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Phytochemical Profiling of Three Medicinal Plants and Their Antimicrobial Potential Against Foodborne Pathogens

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Abstract
Two solvent extracts (ethanolic and aqueous) of three medicinal plants Sage (*Saliva officinalis L.*), Chamomile (*Matricaria chamomile L.),* and Anise (*Pimpinella anisum L.*) were evaluated for their antimicrobial efficacy against several food pathogens and spoilage microorganisms. The results revealed that the ethanolic extract of the three medicinal plants had significant antimicrobial action against all tested microbes compared with aqueous extract. The phytochemical examination of the three medicinal plants revealed the presence of carbohydrates, phenols, flavonoids, tannins, proteins, saponins, and alkaloids in the Sage and Anise plant extracts but an absence of saponins in the ethanolic extract of Chamomile plant. The results showed that plant extracts had a considerable effect on the cell membranes of Gram-positive bacteria. In conclusion, plant extracts are effective natural antimicrobials and show potential as natural food preservatives, warranting further investigation.

Keywords: *Pimpinella anisum* L.; *Matricaria chamomile* L.*; Saliva officinalis* L.; Phytochemical assays, Antimicrobial activities.

التوصيف الكيميائي النباتي لثلاثة نباتات طبية وتقييم فعاليتها المضادة للميكروبات ضد مسببات الامراض المحمولة بالغذاء

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الخلاصة
تم تقييم مستخلصين مذيبين (أيثانول ومائي) من ثلاث نباتات طبية, المريمية (.*Saliva officinalis* L) والبابونج (.*Matricaria chamomile* L) واليانسون (.*Pimpinella anisum* L) لفعاليتهما المضادة للميكروبات ضد العديد من مسببات الأمراض الغذائية والكائنات الحية الدقيقة المسببة للتلف. وكشفت النتائج أن المستخلص الكحولي للنباتات الطبية الثلاثة كان له تأثير مضاد للميكروبات بشكل كبير ضد جميع الميكروبات المختبرة مقارنة بالمستخلص المائي. وكشف الفحص الكيميائي النباتي للنباتات الطبية الثلاثة عن وجود الكربوهيدرات, الفينولات, الفلافونويدات, التانينات, البروتينات, السابونين والقلويدات في مستخلصات نباتات المريمية واليانسون ولكن عدم وجود السابونين في المستخلص الايثانولي لنبات البابونج. وأظهرت النتائج أن المستخلصات النباتية لها تأثير كبير على الأغشية الخلوية للبكتيريا الموجبة لملون غرام. وفي

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الختام، فإن المستخلصات النباتية هي مضادات طبيعية للميكروبات فعالة وتظهر إمكانيات كمواد حافظة طبيعية للأغذية, مما يستحق المزيد من الدراسات .

1. Introduction

Food infection and spoilage are among the major causes of food-borne illness worldwide. The food born microorganisms that cause these illnesses enter food via cross-contamination, inappropriate handling, and temperature abuse. Common microorganisms causing deterioration in many different foods and products are bacteria, yeast, and molds [1]. Once these microbes get into food goods, they absorb nutrients and generate metabolites that cause food spoiling [2]. Infections are commonly caused by foodborne bacteria such as *Staphylococcus aureus* and *Bacillus cereus* of Gram-positive bacteria and other Gram-negative bacteria including *Pseudomonas aeruginosa, Escherichia coli,* and *Salmonella typhi* [3].

Medicinal plants have been employed as food preservatives due to their antimicrobial activity, which reduces the growth of pathogenic bacteria and lengthens the shelf life of food products without the use of chemical preservatives. Recently, several studies have employing plant extracts as effective natural preservatives. These plant extracts are natural sources of antimicrobial compounds that are both nutritionally safe and biodegradable [4]. Crude extracts from various parts of medicinal plants, such as the root, stem, flower, fruit, and twigs, have long been used to treat some human disorders. Medicinal plants include a variety of phytochemicals including terpenoid, tannins, alkaloids, and flavonoids which have antimicrobial antioxidant action [5].

Sage plant (*S. officinalis*) is considered a popular herb that has long been used to make herbal infusions. Incorporating sage infusion into your regular diet can provide numerous benefits, including antibacterial, antifungal, anti-inflammatory, anticancer, and anti-proliferative properties [6] and also Anise plant (*P. anisum*), a fragrant plant from the umbeliferea family, has been utilized in traditional Iranian medicine. It contains antibacterial, antifungal, antiviral, antioxidant, and muscle relaxant properties [7]. The essential oil contains anethole, which has antimicrobial activity. Its extract has antimicrobial effects against a variety of bacteria and fungi [8]. Chamomile (*M. chamomilla)* is a well-known medicinal herb with a global presence. It is commonly used in traditional medicine to treat a wide range of illnesses, including infections, neuropsychiatric, respiratory, digestive, and liver issues, as well as skin, eye, and oral diseases [9]. It also functions as an analgesic, antispasmodic, antibacterial, and antiemetic. Chamomile (*M. chamomilla)* essential oils and extracts exhibit remarkable antioxidant, antibacterial, antifungal, antidiabetic, anticancer, antiparasitic, anti-inflammatory, antidepressant, antipyretic, antiallergic, and analgesic properties [10].

The objective of this study was to investigate the impact of three medicinal plant extracts against some food pathogens and determine their phytochemical content.

2. Materials and Methods

2.1 Collection of the plant samples

Three samples of Sage (*S. officinalis*), Chamomile (*M. chamomilla)*, and Anise (*P. anisum*) plants were collected in (October 2023) from local markets in Diyala Governorate. Two solvents, 96% ethanol alcohol and distilled water, were used to evaluate the antimicrobial activities.

2.2 Extraction of plants

100 ml of ethanol and semi-hot water were used to dissolve 10 g of sample powder separately in conical flasks and kept in a shaking bath at room temperature for 24 h. The muslin fabric was used to filter the extract and finally centrifuge it for 20 min at 6000 rpm. The supernatant, after centrifugation, was collected and kept in the oven at 40 °C for 4 h, and further phytochemical examination of the remaining solution was stored in the refrigerator [11].

2.3 Phytochemical tests of plants

Several tests were performed to establish the chemical composition of the plant extracts produced. The constituents included proteins, carbohydrates, alkaloids, saponins, phenolic compounds, tannins, and flavonoids [12].

2.4 Test for proteins (Biuret test)

Plant extracts were treated with a solution containing 11% sodium hydroxide and 3% copper sulphate to produce the purple-blue color [12].

2.5 Test for carbohydrates (Molisch's test)

In this experiment, 2 ml of plant extracts was used and 2-3 drops of Molisch's reagent was added to the extract, then 2 ml of concentrated sulfuric acid (H_2SO_4) was added dropwise. To indicate the presence of carbohydrates, a purple or blue ring formed between the two layers of liquid [12].

2.6 Test for alkaloids (Mayer's test)

One ml of potassium mercuric iodide (Mayer's indicator) was added to 3 ml of plant extract, which indicated the presence of alkaloid chemical compounds in the creation of a white precipitate [12].

2.7 Test for saponins (Foam test)

A small amount of plant extract was violently shaken with a vortex, and the production of permanent foam indicated the presence of saponins [12].

2.8 Tests for phenolic compounds

2.8.1 Ferric chloride test

The neutral $FeCl_3$ was added to 2 ml of plant extract, and the green color indicates the presence of phenolic chemicals [12].

2.8.2 Lead acetate test

Two ml of lead acetate Pb $(CH_3COO)_2$ solution was added to 2 ml of plant extract, and a yellow precipitate was formed, which suggests the presence of flavonoids and tannins [12].

2.9 Collection and bacteriological identification of tested microorganisms

To determine the antibacterial activities of plant extracts against food spoilage bacteria, samples were collected from various spoilage foods like fast food. The samples were cultivated using several media, including MacConkey agar, nutrient agar, and blood agar. The plates are incubated at 37°C for 18- 24 h. The fungus was cultivated on Sabouraud's dextrose agar and incubated at 37°C for 24- 48 h. Pure bacterial isolates were found and validated based on microscopic examination and biochemical tests. Four types of bacteria were obtained: Gram-positive (*S. aureus*), Gram-negative (*P. aeruginosa, K. pneumonia,* and *E.*

coli), and one of the common yeasts (*C. albicans*) was chosen. The bacteria were left overnight to grow on Mueller–Hinton medium at 37°C for 18-24 h using a spectrophotometer. We collected 5 ml of sterile saline water and diluted the bacterial culture to a concentration of 10^8 CFU/ml at 580nm.

2.10 Microscopic Examination

Using a sterile inoculating loop, a touch of a fresh colony was transferred to a clean glass slide, where it was mixed with distilled water and spread out. The slide was then allowed to dry and fixed using heat on a Bunsen burner. The cells were then stained using the Gram staining method and examined under a compound light microscope to observe the shape of the cells and the nature of their interaction with Gram stain [13].

2.11Biochemical tests

2.11.1 Catalase test

Using sterile wooden sticks, a single colony of the bacterial culture was moved to a clean glass slide and combined with a few drops of 3% H_2O_2 reagent. Bubbles start developing immediately, indicating a positive result [14].

2.11.2 Oxidase test

Using sterile wooden sticks, a single colony of the bacterial culture was spread out on filter papers that had been soaked in 1-2 drops of oxidase reagent. The organism begins to become purple within 30 seconds of coming into touch with the oxidase reagent, indicating a positive result. Bacteria carrying the respiratory enzyme cytochrome c oxidase are identified by the oxidase test [14].

2.11.3 Indole production test

This test was done using a peptone water broth medium inoculated with bacteria after 24 hours of incubation at 37 degrees Celsius. Five drops of Kovac's reagent were added, and a red ring appeared on the surface, indicating a positive result. The indole test identifies bacteria capable of producing indole using the enzyme tryptophanase [14].

2.12 Antibacterial susceptibility assay

The antibacterial activity of the plant extracts was assessed against the test isolates using the agar-well diffusion assay with three replications, following the methodology outlined by Nwinyi *et al.*, [15]. The McFarland's standardized isolates (1.5×10^8 CFU/mL) were spread over the surface of sterile Mueller Hinton Agar (MHA) plates using a sterilized swab stick. Wells were made on the agar plates using a sterile cork borer with a diameter of 6mm. Subsequently, varying concentrations (200, 100, and 50 mg/ml) of the extracts were introduced in a consistent volume (0.1 ml) into the respective wells of the plates. The plates were placed on the bench for a duration of 40 minutes to facilitate pre-diffusion of the extract. Subsequently, they were subjected to incubation at a temperature of 37 ℃ for a period of 24 hours. The diameter of inhibitory zones was measured using a transparent ruler calibrated in millimeters. The zone width of inhibition displayed by the bacterial isolate under investigation at the specified concentration was ascertained by analyzing the measurements. The positive control used in this experiment was ampicillin, while the negative control was dimethylsulfoxide (DMSO).

3. Statistical analysis

The statistical analysis of this study was conducted using an analysis of variables (ANOVA) in line with the SPSS program. The analysis of variance (ANOVA) was employed to assess the differences among many groups, with a minimum statistically significant moral difference of 0.05 or its equivalent. The experiment involving the suppression of artificially generated free radicals was conducted using three repetitions [16].

4. Results and Discussion

4.1 Phytochemical assay

Using chemical reagents, particular chemical components present in the extracts of medicinal plants were identified in this study. The phytochemical assay tests in Tables 1 and 2 showed positive results for tannins, flavonoids, carbohydrates, proteins, saponins, alkaloids, and phenolic compounds for ethanolic and aqueous extract, but absent of alkaloids in Sage aqueous plant extract and saponins in chamomile ethanolic plant extract respectively. Phytochemical assay of the extract of Anise plant demonstrated the presence of all organic components (Table 3), including, the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, phenolic compounds, and cardiac glycosides, which could be responsible for the observed antimicrobial properties. Certain critical active chemicals in plant extracts have important therapeutic significance for treating various ailments. Phytoconstituents are the several types of chemical ingredients that make up plants. These potent substances found in therapeutic plants have physiological and medical implications [17]. These plant-derived components, such as flavonoids, quinines, and terpenoids, play a significant role against a variety of diseases such as antibacterial, antidiarrheal, anticancer, antimutagenic, anti-inflammatory, antioxidant activities, aid in the reabsorption of electrolytes, inhibit specific pathogens, and aid in motility of the intestines [12].

Table 1: The phytochemical assay of Sage (*S. officinalis* L.) plant extracts.

Compounds	Tests	Result	Aqueous extract	Ethanolic extract
Proteins	Biuret test	Purple blue	+ ve	+ ve
Carbohydrates	Molish test	Violet ring	+ ve	+ ve
Alkaloids	Mayer's reagent	White ppt	- ve	+ ve
Phenolic compounds	Ferric chloride test	Green ppt	+ ve	+ ve
Tannins and Flavonoids	Lead acetate	Yellow white ppt	+ ve	+ ve
Saponins	Fast stirring	Dense foam for a long time	+ ve	+ ve

Table 2: The phytochemical assay of Chamomile (*M. chamomilla* L.) plant extracts.

Compounds	Tests	Result	Aqueous extract	Ethanolic extract
Proteins	Biuret test	Purple blue	+ ve	+ ve
Carbohydrates	Molish test	Violet ring	+ ve	+ ve
Alkaloids	Mayer's reagent	White ppt	+ ve	+ ve
Phenolic compounds	Ferric chloride test	Green ppt	+ ve	+ ve
Tannins and Flavonoids	Lead acetate	Yellow white ppt	+ ve	+ ve
Saponins	Fast stirring	Dense foam for a long time	+ ve	- ve

Table 3: The phytochemical assay of Anise *(P. anisum* L.) plant extracts.

Compounds	Tests	Detection result	Aqueous extract	Ethanolic extract
Proteins	Biuret test	Purple blue	+ ve	+ ve
Carbohydrates	Molish test	Violet ring	+ ve	+ ve
Alkaloids	Mayer's reagent	White ppt	+ ve	+ ve
Phenolic compounds	Ferric chloride test	Green ppt	+ ve	+ ve
Tannins and Flavonoids	Lead acetate	Yellow white ppt	+ ve	+ ve
Saponins	Fast stirring	Dense foam for a long time	+ ve	+ ve

4.2 Bacterial and Fungal Isolation and Identification

Bacterial isolates were cultured on blood agar, MacConkey agar, and nutrient agar at 37°C for 24 hours, whereas *C.albicans* were grown on Sabouraud dextrose agar and incubated at 37°C for 24 to 48 hours.

On MacConkey agar, *E. coli* appears in pink colonies due to lactose fermentation, *K. Pneumonia* appears as pink colonies with mucoid colonies, whereas *P. aeruginosa* colonies appear as pale its lactose non- fermenter [18]. *P. aeruginosa*, *E. coli,* and *S. aureus* colonies on blood agar plates show beta-hemolysis. On Sabouraud dextrose agar, *C. albicans* appears white and smooth. According to Cappuccino and Welsh [19], the microscopic examination of isolates stained with Gram stain showed that the cells of *K. pneumonia, E. coli*, and *P. aeruginosa* appeared as small, red bacilli**,** indicating that these bacteria are negative for the Gram stain. *S. aureus* appears purple, indicating that the organism is Gram-positive [20]. *Candida* yeasts appear dark blue (Gram stain). Biochemical testing was performed on all isolates, including oxidase, catalase, and indole tests *S. aureus, E. coli,* and *K. pneumoniae* had negative oxidase test findings. *P.aeruginosa* was discovered by a positive oxidase test, and the appearance of dark purple on the surface of colonies, indicating the production of cytochrome oxidase. It was also used to identify the bacteria that produce the enzyme. All isolates proved positive for the catalase test, which uses bubble production to detect organisms that generate the enzyme catalase. Other isolates had negative results except for the *E. coli*, which produced a positive result for the indole test. It differs from the other Gram-negative isolates utilized in this investigation because of this (Table 4).

Table 4: The results of diagnostic tests for bacteria.

Test	*S. aureus*	*K. Pneumoniae*	*P. aeruginosa*	*E. coli*
Oxidase	-	-	+	-
Catalase	+	+	+	+
Indole	-	-	-	+
Growth on Blood agar	Beta-hemolytic	non-hemolytic	Beta-hemolytic	Beta-hemolytic
Growth on MacConkey agar	-	+	+	+
Lactose Fermentation	-	+	-	+
Gram Stain	+	-	-	-

* - Negative

* + Positive

4.3 Antibacterial activity of plant extract

This study evaluated the antimicrobial activities of alcoholic and aqueous extracts of Sage, Anise, and chamomile plants **(**Table 5) against microorganisms that cause food spoilage. Two plant extracts (aqueous and alcoholic extract) were tested against three strains of Gram-negative bacteria (*P. aeroginosa, E. coli*, and *K. Pneumoniae*) and one strain of Gram-positive bacteria (*S. aureus*) as well as one pathogenic fungus (*C. albicans*). The well diffusion method was used to assess microorganisms' susceptibility. The results indicated that the ethanolic and aqueous extracts of chosen plants effectively suppressed the growth of food-pathogenic microorganisms with varying efficacy. *S. aureus* was one of the microorganisms that exhibited higher sensitivity to both extracts (aqueous and alcoholic), and sage extract showed greater efficacy against *S. aureus* than chamomile, as shown in (Figures 1 and 2). Ethanolic extract of the Anise plant demonstrated the highest inhibitory action against microorganisms, especially *E. coli* (Figure 4).

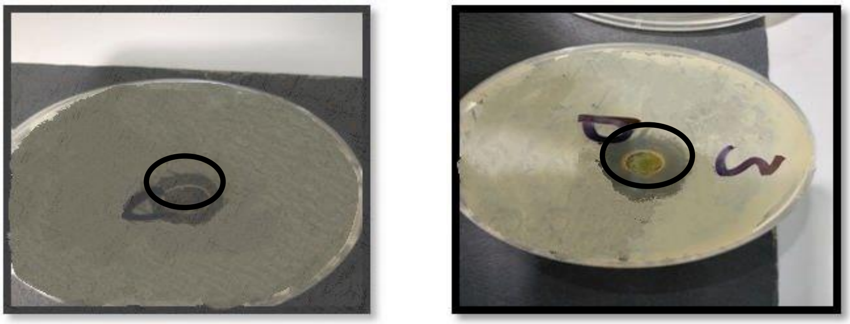
Table 5 : Inhibition zone diameters (mm) for all microorganisms.

Microorganism	Aqueous	Ethanolic	Aqueous	Ethanolic	Aqueous	Ethanolic
	Sage		Anise		Chamomile	
S. aureus	20-25 mm	25-30 mm	12-16 mm	15-20 mm	15-20 mm	20-25 mm
E. coli	12-18 mm	18-22 mm	22-28 mm	30-35 mm	10-14 mm	15-18 mm
P. aeruginosa	10-12 mm	12-15 mm	15-20 mm	10-15 mm	8-10 mm	10-12 mm
K. pneumoniae	12-15 mm	14-18 mm	11-18mm	12-16 mm	10-14 mm	12-16 mm
C. albicans	18-22 mm	20-25 mm	7 mm	4 mm	12-16 mm	15-20 mm

Ethanolic extracts enhance the solubility of bioactive compounds, making them more effective against Gram-positive bacteria *(S. aureus*). These extracts contain phenolic compounds, flavonoids, and essential oils that can disrupt bacterial membranes. The results obtained aligned with the findings of Al-Qaysi and Al Tulaibawi [21], who discovered that an ethanolic extract of *Salvia officinalis* had potent antibacterial action against Gram-positive. Furthermore, Bensebia *et al*., [22] and Ghareeb *et al*., [23] observed that extracts exhibited greater efficacy against Gram-positive bacteria in comparison to Gram-negative bacteria. Figure 4 shows that *E. coli* is more susceptible to Anise extracts (aqueous and ethanolic) than other pathogenic microorganisms. This result was in agreement with the findings of Satish *et al*., [24]. The ethanolic composition of the extract could play a role in their high activity against the tested isolates compared to the aqueous extract. The hydroxyl group present in the composition makes it possible for this extract to enter the cell and permeate the cytoplasmic membrane, which leads to metabolic disorders [17]. Plant extracts can inhibit bacterial development by penetrating bacterial cell walls or affecting essential bacterial cell components like cytoplasm, ribosomes, or DNA. This is achieved by creating hydrogen bonds with proteins, which demolish the bacterial cell's protein structure and stop the growth of the bacteria. It is also because the gram stain's negative bacteria lack the peptidoglycan layer [25]. This study suggests that potentially helpful plant extracts can be utilized as natural preservatives to preserve food without posing a health risk to humans and to prevent food poisoning illnesses.

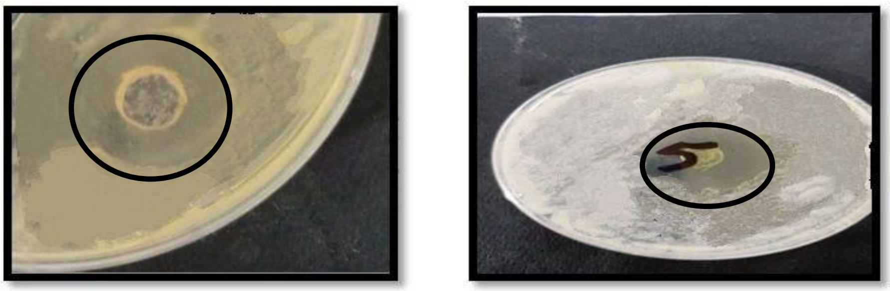
M. chamomilla *S. officinalis*

Figure 1: Inhibition zone of *S. aureus* caused by aqueous extracts of *M. chamomilla*(A) and *S. officinalis* (B).



M. chamomilla *S. officinalis*

Figure 2: Inhibition zone of *S. aureus* caused by ethanolic plant extracts of *M. chamomilla*(A) and *S. officinalis* (B).



M. chamomilla *S. officinalis*

Figure 3: Inhibition zone of pathogenic fungus (*C. albicans*) caused by ethanolic extracts of *M. chamomilla* and *S. officinalis*.

Figure 4: Antibacterial activity of aqueous and ethanolic plant extracts of *P. anisum* against pathogenic microorganisms which picture belong to *S. aureus*, *P.aeroginosa, E. coli K. Pneumoniae* and *C. albicans* microorganisms *1 Aqueous extract *2 Alcoholic extract

5. Conclusions

The ethanolic extracts demonstrated strong inhibitory effects against microorganisms, particularly *S. aureus* and *E. coli*. This suggests that the active compounds in these extracts may possess antimicrobial properties, making them potential alternatives to conventional antibiotics. Further studies are needed to explore their in vivo efficacy to assess their effects on living organisms, determine appropriate dosages, and ensure safety. These findings could contribute to developing of new natural treatments for bacterial infections, highlighting the need for more research on mechanisms of action and long-term effects.

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Conflict of interest.

The authors have not revealed any conflicts of interest.

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