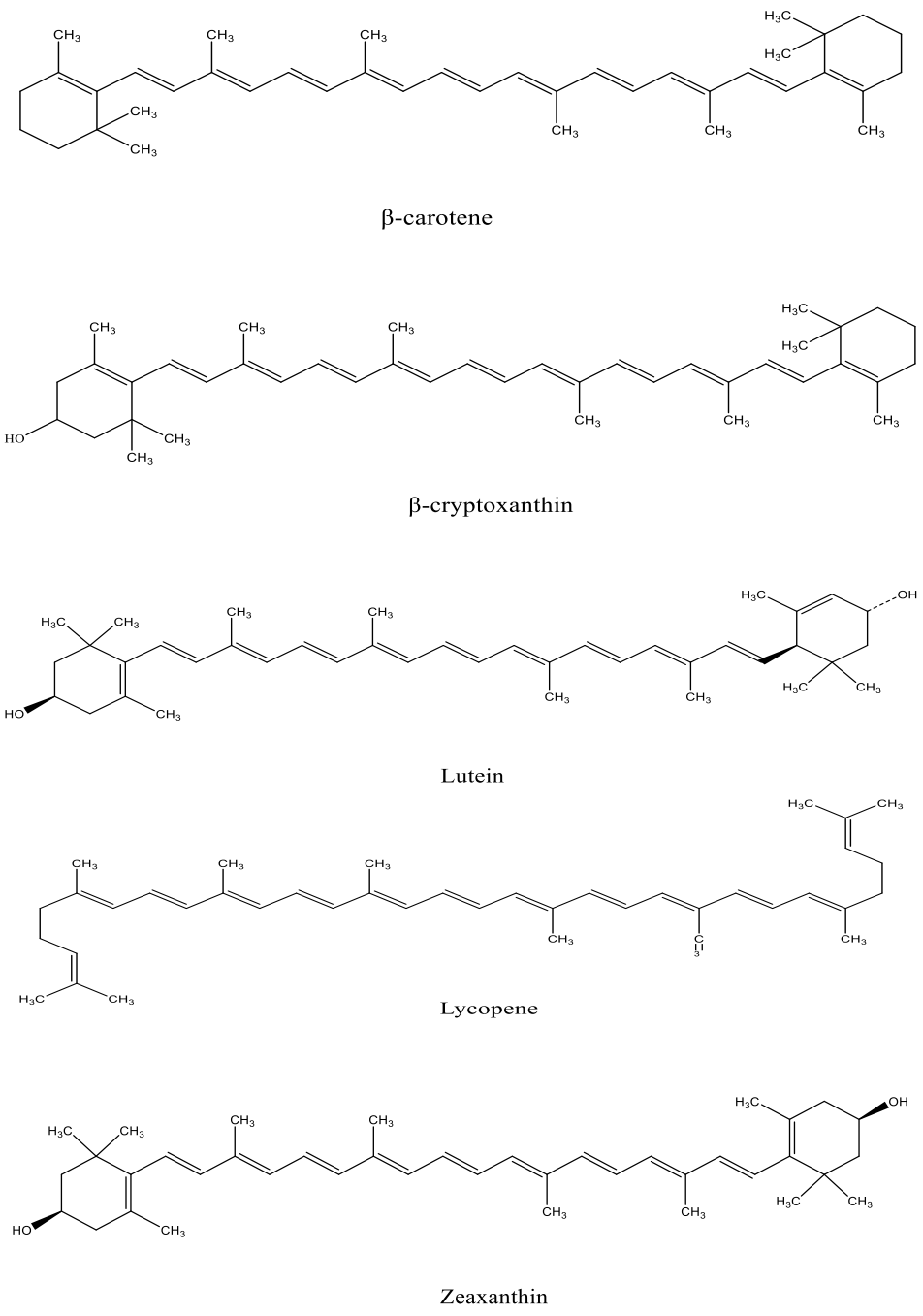


Figure 4. Chemical structures of some citrus carotenoids

Table 1. Chemical composition of *Citrus* sp. (major components)

Latin name	English name	Phytochemical Screening					References
		Carotenoids	Coumarins	Flavonoids	Terpenes	Others	
***Citrus* × *aurantium* L.**	Bitter orange		Osthol **Furanocoumarin**: Bergapten, epoxybergamottin, 6' 7'-dihydroxybergamottin	**Flavanones**: Hesperetin, naringenin **Flavones:** Apigenin, diosmetin, luteolin, nobiletin and tangeretin **Flavonol**: Kaempferol and quercetin	**Limonoids**: Deacetylnomilin, ichangin, limonin, nomilin, obacunone **Monoterpenes:** *β*-Myrcene, limonene, linalool	**Alkaloids**: p-Synephrine,	(Dugrand-Judek et al., 2015)
***Citrus aurantiifolia* (Christm.) Swingle**	Lime		5-Geranyloxy-7-methoxycoumarin, citropten (5,7-dimethoxycoumarin), isopimpinellin, herniarin, umbelliferone, xanthotoxol **Furanocoumarin**: Bergamottin, bergapten		**Monoterpenes:** α-Terpineol, α-thujene, *p*-cymene, camphene, carvone, citral, fenchene, linalool, terpinen-4-ol, geranial, geraniol, limonene, sabinene **Sesquiterpenes:** Bergamotene, caryophyllene, spathulenol *trans*-α-bisabolene	1-methoxy-cyclohexene, 3,7-dimethyl-(Z)-2,6-octadienal, 3-methyl-1,2-cyclopentanedione, methyl cyclopentenolone, palmitic acid, 2-methyltetrahydropyran, 3-methyl-3-penten-2-one, 3-hexen-2-one, 4-hexen-3-one, crysantenile, pinacol resorcinol	(Sandoval-Montemayor et al., 2012)
***Citrus limon* (L.) Osbeck**	Lemon		Citropten, scopoletin **Furanocoumarin**: Bergamottin, bergapten	**Flavones**: Apigenin, diosmetin, diosmin, homoorientin, luteolin, orientin, vitexin (whole fruit)	**Limonoids**: Limonin, nomilin (whole fruit)	**Carboxylic acids:** Citric acid, galacturonic acid, glucuronic acid, glutaric acid, homocitric acid, 3-hydroxymethylglutaric acid, isocitric acid, malic acid, quinic acid **Phenolic acids:** Dihydroferulic acid, p-hydroxybenzoic acid, 3-(2-hydroxy-4-methoxyphenyl) propanoic acid, synapic acid (whole fruit)	(Klimek-Szczykutowicz et al., 2020)

Table 1. Chemical composition of *Citrus* sp. (major components) (continued)

***Citrus maxima* (Burm.) Merr. (syn. *Citrus grandis* (L.) Osbeck)**	Pomelo	Carotene, roseoside	**5-Geranyloxy-7-methoxycoumarin, auraptene, bergamottin (peel), 5-methoxy seselin, 5-methyltodannol, 6-hydroxy methylherniarin (roots, stem bark)**	**Flavones: Acacetin, cosmosiin, diosmetin, diosmin, hesperidin, eriocitrin, naringin, rutin, tangeretin**	**Monoterpenes**:α-Pinene, α-terpineol, anethole, β-pinene, camphene, camphor, citral, citronellal, citroonellol, farnesol, geraniol, myrcene, neral, terpinene **Sesquiterpenes:** α-Bisabolol, α-cadinene, α-copaene, elemol	**Steroids:** β-Sitosterol, campesterol, daucosterol, stigmasterol	(Vijayalakshmi & Radha, 2016)
***Citrus medica* L.**	Citron	*β*-Cryptoxanthin, *β*-carotene, *cis*-violaxanthin, lycopene, lutein, xanthin, violaxanthin, zeaxanthin	Citropten, scoparone, scopoletin, umbelliferone (peel)	**Flavanones**: Hesperidin, 3,4',5-Trihydroxy-3',6,7- trimethoxyflavone neoeriocitrin, neohesperidin, naringin **Flavones:** Apigenin, chrysoeriol, diosmetin	**Monoterpenes**: β-Pinene, γ-terpinene, α-terpinolene, citral (geranial and neral), citronellol, limonene, linalool, geraniol, geranyl acetate, neryl acetate, mycrene, trans-α-bergamotene **Sesquiterpenes:** Valencene	**Steroids:** β-sitosterol	(Favela-Hernández et al., 2016)
***Citrus paradisi* Macfad**	Grapefruit		5-Geranyloxy-7-methoxycoumarin, 6'7'-dihydroxybergamottin (DHB), auraptene, bergamottin, epoxyauraptene, epoxybergamottin, meranzin	**Flavanones:** Isosakuranetin, hesperidin, neohesperidin, naringin, naringenin **Flavones:** 3,3',4',5,6,7,8-Heptamethoxyflavone **Flavonols:** Quercetin	**Limonoids**: limonin, **Monoterpenes**: α-pinene, limonene, myrcene, octanal, sabinene **Sesquiterpenes:** α-copaene, α-farnesene, trans-caryophyllene		(El Kamali et al., 2015; Uckoo et al., 2012; Wangensteen et al., 2003)
***Citrus reticulata* Blanco**	Tangerine		5-Geranyloxy-7-methoxycoumarin, 7-Geranyloxy-6-methoxycoumarin, xanthotoxol, xanthotoxin	**Flavanones**: Didymin, eriodictyol, hesperidin, naringin, naringenin, neohesperidin, poncirin **Flavones:** 5-Demethylnobiletin, diosmetin, luteolin, nobiletin, rhoifolin, sinensetin, tangeretin, quercitrin **Flavonols:** Kaempferol, quercetin	**Diterpenes:** Dehydroabietic acid **Monoterpenes**: α-Pinene, limonene, citronellyl acetate, citronellyl laurate, citronellyl valerate **Tetracylic terpenes:** Cholestane **Triterpenes**: 4,4-Dimethyl-14alpha-formyl-5alpha-cholesta-8,24-dien-3beta-ol, cholest-16-en-3-ol	β-stigmasterol, γ-sitosterol, 2-Methoxy-4-vinylphenol, 3,7,11,15-tetramethyl-2-hexadecene, caffeic acid, citronellyl valerate, ferulic acid, ethyl palmitate, methyl palmitate, linoleic acid, oleic acid, tetracosanoic acid, quinic acid	(Ferreira et al., 2018; Kaushal et al., 2022; Saleem et al., 2005; Zhang et al., 2014)

Table 2. Chemical composition of *Citrus* sp. essential oil (major components)

Latin name	English name	Phytochemical Screening		References
		Leaves	**Peels**	
***Citrus* × *aurantium* L.**	Bitter orange	Eucalyptol, sabinene, β-linalool, α-terpineol, α-pinene, β-myrcene, 4-terpineol, β-pinene, D-limonene, O-cymene, 4-carvomenthenol, linalool, linalyl acetate, 6,9,12,15-docosatetraenoic acid methyl ester, tetraneurin-α-diol	Limonene, linalool, linalyl acetate, myrcene, geranial, β-myrcene, neral, β-pinene, γ-terpinene, sabinene, geranyl acetate, β-caryophyllene, α-terpineol, α-pinene	(Maksoud et al., 2021; Okla et al., 2019)
***Citrus aurantiifolia* (Christm.) Swingle**	Lime	(Z)-Hex-3-en-1-ol, myrcene, limonene, trans-β-ocimene, linalool, citronellal, citronellol, neral, geraniol, geranial, citronellyl acetate, trans-β-caryophyllene	α-Thujene, α-pinene, sabinene, β-pinene, β-myrcene, α-terpinene, o-cymene, limonene, β-ocimene, γ-terpinene, α-terpinolene, α-terpineol, linalool, nerol, geraniol, α-bisabolol, α-citral, β-citral, neryl acetate, trans-α-bergamotene, β-bisabolene	(Lemes et al., 2018; Lin et al., 2019)
***Citrus limon* (L.) Osbeck**	Lemon	Myrcene, limonene, 3-carene, β-ocimene, gamma-terpinene, linalool, 6-octenal,7-methyl-3-methylene, citronellal, isoneral, terpinen-4-ol, isogeranial, α-terpineol, nerol, citronellol, neral, geraniol, geranial, citronellyl-propanoate, neryl acetate, geranyl acetate, z-caryophyllene	Limonene, neral, linalool, nonanal, *trans*-verbenol, decanal, geraniol, *cis*-α-bergamotene, ethyl cinnamate, ethyl p-methoxycinnamate, monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpenes, ketone	(Paw et al., 2020; Petretto et al., 2023)
***Citrus maxima* (Burm.) Merr.-*Citrus grandis* (L.) Osbeck**	Pomelo	α-Pinene, trans-isolimonene, delta-carene, trans-ocimene, nerol, citronellol, β-caryophyllene, calarene, α-humulene, patchoulene, allo-aromadendrene, germacrene D, β-ionone α-selinene α-farnesene, delta-cadinene, nerolidol, lauric acid, caryophyllene oxide, spathulenol, heptadecane, myristic acid, loliolide neophytadiene, palmitic acid, margaric acid, phytol, ethyl linoleolate, stearic acid, 9,12,15-octadecatrienoic acid, methyl ester	3-carene, cyclohexene, 4-methylene-1-(1-methylethyl), α-pinene, D-limonene, trans-linalool oxide (furanoid), cis-linaloloxide, 1,6-octadien-3-ol, 3,7-dimethyl, α-terpineol, 2,6-octadienal, 3,7-dimethyl-, (Z), citral, 2-carene, geranyl acetate, caryophyllen, α-cubebene, α-guaiene, stigmasterol, desmosterol, (3α,22E) 3-methoxy-stigmasta-5,22-diene, campesterol, α-sitosterol, β-sitosterol, 24-propylidene-, (3á) cholest-5-en-3-ol, allopregnane-3á,7à, 11à-triol-20-one, 9,19-Cyclolanost-24-en-3-ol	(Susandarini et al., 2016; Visakh et al., 2022)
***Citrus medica* L.**	Citron	Limonene,7-oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-, 6-octenal, 3,7-dimethyl- cyclohexanone, 2-methyl-5-(1-methylethenyl)-, 1-monolinoleoylglycerol trimethylsilyl ether, 6-octen-1-ol, 3,7-dimethyl-, n-pentyl(1-propenyl)dimethylsilane, citral, 2-octen-1-ol, 3,7-dimethyl-, isobutyrate, (Z)-, 2-oxocycloheptyl acetate, 2,4-dodecadienoic acid, 11-methoxy-3,7,11-trimethyl-, methyl ester, (E,E)-, methoprene, geranyl methyl ether, 13-heptadecyn-1-ol, 1,2-cyclohexanediol, 1-methyl-4-(1-methylethenyl)-, 2,6-octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-, mehp, 3,7-nonadien-2-ol, 4,8-dimethyl-, erucylamide	1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene, α-pinene, 3-octyn-2-ol, β-myrcene, 2-acetyl-5-methylfuran, cyclooctyl alcohol, limonene, isolimonene, 1,3,6-octatriene, 3,7-dimethyl-, (Z)-, 1-heptanol, 3-methyl-, linalool, nonanal, trans-p-mentha-2,8-dienol, 7-oxabicyclo[4.1.0]heptane, 1-methyl-4-(1-methylethenyl)-, citral, 6-octenal, 3,7-dimethyl-, cis-verbenol, carane, 4,5-epoxy-, trans, 1,2-cyclohexanediol, 1-methyl-4-(1-methylethyl)-, 4-terpineol, terpinyl acetate, β-terpinyl acetate, decanal, neryl acetate, neryl alcohol, undecanal, neryl acetate, dodecanal, (Z,E)-α-farnesene, caryophyllene, α-bergamotene, 1,6,10-dodecatriene, 7,11-dimethyl-3-methylene-, (Z)-, tetrakis(trimethylsiloxy)silane, α-caryophyllene, germacrene D, cis-.α-bisabolene, γ-elemene, β-bisabolene, δ-cadinene, tetradecanal, neoisolongifolane, hydroxy-, β-bisabolol, n-hexadecanoic acid	(Bhuiyan et al., 2009)

Table 2. Chemical composition of *Citrus* sp. essential oil (major components) (continued)

***Citrus sinensis* (L.) Osbeck**	Sweet orange	Sabinene, delta-3-carene, (E)-beta-ocimene, linalool, terpinen-4-ol	α-Pinene, β-pinene, myrcene, terpinolene, D-limonene, aldehyde C8, citronellol, linalool	(Ghadiri et al., 2020; Kasali et al., 2011)
***Citrus paradisi* Macfad.**	Grapefruit	Sabinene, β-pinene, myrcene,limonene, (E)-β-ocimene, γ-terpinene, terpinolene, linalool, terpinen-4-ol, β-sinensal, β-elemene	α-Pinene, β-pinene, myrcene, limonene, n-decanal, (+)-(S)-carvone, 1-cyclohexene-1-carboxaldehyde, alpha-farnesene, α-copaene, trans-caryophyllene, palmitic acid	(El Kamali et al., 2015; Paoli et al., 2016)
***Citrus reticulata* Blanco**	Tangerine	α- Fenchene, sabinene, β-myrcene, iso- sylvestrene, limonene, (E)-β-ocimene, α-cis- bergamotene, γ-terpinene, γ- elemene, linalool, α- selinene δ- elemene, germacrene b, terpinen-4-ol, thymol, methyl ether, β-sinensal, α-sinensal	α-Fenchene, β-pinene, sabinene, limonene, γ-terpinene, linalool, terpinen-4-ol	(Hamdan et al., 2016)

3. Breast Cancer

The 21st century faces a rising flood of chronic diseases, led by cancer, with breast cancer taking center stage despite advances in healthcare (Chhikara & Parang, 2023; Miller et al., 2022). Its complexity lies in its multi-stage progression, inherent heterogeneity, and metastatic potential (Benson et al., 2009; Waks & Winer, 2019). Yet, a glimmer of hope exists in decreasing mortality rates due to improved management strategies (Giaquinto et al., 2022). Recognizing its dual nature, requiring both local and systemic approaches, has sparked renewed interest in natural resources like citrus fruits for therapeutic intervention (Senkus et al., 2015).

Natural products offer compelling potential, with over half of FDA-approved anticancer drugs since 1960 being natural derivatives (Mayer & Gustafson, 2004). *Citrus* species, rich in diverse phytochemicals like coumarins, flavonoids, and limonoids, emerge as promising candidates. These bioactive compounds not only exhibit anticarcinogenic effects but also boast better tolerability compared to synthetic drugs, further propelling research efforts (Silalahi, 2002).

4. Recent Breast Cancer Studies on *Citrus* sp.

Once considered waste, citrus peels are rewriting their narrative as a readily available resource with remarkable potential in breast cancer prevention and treatment. Their diverse bioactivities offer a multifaceted attack:

Dose-Dependent cytotoxicity:

Extracts from various citrus peels exhibit a potent, dose-dependent ability to suppress the proliferation of breast cancer cell lines (MCF-7, MDA-MB-231) (El-Kersh et al., 2021; Narayanankutty et al., 2022).

Apoptosis induction:

Beyond mere growth inhibition, citrus peels actively trigger programmed cell death (apoptosis) in cancer cells. Compounds like naringenin, naringin, quercetin, and *C. limon* extract effectively induce apoptosis through mechanisms like BAX-Caspase 3 activation (Alshatwi et al., 2011; El-Kersh et al., 2021).

Antioxidant shield:

Rich in flavonoids and limonoids, citrus peels act as potent antioxidant shields, scavenging free radicals, limiting oxidative damage, and potentially preventing cancer initiation (Oyebadejo et al., 2019; Saranya et al., 2017).

Angiogenesis suppression and tumor reduction:

Citrus peels show promising effects beyond cellular targets. A lemon and ginger infusion significantly suppressed angiogenesis and induced apoptosis *in vivo*, while also achieving a 50% tumor-free rate and 32.8% tumor reduction in mice (Al-Ataby & Talib, 2022).

Targeted arsenal for personalized therapies:

Beyond broad benefits, specific peel components, like acridone alkaloids, exhibit targeted cytotoxicity against specific breast cancer cell lines (Segun et al., 2018). Citrus peel extract nanoparticles also show

enhanced cytotoxicity, opening doors for personalized drug delivery strategies (Amalina & Wahyuni, 2021).

5. Conclusion

Once discarded as waste, citrus peels are rewriting their narrative as promising allies against breast cancer. Their diverse bioactivities and encouraging *in vivo* results position them as valuable resources for novel cancer prevention and treatment strategies.

However, unlocking their full potential requires further research. A deeper understanding of their anticancer mechanisms is crucial for optimizing therapeutic application. Rigorous clinical trials are essential to bridge the gap between preclinical findings and safe, effective human therapies. Furthermore, exploring synergies between citrus peels and established treatments could lead to more potent and multifaceted regimens.

In conclusion, while citrus peels hold immense promise, continued research and rigorous clinical trials are key to transforming these readily available natural weapons into viable cancer-fighting tools, bringing us closer to a future where they not only nourish but also protect.

Ethical Statement

There is no need to obtain ethics committee permission for this study due to the use of open access sources. However, the study was conducted in accordance with ethical principles.

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Presentation Information

The findings of this study have not been presented at any conference or journal.

Conflicts of Interest

The authors declare no conflicts of interest regarding this study. Any institution or organization providing funding for this research did not have any role in the design, data collection, analysis, interpretation, or publication to influence or distort the findings.

Author Contributions

All authers contributed to literature search, data collecting and editing the report.

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