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Natural Preservatives as Medicinal Aromatic Plants: Implications for Sustainable and Functional Bread

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ABSTRACT

In this study, the plants *Melissa officinalis* (*Melissa*), *Elaeagnus angustifolia* (*Elaeagnus*), *Styrax officinalis* (*Styrax*) and *Echinops ritro* (*Echinops*) were firstly used to prepare enriched bread and to study their effects on the shelf life of bread. Water and alcohol extracts of the plants were also prepared to determine their antibacterial and antifungal activities *in-vitro*. The focus is on their potential applications as natural preservatives in sustainable functional bread production. The antimicrobial activity was evaluated using the agar well diffusion assay. Results showed that alcohol extracts of medicinal and aromatic plants exhibited significantly higher antimicrobial activity than water extracts, with inhibition zones diameters ranging from 15-22 mm for alcohol extracts compared to 8-13 mm for water extracts. Gram-negative bacteria, such as *Salmonella* Paratyphi A, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, showed resistance, with inhibition zone diameters below 10 mm. However, alcohol extracts from *Styrax* and *Elaeagnus* achieved inhibition zone diameters of 12-15 mm against these pathogens. Gram-positive bacteria, including *Staphylococcus aureus*, showed promising results, with alcohol extracts zones averaging 20 mm and water extracts inhibition of zone diameters averaging 14 mm. Fungal inhibition zone diameters was effective, with extracts reducing *Aspergillus niger* growth by 85%. A shelf life experiment revealed that bread enriched with *Elaeagnus* and *Melissa* extracts remained mold-free for 7 days, while control samples developed mold within 3-4 days. Sensory analysis indicated that 80% of participants preferred the taste and aroma of *Elaeagnus* seed bread, with an average score of 4.5-5. The incorporation of medicinal and aromatics plants not only enhances bread flavour but also provides health benefits besides sell life of bread. These plants serve as valuable natural preservatives, improving nutritional value, extending shelf life, and inhibiting harmful microorganisms in sustainable bread production.

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Introduction

The history of medicinal and aromatic plants (MAP) is as old as human history. Sumerians and Assyrians used these plants for therapeutic purposes in 5000-3000 BC. Following this, it is stated that Greeks, Egyptians, Hittites used a total of 250 different medicinal plants (Giesecke, 2023). According to The World Health Organization (WHO), there are around 20.000 medicinal plants in the world (Yücel et al., 2019). In Turkiye 3.035 of the 11.707 taxa growing naturally are in the endemic plant group (Demiray, 2021; MNPS, 2021). Around 80% of people in developing countries rely on traditional herbal remedies, according to the WHO. Out of the 374,000 species of plants (MNPS, 2021), over than 28,000 are used for medicinal purposes, on the other hand about to 30,000 of these species showing antimicrobial effect are identified. (Tunca-Pinarli, et al., 2023). Medicinal plants are an important source of new drugs, and over 100 countries regulate about to their use. Ethnomedicinal knowledge has led to the discovery of 74% of bioactive plant compounds (Picking, 2024). Recently, most researchers have focused on the effect of antimicrobial activity of medicinal plants (Castronovo et al., 2021; Al-Qura'n, 2009).

Antimicrobial Activity of Medicinal Plant Extracts

Medicinal plants are those that contain various parts or bioactive components, which can be directly obtained and used either internally or externally to treat diseases in humans and animals. (Stéphane et al., 2021; Özdemir et al., 2024) Today, it is widely acknowledged that MAPs contain compounds with antimicrobial properties (Vaou et al., 2021). The MAPs contain essential oils or alkaloids, flavonoids, sesquiterpene, lactones, diterpenes, triterpenes or naphthoquinones (Azeem et al., 2020; Karthikeyan et al., 2020; Ohiagu et al., 2021), and are popular both in the medical field such as potential antimicrobial, anticancer and actively function in many fields. This compounds belonging antimicrobial properties, inhibit bacteria, fungi, viruses and protozoa with different mechanisms than conventional drugs. On the other hand, the compounds extracted from plants offer potential in the treatment of antimicrobial resistant strains and may either have intrinsic antimicrobial effects or enhance th e efficacy of antimicrobials by modifying resistance mechanisms (Álvarez Martinez, et al., 2020; Biharee et al., 2020; Özdemir et al., 2023; Tehranizadeh et al., 2016). Although the some of them can not effect on their own, they can work in synergy with antibiotics to overcome bacterial resistance (Onyancha et al., 2021).

Herbal treatments are generally safer than synthetic drugs, with fewer side effects and a lower risk of resistance development. However, similar to antibiotics, if only one compound targets a specific pathway, resistance can still occur (Tehranizadeh et al., 2016; Umaru, et al., 2020). The efficacy of medicinal plant extracts depends on the synergistic interactions of multiple active compounds, which increase the bioavailability, solubility and absorption of the drug while suppressing bacterial resistance and minimising toxicity (Choudhury, 2022). There is a need to better elucidate mechanisms of resistance and optimal use of medicinal plants to control microbial infectious diseases (Tiwari et al., 2023).

Styrax officinalis L. (*Sytrax*) and *Elaeagnus* composed a Important compounds such as egonol, egonol oleate, americanin, various phenolic acids and benzofuran derivatives have been isolated from its leaves, fruits, seeds, flowers and stems. The extracts containing these compounds exhibit significant biological activities, including antibacterial, antifungal, antioxidant, antitumour, anti-leukaemic, haemolytic and tyrosinase inhibitory effects. In addition to these, for therapeutic applications, both natural compounds and their synthetic derivatives are used (Demiray, 2021; Jaradat, 2020). In Turkiye, *Elaeagnus* is employed in traditional medicine for its tonic and antipyretic properties, as well as for the treatment of diarrhea and kidney disorders (Altundag & Ozturk, 2011). Okmen and Turkcan (2014) reported that methanol extracts of *Elaeagnus*, while having no effect on *Escherichia coli* ATCC 1122 and *C. albicans* RSKK 02029, showed the highest antimicrobial activity with a 16 mm inhibition zone diameters against *Yersinia enterocolitica*. The minimum inhibitory concentration (MIC) for the extract was 3.5 mg/mL for all bacterial strains tested except *Y. enterocolitica* NCTC 11174. The *Rheum ribes* chloroform extract exhibited strong antimicrobial activity, with MIC values of 0.89 mg/L for *S.* Paratyphi A (50.81% inhibition) and up to 14.17 mg/L for *B. subtilis* (96.04% inhibition), demonstrating higher efficacy than tetracycline against several pathogens and varying effects on Gram-positive, Gram-negative, and probiotic bacteria due to potential surface tension interactions (Özdemir et al., 2022-2023).

Melissa contains bioactive compounds such as volatile compounds (monoterpenes, sesquiterpenes, and monoterpene alcohols, giving it a lemon-like scent), triterpenes, phenolic acids (benzoic acid derivatives (e.g., gallic acid) and cinnamic acid derivatives) and flavonoid being a major component (Mabrouki & Duarte, 2018; Mencherini et al., 2007; Moradkhani et al., 2010).

Echinops species have been traditionally used to treat a variety of ailments. These include bacterial and fungal infections, fever, respiratory and cardiac problems (Bitew & Hymete, 2019). Phytochemical analysis suggests *Echinops* has antioxidant, antimicrobial and immunomodulatory properties (Falah et al., 2021). This genus, which belongs to the Asteraceae family, comprises over 120 species characterised by uniflorous capitula arranged in spherical or oval heads (Bitew & Hymete, 2019). Chemical profiling of *Echinops* roots reveals a predominance of thiophenes, such as α-terthiophene, while flavonoids isolated from *E. grijsii* roots and *E. echinatus* plants may contribute to hepatoprotective effects (Wang et al., 2015). Studies on *E. heterophyllus* extracts have shown significant hepatoprotective activity against methotrexate-induced hepatotoxicity. Ethanolic extracts were superior to flavonoid fractions (Abdulmohsin et al., 2019). In vitro evaluations of extracts from *E. giganteus*, *E. ritro* and *E. tournefortii* showed remarkable free radical scavenging effects, but current assessments of antioxidant activity lack comprehensive in vivo models (Anvari & Jamei, 2018; Sytar et al., 2022).

This study aims to evaluate the *in vitro* antimicrobial properties of selected MAPs such as *Melissa*, *Elaeagnus*, *Styrax* and *Echinops* and investigate their potential as natural bread preservatives. This research seeks to enhance the shelf life, safety, and sensory quality of functional bread, contributing to sustainable and innovative food preservation methods.

Materials and Methods

Indicator Microorganism

All of the indicator microorganisms were obtained from Cukurova University, Biotechnology Research and Application Center. Gram negative bacteria were *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella* Parathypi A NCTC13, *E. coli* ATCC 25922, Gram positive bacteria were *Listeria monocytogenes* ATCC 7677, *Bacillus subtilus* B-354, *Staphylococcus aureus* ATCC 29213, Molds were *Aspergilus niger* 10 ph k, *Aspergilus niger* ATCC 1015, *Candida utilis, Candida sakazakii, Candida albicans, and Yeasts were Saccharomyces cerevisiae*_sauch_VL1, *Saccharomyces cerevisiae_zymaflore and* Pneumonia-Associated Respiratory Pathogens (*Klebsiella pneumonia*-1, *Acinetobacter baumannii*-5, *Pseudomonas aeruginosa*-3).

Medicinal and Aromatic Plants

Melissa, *Elaeagnus, Styrax* and *Echinops* plants were collected from the Çukurova region of Turkey. Different organs (whole plant, seed, fruit, leaf) of these plants were used in the experiment. These samples were *Eleganus* seeds and fruit, *Echinops* fruit, *Styrax* seeds and fruit, *Melissa* whole and *Melissa* and *Styrax* juices. The collected MAPs (Figure 1) were immediately stored in a -80°C cold chain until analysis was performed.

Figure 1. Images of medicinal and aromatic plants (MAP)s used in the study

Figure 2. The dough was prepared with the incorporation of seeds of *Melissa* with *Echinops* and fruit organs of *Echinops, Styrax and Melissa*

Solvent Extracts of MAPs

The extracts were prepared using ethyl alcohol and water as solvents for the different plant organs. The seeds of *Echinops* and *Styrax,* flowers of *Echinops*, leaf of *Melissa* were ground in a blender, the seeds of *Styrax* and *E. angustifolia* were separated from the fruit, dried in an oven, ground in a mill and pulverised. For this purpose, extraction solutions containing 5 g of each plant and 45 mL of solvent were prepared. The same procedure was followed for the second extract, but ethyl alcohol was used as solvent (Tufekci et al., 2018). The solutions were filtered and used in the study as alcohol and water extracts of the plants. Only the extracts of *Styrax* and *Melissa* were obtained.

As a result, (Table 2) the 10% and 5% water extract (WE) and alcohol extracts (AE) of *Melissa* leaves, *Echinops flowers*, *Styrax* (seed and fruit), *Elaeagnus* (seed and fruit) were prepared. Determination of antimicrobial effect of water and alcohol extracts of aromatic plants on gram-positive bacteria (inhibition zone diameter (mm) measurement results. Water extract groups (1-6): 1- M*elissa.* 2- *Elaeagnus* seed, 3- *Elaeagnus* fruit, 4- *Styrax* seed, 5- *Sytrax* fruit, 6- *Echinops*, Alcohol extract groups (7-12) :7- M*elissa,* 8- *Elaeagnus* seed, 9- *Elaeagnus* fruit,10- *Sytrax* seed, 11- *Styrax* fruits, 12- *Echinops*, 13- *Sytrax* juice, 14- M*elissa* juice.

Determination of Antibacterial Activity

The well diffusion agar assay test that was performed according to the methods designed and modified by Moghimi et al. (2016). Indicator bacteria subcultured overnight in triptic soy broth at 37 °C. It was poured into 90 mm diameter petri dishes with 20 mL Mueller-Hinton agar (Merck 1.05437) and added 0.5 Mc-Farland 1 mL fresh indicator bacteria and kept at room temperature for 30 minutes. Later, aseptically wells with a diameter of 6 mm were opened to frozen agar petries. 100 µL each plant extract were added to each well. Then inhibition zone diameters formed around the wells as a result of the incubation process of the indicator bacteria were measured in millimeters with the digital caliper (Mitutoyo 500-181-30, 0-150 mm) and interpreted CLSL 2018.

Preparation of Bread with MAPs Additive

Visuals of the plants used in the experiments were taken and shown in Figure 2. To ensure optimal incorporation of MAPs into the dough, the preparation process for herb-enriched bread included several steps. First, the aromatic plants were carefully washed to remove any impurities. The plants were then ground in a food processor. This was done until a homogeneous mixture was obtained. The seeds of *Styrax* and *Elaeagnus* were separated from the fruit before processing. The seeds were then dried in an oven to remove moisture. They were then ground to a fine powder in a mill. To produce experimental bread samples, the prepared plant powders were incorporated into the dough mix in specific proportions. For each bread, 5 g of MAP fruits and seeds were added to 200 g of flour by weighing 5 g on a precision balance. The dough was left to rest and then baked in an oven at 180 °C (Figure 2). This ensures even heat distribution for consistent texture and flavour development.

After baking, the bread samples contained with / without herbal additives were sliced. Following, they were exposed to air and placed in polyethylene bags and stored at room temperature in a dark environment to assess their shelf life. A portion of the bread was also cut into small pieces to facilitate the sensory evaluation, in which the participants rated the taste and texture.

Results

MAPs were rich in potential antioxidants and antimicrobial compounds, including carotenoids, phenolics, phenols, and flavonoids (Özdemir et al., 2022; Sarkar, 2020). Each plant’s biological effect is related to its chemical profile, hence chemical composition. The inhibition zone diameters formed in plate assays of water and alcohol extracts of MAPs against Gram-negative bacteria, Gram-positive bacteria, molds, and yeasts were measured and calculated.

***In-vitro* Antibacterial Activiy of MAPs**

Gram-negative pathogens

Inhibition zone diameters measurements of water extracts of aromatic plants numbered from 1 to 6, alcohol extracts from 7 to 12 and plant juices such as *Melissa* and *Styrax* from 13 to 14 are as shown in Figure 3- 5b.

Melissa officinalis extracts (except water extract of whole plant) suppressed *S.* Paratyphi A and were highly effective against *Klebsiella* spp., *E. coli*, and *K. pneumonia*-1 with the alcohol extract showing the highest effect. Both alcohol and water extracts of *Styrax* seed inhibited *K. pneumonia*-1, *P. aeruginosa*, and *S.* Paratyphi A, while only alcohol extract of *Echinops* and *Elaeagnus* were effective against *E. coli*. The alcohol extracts of all plants showed inhibition against *A. baumannii*-5, while both water and alcohol extracts were effective against *S.* Paratyphi A. The presence of triterpenoids, tannins and saponins was identified in an initial phytochemical analysis of the fruit pericarp extract of *Styrax*. Tayoub et al. (2006) reported that bioactivity study evaluated the plant's potential as a biopesticide, with essential oil ratios ranging from 0.01-0.02%, lower than other studies. Key components identified in three development stages included (E)-2-hexenal, geraniol, octanol, nonanal, α-terpineol, tridecanal, trans-cubebol, and geranyl acetone. Another studies, infusions from the leaves and flowers of the plant have been used to treat coughs, diphtheria, and leucorrhoea, while the plant also possesses antibacterial, antifungal, and wound-healing properties and is used in India for conditions such as scabies and skin ulcers. Additionally, a tincture of the plant can serve as a mouthwash for asthma and is applied in the treatment of coughs, gonorrhoea, oedema, and tuberculosis (Al-Qura'n, 2015; Paşa, 2023).

Gram-positive pathogens

All groups showed inhibitory effect on *Listeria monocytogenes* except juice of *Melissa* and *Styrax* fruit. Inhibitory effect was observed more on *Bacillus. Styrax* juice did not show any inhibitory effect on any gram positive organism (Figure 4).

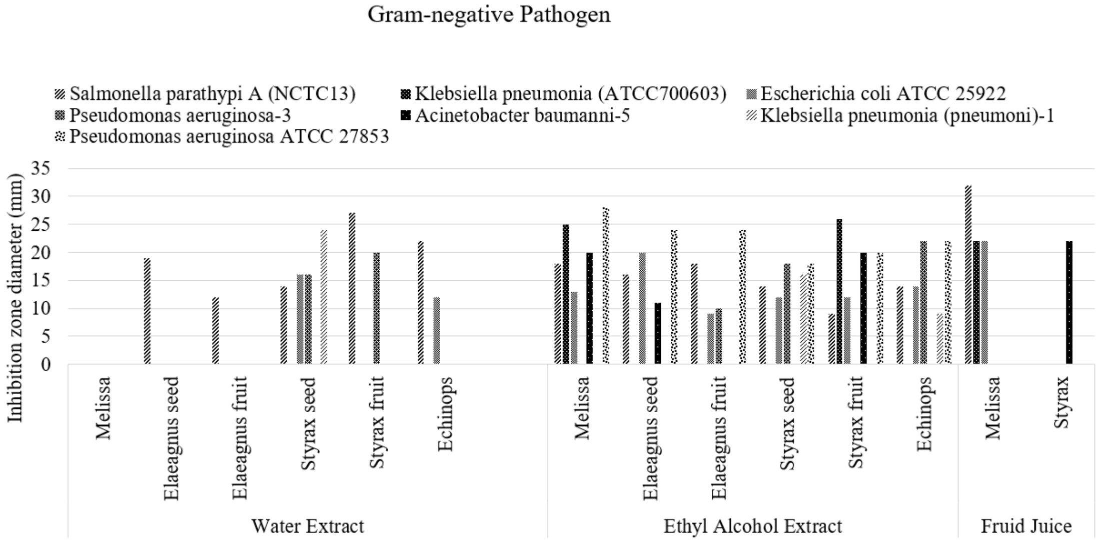


Figure 3. Inhibition zone measurement of MAPs on Gam-negative bacteria

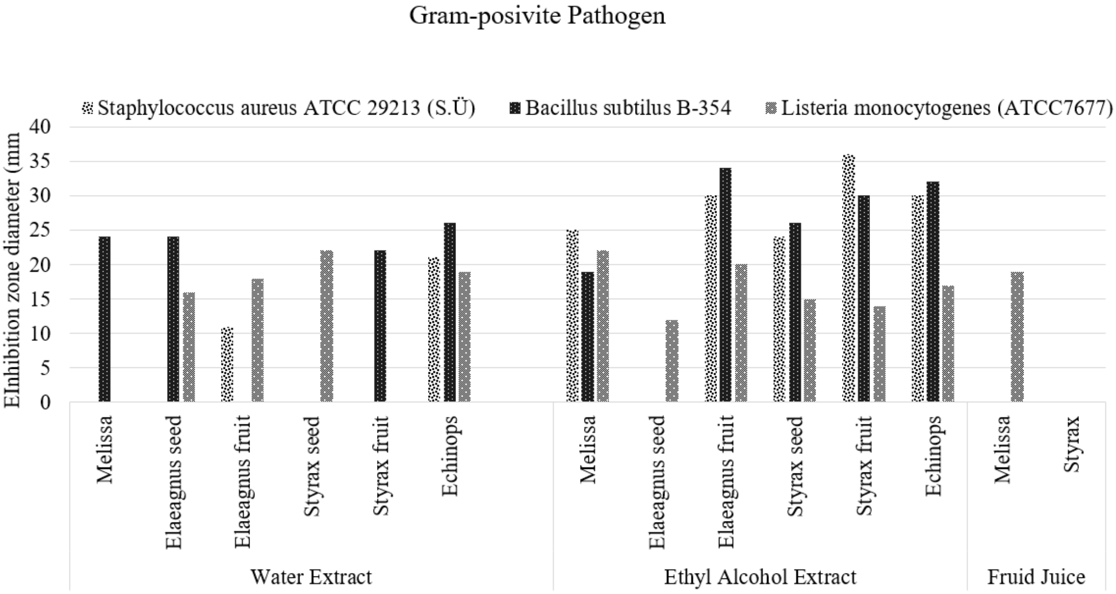
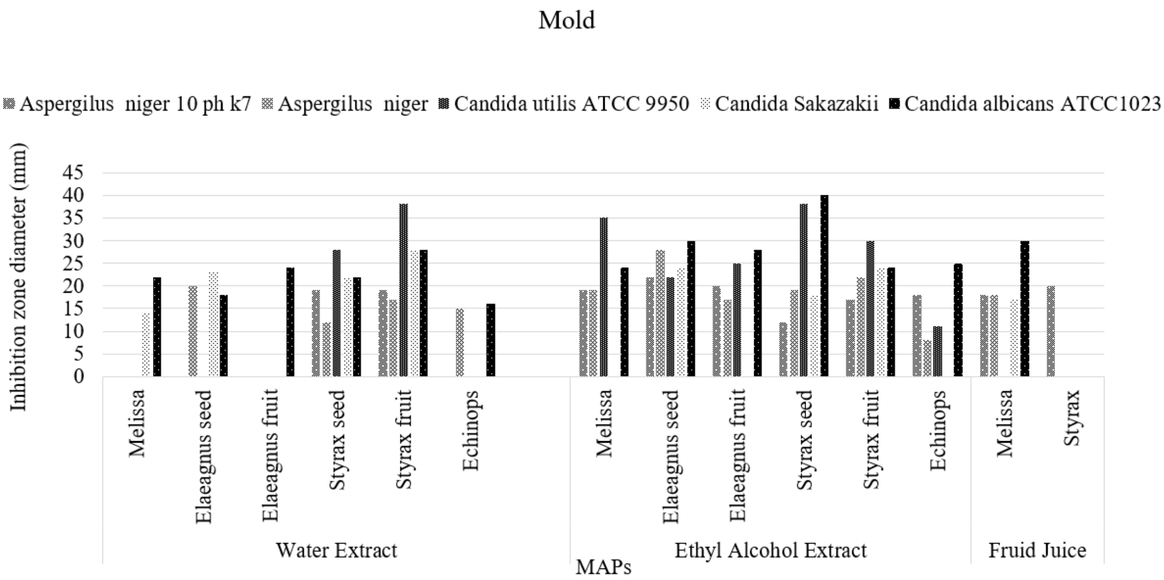


Figure 4. Inhibition zone measurement of MAPs on Gam-positive bacteria



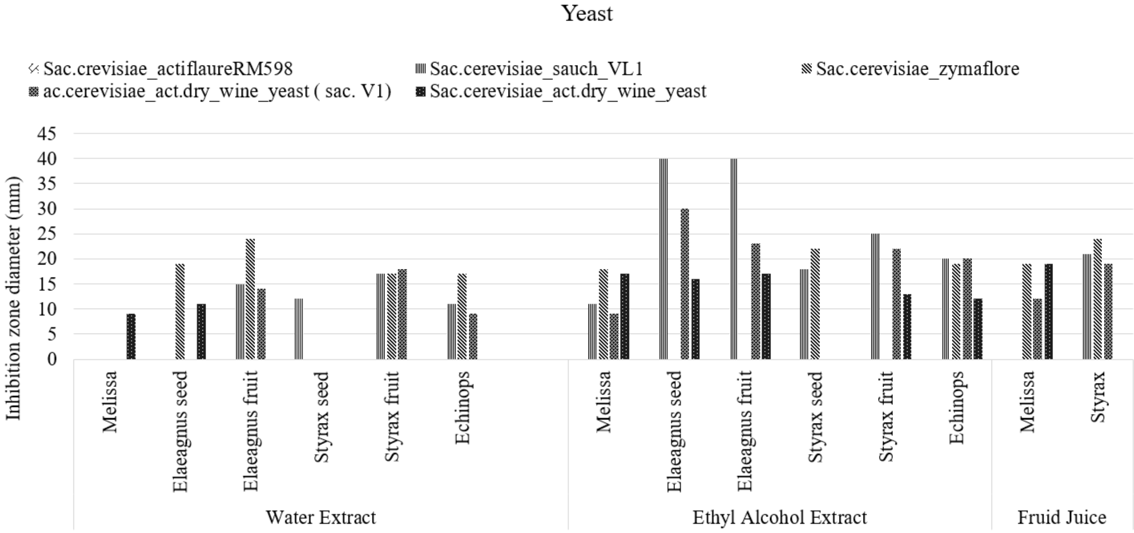


Figure 5b. Inhibition zone measurement of aromatic plants on yeast.

Bertanha et al. (2013) and Öztürk et al. (2008) reported the antibacterial activity of *Melissa* compounds (Egonol, Homoegonol, Americanin) against *Bacillus subtilis*, *E. coli*, *S. aureus*, *Haemophilus influenzae*, *K. pneumoniae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*. Celebi et al. (2021) assessed the antimicrobial efficacy of *Elaeagnus* extracts against *Proteus mirabilis* (≥ 12.500 µg/mL), *C. albicans* (1.562 µg/mL), *Enterococcus faecalis* (1.562 µg/mL), *S. aureus* (3.125 µg/mL), *S. epidermidis* (1.562 µg/mL), *E. coli* (≥ 12.500 µg/mL), and *P. aeruginosa* (≥ 12.500 µg/mL), showing strong activity, especially against gram-positive bacteria and yeast. Additionally, ZnO nanoparticles with plant extract *Eleagnus angustifolia* showed antimicrobial activity with MIC values of ≥ 1.6 mg/mL.

Echinops species are traditionally used for bacterial and fungal infections, fever, and respiratory and heart problems (Bitew & Hymete, 2019). They have antioxidant, antimicrobial, and therapeutic properties, with thiophenes acting against fungi, bacteria, and insects, and terpenes and flavonoids offering anti-inflammatory and liver protection effects (Bitew & Hymete, 2019; Erenler et al., 2014). Extracts from *E. grijsii* root (300 mg/kg) and *E. echinatus* aerial parts (500 and 750 mg/kg) reduced liver function markers ASAT and ALAT. Aqueous methanol extracts of *E. ritro* L. were also studied.

The study by Hosseini & Gholipour (2020) tested plant extracts from *Quercus brantii*, *Elaeagnus angustifolia*, *Satureja montana*, *Tragopogon dubius*, and *Sonchus asper* for antimicrobial and anti-biofilm properties. *Quercus brantii* showed the strongest antibacterial and anti-biofilm effects, including against *Pseudomonas* and *Staphylococcus*. *Tragopogon dubius* and *Sonchus asper* had limited anti-biofilm activity. Özdemir et al. (2024) reported that *Rheum ribes* extract had strong antimicrobial effects. It inhibited *K. pneumoniae* (Pneumonia-Associated Respiratory Pathogens) and *P. aeruginosa* at 0.5 ppm, with inhibition rates of 94.88–100% against *K. pneumoniae* isolates and 82.82% against *P. aeruginosa*. Jalal et al. (2015) tested the antibacterial activity of essential oils against *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Citrobacter koseri*. The oil showed strong antibacterial activity, with citronellal, β-caryophyllene oxide, and geraniol acetate as the main components.

***In-Vitro* Antifungal Activity of MAPs**

Figure 5a and 5b showed the zone measurement (mm) graph on mould and yeast in water and alcohol extracts of MAPs. The MAPs tested displayed varying degrees of antifungal activity, with alcohol extracts generally outperforming water extracts in inhibiting mould (Figure 5a) and yeast growth (Figure 5b).

Alcohol extracts (AEs) of MAPs strongly inhibited *Candida albicans* and *Aspergillus niger*, while water extracts (WEs) showed milder effects. *Styrax*, *Melissa*, *Echinops*, and *Elaeagnus* displayed strong antifungal properties, with *Elaeagnus* being especially effective against *Aspergillus* spp. *Styrax* contains triterpenoids, tannins, saponins, and essential oils like geraniol, and is traditionally used for coughs, diphtheria, and skin conditions (Dehghan et al., 2014; Al-Qura'n, 2015; Paşa, 2023). MAPs, rich in essential oils and flavonoids, show antimicrobial effects, including against resistant strains, with *Styrax* and *Melissa* having antifungal activity (Jaradat, 2020).

Echinops essential oil showed strong antibacterial and antifungal activity, with low MIC values against *E. coli*, *S. aureus*, and *S. enteritidis* (Jiang et al., 2017). *Styrax suberifolius* bark inhibited *Phomopsis cytospore*, *Fusarium oxysporum*, and *Alternaria solani* (Zheleva-Dimitrova et al., 2023). *Styrax* saponins, including saponin A, showed antifungal activity against *Trichoderma viride*, *Fusarium oxysporum*, *A. niger*, and *Rhizopus mucor* (Mansour et al., 2016; Sak et al., 2024). *Elaeagnus* demonstrated antibacterial and antifungal activity, particularly against *Alternaria solani*, *Botrytis cinerea*, and *Aspergillus* species (Khan et al., 2016). *Styrax* species are known for over 150 bioactive compounds with insecticidal, antibacterial, and anti-inflammatory properties (Liu et al., 2018).

Elaeagnus angustifolia is rich in nutrients, antioxidants, and minerals, with antimicrobial activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *Y. enterocolitica*, coagulase-negative *Staphylococci*, and fungal pathogens like *Alternaria solani* and *Aspergillus* species (Farzaei et al., 2015a; Khan et al., 2016; Okmen & Turkcan, 2014; Bahraminejad et al., 2015). High levels of caffeoylquinic acids and flavonoids were found in Elaeagnus (Zengin et al., 2022), and 95 secondary metabolites were identified, including chlorogenic acid, apigenin, and hyperoside (Bitew & Hymete, 2019), with antimicrobial activities Uzelac et al., 2023). *Elaeagnus* grows in dry, stony habitats in southern and eastern Europe and western Asia (Loizeau & Jackson, 2017), *Melissa* which extensively distributed in the Mediterranean region (Pouyanfar et al., 2018), *Styrax* species habitats in the Asian region (Paparella et al., 2024) Echinops habitas in primarily in Africa and Asia (Elserag et al., 2024).

Shelf Life and Sensory Testing of Bread Enriched With MAPS

The shelf life of unadulterated bread and bread made from TAP were observed for 7 days in two different conditions as in polythene packaging (Table 1) and outdoor (Table 2). The shelf life of bread with MAPs was tested over 7 days. The results of the functional bread trial; these plants were used in bread making and the results are given in Table 1.

Table 1. Mould formation on the functional bread by MAPs kept in the polyethylene packaging and out-door for 1 week

Fonctional Bread	Additive MAP form	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day7
In-door (polythene packaging)								
Control	-	-*	-	X				
Styrax	Fruit	-	-	-	-	X	-	-
	Seed	-	-	X		-		
Elaeagnus	Fruit	-	-	-	X	-		
	Seed	-	-	-	-	-	-	X
Melissa	Leaf	-	-	-	-	-	-	X
Echinops	Flower	-	-	-	-	X		
Out-door								
Control	-	-*	-	-	-	-	-	-
Styrax	Fruit	-	-	-	-	X	-	-
	Seed	-	-	-	-	X	-	-
Elaeagnus	Fruit	-	-	-	-	-	-	-
	Seed	-	-	-	-	-	-	-
Melissa	Leaf	-	-	-	-	-	-	-
Echinops	Flower	-	-	-	-	-	X	-

*: Not mouldy (-), Mouldy (X).

The shelf life of unadulterated bread and bread made with TAP was monitored over 7 days under two conditions: polythene packaging and outdoor exposure (Table 1). Outdoors, additive-free bread developed mould on day 3, *Styrax* fruit extract delayed mould to day 5, and seed extract showed mould on day 3. *Elaeagnus* fruit extract prevented mould until day 4, while the seed extract and *Melissa* remained mould-free for 7 days. *Echinops* developed mould on day 5.

In open air, additive-free bread developed mould on the 1st day. Bread with *Styrax* extracts developed mould on day 5, while *Elaeagnus* and *Melissa* prevented mould throughout the 7-day period. *Echinops* showed mould on day 6. Overall, *Elaeagnus* and *Melissa* were the most effective, particularly outdoors. Additive-free bread was the most susceptible to mould. Plant extracts, especially seeds, enhanced mould resistance, with better results in indoor storage.

Table 2. Sensory test of functional bread enriched with MAPs in comparison with a standard bread. 12 Participants and percent likeability

	MAPs	How did the bread taste?				
MAPs		5*	4	3	2	1
Styrax	Fruit	25	58	17		
	Seed	42	58	-	-	-
Elaeagnus	Fruit	17	83	-	-	-
	Seed	25	67	8	-	-
Melissa	Leaf	67	33	-	-	-
Echinops	Flower	25	42	-	33	-

** I like it very much: 5, I like it: 4, Undecided: 3, I don't like it: 2, I don't like it at all: 1

Elaeagnus and *Melissa* emerged as the most effective and preferred components for both shelf life enhancement and sensory qualities. They demonstrated strong mould resistance, especially outdoors, and received high ratings from participants. Additive-free bread was the least durable and received moderate sensory acceptance, highlighting the potential of plant extracts as functional additives in bread formulations. Mahboubi (2018) found *E. angustifolia* extracts (200–600 mg daily) beneficial for osteoarthritis. *Melissa* (Lemon balm) has antibacterial, sedative, and antispasmodic properties, aiding with stress and gastrointestinal issues (Zam et al., 2022; Ohiagu et al., 2021).

The observations of the breads evaluated in terms of shelf life, which continued for 7 days by looking at the breads 2 times every day in the morning and evening to see whether mould formed on them or not, are given in Tables 2

The breads with herbs were firstly subjected to sensory test. Twelve person participated in the sensory test of bread enriched with MAPs. *Styrax* seeds were similarly well received, with 42% giving them a 5 and 58% a 4. Bread with *Elaeagnus* seeds had the highest acceptance, with 17% rating it 5 and 83% rating it 4. The seeds of *Elaeagnus* received a slightly lower score, with 25% giving it a 5, 67% giving it a 4 and 8% being undecided. Bread made with *Melissa* received very positive feedback, with 67% of participants giving it a 5 and 33% a 4. However, bread made with *Echinops* showed mixed reactions, with only 25% giving it a 5, 42% giving it a 4 and 33% giving it a 1 ("don't like it at all"). The results show the most favourable components to be *Elaeagnus* fruit and *Melissa.*

Melissa officinalis, known for its antimicrobial activities, shows potential as a natural food preservative due to its ability to suppress several foodborne pathogens. Studies indicate its efficacy in various food matrices, suggesting that it could extend shelf life and provide a safer alternative for food preservation (Carvalh et al., 2021). Similarly, phytochemical investigations of Styrax species have revealed lignans and triterpenoids with diverse biomedical properties, such as antifungal activity against Candida albicans and reduction of DNA damage in liver cells. These findings suggest that Styrax species could also be used as food additives (Son et al., 2021).

In addition, Elaeagnus seeds, which contain 50.87% oil and 36.27% protein, are rich in unsaturated fatty acids, particularly linoleic and oleic acids. Its proteins, especially alkaline protein, have essential amino acid profiles and functional properties comparable to those of soy protein isolate. On the other hand , *Echinops* setifer extract (ESE) is a bioactive source with health benefits. When added to yoghurt, it improved texture, mouth feel and functional properties. Extracts at concentrations of 30% and 40% maintained high viable counts and overall quality during 15 days of storage, highlighting their potential to improve both the nutritional value and functionality of yoghurt (Shirani et al., 2022).

Conclusion

Studies have highlighted the significant potential of aromatic and medicinal plants such as *Melissa, Elaeagnus*, *Echinops* and *Styrax* in the improvement of food quality and safety. Their essential oils and extracts are valuable for improving shelf life and ensuring food safety, as they possess potent antibacterial, antioxidant and antifungal properties. These plants can act as functional ingredients in foods, providing natural preservatives that extend product life while inhibiting the growth of harmful microorganisms. When incorporated into functional foods such as bread, these plants offer not only nutritional benefits but also psychobiotic, therapeutic and protective properties, in line with advances in biotechnology to create versatile foods that contribute to overall health and well-being. Optimising the extraction and application of bioactive compounds from these plants, improving their scalability and ensuring their efficacy in food preservation, safety and quality will be the focus of future biotechnology developments. Medicinal and aromatic plants have the potential to revolutionize the food industry by providing safer, more sustainable, and natural alternatives to synthetic additives and promoting healthier, longer-lasting foods.

Declarations

Ethical Approval Certificate

The authors declare that ethical approval is not required for this research.

Author Contribution Statement

Nurten Yılmaz.: Supervision, Data collection, investigation, formal analysis, and writing the original draft review and editing.

Fund Statement Conflict of Interest

All authors declare that there is no conflict of interest related to this article.

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