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Evaluation of the Antibacterial and Synergistic Activities of Aqueous and Alcoholic Extracts of Chamomile (*Matricaria chamomilla* L.) Against Selected Pathogenic Bacterial Strains

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ABSTRACT

The escalating global health crisis posed by antibiotic-resistant bacteria has prompted researchers to seek effective natural alternatives to combat these pathogens. Chamomile, a medicinal plant with recognized antibacterial properties, has emerged as a promising candidate. This study aimed to evaluate the impact of chamomile extracts (aqueous and alcoholic) on the growth of select pathogenic bacteria and investigate their synergistic effect with antibiotics on the susceptibility of these bacterial strains**.** The antibacterial activity of aqueous and alcoholic chamomile extracts was assessed using the disc diffusion method against six pathogenic bacteria: Escherichia coli, Salmonella Typhimurium, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Enterobacter cloacae. The synergistic effect of combining these extracts with antibiotics on the efficacy of the latter was also examined. Chemical analysis of the extracts was conducted to identify their active constituents. The aqueous extract exhibited no antibacterial activity, while the alcoholic extract demonstrated efficacy against Staphylococcus aureus. When combined with certain antibiotics, both aqueous and alcoholic extracts enhanced the susceptibility of most bacteria to the tested antibiotics, with the exception of Klebsiella pneumoniae, which exhibited increased resistance to the antibiotic tetracycline. Chemical analysis of the alcoholic extract revealed the presence of active compounds (alkaloids, saponins, glycosides, phenols) that could potentially account for its antibacterial effect. Chamomile exhibits an inhibitory effect on certain bacterial strains and demonstrates a synergistic effect with some antibiotics.

تقييم الفعالية المستضدية والتآزرية مع المضادات الحيوية لمستخلصات نبات البابونج (Matricaria chamomilla L.)ضد بعض السلالات البكتيرية المسببة للأمراض

أحمد صالح ضو[1]، آمنة علي الفتحي المجدوب[1]، مريم بشر فضل[1]، هند محمد خلف الله[2]

تعد مقاومة البكتيريا للمضادات الحيوية مشكلة صحية عالمية متنامية. تسعى الدراسات إلى إيجاد بدائل طبيعية فعالة لمكافحة هذه البكتيريا. يُعد نبات البابونج من النباتات الطبية ذات الخصائص المضادة للبكتيريا. هدفت هذه الدراسة إلى تقييم تأثير مستخلص البابونج (المائي والكحولي) على نمو بعض البكتيريا المسببة للأمراض، واختبار تأثيره التآزري مع للمضادات الحيوية على حساسية الأنواع البكتيريا المدروسة. حيث تم اختبار فعالية المستخلصات المائية والكحولية للنبات باستخدام طريقة القرص المشبع على ستة أنواع من البكتيريا الممرضة: الإشريكية القولونية، السالمونيلا، الزائفة، الكليبسيلا، المكورات العنقودية الذهبية، المتقلبة. كما تم اختبار تأثير خلط المستخلصات المائية والكحولية مع المضادات الحيوية على فعالية هذه المضادات أيضا تم تحليل المكونات الكيميائية للمستخلصات لتحديد المواد الفعالة هذا النبات. أظهرت النتائج ان المستخلص المائي أي تأثير مضاد للبكتيريا، بينما أظهر المستخلص الكحولي فعالية

ضد المكورات العنقودية. أظهر المستخلص المائي والكحولي عند خلطها مع بعض المضادات الحيوية زيادة في حساسية معظم البكتيريا للمضادات الحيوية المختلفة، باستثناء Klebsiella التي أظهرت زيادة في المقاومة للمضادات الحيوية TE أظهر تحليل المكونات الكيميائية للمستخلص الكحولي وجود مواد فعالة (قلويدات، صابونين، جليكوسيدات، فينولات) قد تكون مسؤولة عن تأثيره المضاد للبكتيريا. الاستنتاج: لنبات البابونج تأثيرًا مثبطًا على بعض أنواع البكتيريا، بالإضافة إلى تأثير التآزري مع بعض المضادات الحيوية .

INTRODUCTION

Medicinal and aromatic plants are among the most important plants to man since ancient times.(Al-Hamali, 2022). Undoubtedly, the Libyans through successive eras depended on plants for food, fuel, fiber, construction, and folk medicine(El-Mokasabi, 2014).

Chamomile (Matricaria chamomilla L.) is a widely used herb in traditional medicine. It brings great economic value due to its numerous pharmacological effects and traditional uses(Dai et al., 2023). M. chamomilla is used as tonic of the appetite before the meal, to facilitate digestion after the meals, to fight against the aerophagia, flatulence. It is also useful to calm the headaches, the various pains, the aches, the tooth aches, to facilitate the menstruation and to relieve the pains of the rules(Abdoul-Latif et al., 2011). An annual or perennial plant in the Asteraceae family is chamomile. (Rehmat et al., 2020). Growing up to 30 inches (76 cm) in height, chamomile is an herbaceous annual plant with ferny, fragrant leaves. It has heterogamous (having multiple sexes) inflorescences with tubular yellow disc florets surrounded by white ray flowers. (Rizwana et al., 2016).Chamomile is not fastidious of soil types but thrives best on a well-drained, sandy or sandy-loam soils and tolerates pH from 4.8 to 8.5. It will also grow on clayey lime soils as it has a great tolerance to soil alkalinity (Lim, 2013). It grows both wild and cultivated in Southern and Eastern Europe healing , North Africa, West Asia, North and South America and Australia(Rehmat et al., 2020, Aleksieiev et al., 2022).

Several studies demonstrate the therapeutic effect of this plant on a several diseases, including nervous diseases, reproductive diseases, diabetes, obesity and related metabolic disorders, cardiovascular diseases, gastrointestinal diseases, allergies, skin diseases, eye diseases, and mouth problems. The plant also allowed pain-relieving, and wound and acted as a protective agent for the kidney and liver, gastrointestinal, and reproductive systems(El Mihyaoui et al., 2022, Sharifi-Rad et al., 2018).

M. chamomilla has been displayed to have strong antibacterial potential opposed to Gram-positive and Gram-negative bacteria(Kameri et al., 2023, Ismail et al., 2013). The biological activity of chamomile is mainly due to the flavonoids apigenin, luteolin, quercetin, patulin, and essential oil constituents such as α-bisabolol and its oxides and azulenes.(Sharafzadeh and Alizadeh, 2011, Sotiropoulou et al., 2020).Antimicrobial impedance become the biggest health risk in many places in the world, which damages human health and increases the mortality rate and disease risk associated with major, life-threatening conditions (Sofy et al., 2020). The recent emergence of bacterial strains with reduced a ntibiotic susceptibility raises the specter of incurable ba cterial infections and adds urgency to the search for new infection control strategies.(Muhaisen et al., 2016). Recently much attention has been paid to extracts and biologically active compounds isolated from plant species in herbal medicine. Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials needs to occur(Bakkiyaraj and Pandiyaraj, 2011).The antibacterial and antifungal properties of plants against multi-drug resistant bacteria are therefore being studied by numerous specialists from various nations in a contemporary manner(Sofy et al., 2020).

This study aimed to evaluate the impact of chamomile extracts (aqueous and alcoholic) on the growth of select pathogenic bacteria and investigate their synergistic effect with antibiotics on the susceptibility of these bacterial strains

MATERIALS AND METHODS:

A dried chamomile plant sample was collected from commercial markets in the Wadi Al-Shati area, ground, and placed in clean, sterile glass containers until the extracts were prepared in the laboratory

Specific chemical detection test for the active substances in the aqueous and alcoholic extract of the chamomile plant

The presence of tannins was detected using a 1% lead acetate solution and a 1% ferric chloride solution according to the method(Al-Dalali and Al-Hakim, 1987), and glycosides were detected using Fehling's reagent and Benedict's reagent, and resins, saponins, and flavonoids were detected. by the method of(Ayoola et al., 2008), and also detection of phenols according to the method of(Harborne, 1984).

Bacterial strains

Clinical bacterial isolates were obtained from the Microbiology Laboratory of the Department of Medical Laboratories, College of Medical Technology, Wadi Al-Shati University, the isolates included the following types:

- Staphylococcus aureus
- Escherichia coli
- Klebsiella sp.
- Pseudomonas sp.
- Salmonella sp.
- Protus sp.

The bacteria were activated by culturing them in nutrient broth and then subculturing them on nutrient agar**.**

Preparation of aqueous extract of chamomile plant

500 millilitres of sterile distilled water were combined with 100 grams of ground chamomile plant powder, stirred, and allowed to sit for a full day before being filtered through sterile Whatman No. 1 filter paper. The filtrate was collected and placed in sterile glass Petri dishes and placed in a drying oven at 40 degrees. Celsius for a period of 24 to 48 hours until it dries. It is then scraped off with a sterile scalpel and the resulting product is stored in the refrigerator until use (Khan et al., 2013)

Preparing the alcoholic extract of the chamomile plant

100 grams of ground chamomile plant powder was dissolved by adding 500 ml of 75% ethyl alcohol, soaked in a 1000 ml opaque glass beaker for 24 hours at room condition, and filtered applying Whatman No.1 filter paper. The supernatant filtrate was collected and placed In sterile glass Petri dishes and placed in a drying oven at a temperature of 40 degrees Celsius for 24 to 48 hours, until it dries, it is scraped off with a sterile scalpel and the resulting product is stored in the refrigerator until use (Khan et al., 2013).

Antibacterial Activity Assay:

The antibacterial activity of aqueous and alcoholic chamomile extracts was evaluated using the disc diffusion method. Turbidities of the bacterial suspension was adjusted to match the 0.5 McFarland standard, confirming a consistent bacterial concentration across all test plates. The Mueller-Hinton agar plates were then uniformly covered with this standardized suspension. The agar surface was covered with sterile paper discs (5 mm in diameter) that had been saturated with each extract at concentrations of 5, 10, 20, and 40 mg/ml. (All treatments were repeated three times). The dishes were incubated at 37°C for 24 hours.

For the antibiotic assay, a similar disc diffusion method was employed. However, instead of chamomile extracts, antibiotic tablets were placed directly on the agar surface after the bacterial suspension was spread. All treatments were repeated three times. The plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition around each disc were measured and recorded in millimetres (mm). The size of these zones reflects the extent of bacterial growth inhibition by the chamomile extracts at different concentrations or by antibiotics (Harley, 2008).

Testing the Synergistic Effect of Chamomile Extracts with Antibiotics

A uniform layer of bacteria is spread onto the superficial of Muller Hinton Agar plates using a sterile cotton swab. Prepared antibiotic discs are then saturated with 20 microliters of chamomile extract using semi-automatic pipettes. These extract-soaked discs are placed onto the agar plates teeming with bacteria. After that, the dishes are incubated at 37°C for 18 to 24 hours.

After incubation, the diameter of the inhibition zone (th e area where there is no bacterial growth) around each disk was measured. This zone represents the area where the chamomile extract inhibited bacterial growth. Finally, the size of the chamomile extract zone of inhibition is compared to the zone produced by the unsaturated discs (containing only antibiotics, not chamomile extract) (Harley, 2008) .

Statistical analysis of the data was performed using the Social Sciences Statistical Package (SPSS, version 27). A one-way ANOVA test was attend to assess the antibacterial activity of different extract concentrations compared to the activity of specific antimicrobials. Post hoc tests were then performed to identify which groups differed significantly from each other.

Table (1) List of antibiotic discs containing different concentration that was used in the antibiotic sensitivity test.

Antibiotics	symbol	Concentration
Ampicillin	(AMl)	10µg /disk
Erythromycin	(E)	15µg /disk
Ceftazidime	(CAZ)	**30** µg /disk
Chloramphenicol	(C)	30 µg /disk
Tetracycline	(Te)	10µg /disk
Ceftriaxone	(CRO)	30µg /disk
Nitrofurantion	(F)	300 µg /disk
Amikacin	(AK)	30 µg /disk
Aztreonam	(ATM)	30 µg /disk

RESULTS

The aqueous extract of Chamomile aurea leaves did not show any inhibitory effect on any of the bacterial species studied at any of the concentrations used.

Table (2) Antimicrobial activity of chamomile water extract against tested organisms.

No		Zone of inhibition (mm)			
		5%	10%	20%	40%
1	S. aureus	0	0	0	0
2	Salmonella sp.	0	0	0	0
3	E. coli	0	0	0	0
4	Pseudomonas sp.	0	0	0	0
5	Klebsiella sp.	0	0	0	0
6	Proteus sp.	0	0	0	0

An alcohol extract shows a significant inhibitory effect against S. aureus bacteria at concentrations of 5% and this concentration, the extract completely suppresses visible bacterial growth.40%. The minimum inhibitory concentration (MIC) for S. aureus is 5% alcohol extract. This means that at The alcohol extract has no inhibitory effect on other bacterial species tested in the study (Table 3).

The information in (Table 4) displayed a significant increase ($P < 0.05$) in the sensitivity of S. aureus to E15, AML, and C30 antibiotics at all tested concentrations (5%, 10%, and 40%) compared to the control (antibiotic alone). Similarly, Salmonella displayed increased sensitivity to the E15 antibiotic at 40% concentration ($P < 0.05$) and to the CRO antibiotic at 5% concentration ($P < 0.05$) compared to the control. E. coli also exhibited increased sensitivity ($P < 0.05$) to both TE and F300 antibiotics at all tested concentrations (5%, 10%, 20%, and 40%). Pseudomonas showed a similar trend with increased sensitivity to the CRO antibiotic at all tested concentrations ($P < 0.05$).

Table (3) Antimicrobial activity of Alcoholic extract of Chamomile against test organism

No.		Zone of inhibition (mm)			
		5%	10%	20 %	40 %
1	S. aureus	9*	0	0	13*
2	Salmonella sp.	0	0	0	0
3	E. coli	0	0	0	0
4	Pseudomonas sp.	0	0	0	0
5	Klebsiella sp.	0	0	0	0
6	Proteus sp.	0	0	0	0

Interestingly, Klebsiella results differed from the other bacteria. It showed an inverse effect on the TE antibiotic, where its resistance increased gradually with all tested concentrations ($P < 0.05$). However, its sensitivity increased to the CRO antibiotic at 5% concentration ($P < 0.05$). Proteus, on the other hand, displayed no sensitivity to any of the antibiotics impregnated with the aqueous extract at any tested concentration

Table (4) showing the sensitivity of bacteria to antibiotics saturated with aqueous extract

No.	Bacteria Type			inhibition Zone diameter (mm)			
1	Staphylococcus aureus	Antibiotic type		TE	AML	E15	C30
				38	0	8	29
		Extracts conc.	5%	30	11	37	42*
			10%	38	15	12	44*
			20%	38	10	8	39*
			40%	39*	22*	37	29
2	Salmonella sp.	Antibiotic type					
				14	12	0	2
		Extracts conc.	5%	15	0	0	13*
			10%	15	0	0	11
			20%	15	0	0	12
			40%	16*	0	0	11
3	E. coli	Antibiotic type					
				14	0	17	7

		Extracts conc.	5%	23*	0	0	7*
			10%	23*	0	0	7
			20%	23*	0	0	7
			40%	24*	0	0	7
4	Pseudomonas sp.	Antibiotic type					
				0	0	0	0
		Extracts conc.	5%	7*	0	0	0
			10%	9*	0	0	0
			20%	7*	0	0	0
			40%	2*	0	0	0
5	Klebsiella sp.	Antibiotic type					
				20	10	0	6
		Extracts conc.	5%	17*	13*	0	0
			10%	17*	11	0	0
			20%	13*	11	0	0
			40%	9*	9	0	0
6	Proteus sp.	Antibiotic type					
				8	4	0	0
		Extracts conc.	5%	13	9	0	0
			10%	13	13	0	0
			20%	14	11	0	0
			40%	13	11	0	0

Table (5) Table showing the sensitivity of bacteria to antibiotics saturated with alcoholic extract

No.	Bacteria Type			inhibition Zone diameter (mm)			
1	Staphylococcus aureus	Antibiotic type		TE	AML	E15	C30
				38	0	8	29
		Extracts conc.	5%	31	11	31*	7*
			10%	33	9	33*	31
			20%	8*	15	8	3
			40%	8*	17*	8	31
2	Salmonella sp.	Antibiotic type					
				14	12	0	2
		Extracts conc.	5%	15	0	0	11*
			10%	17*	0	0	6
			20%	19*	0	0	9
			40%	15	0	0	7
3	E. coli	Antibiotic type					
				14	0	17	7
		Extracts conc.	5%	19*	0	23*	7
			10%	17*	0	23*	23*
			20%	21*	0	7	23*

			40%	17*	0	23*	23*
4	Pseudomonas sp.	Antibiotic type					
				0	0	0	0
		Extracts conc.	5%	7*	0	0	0
			10%	6*	0	0	7*
			20%	6*	0	0	6*
			40%	7*	0	0	0
5	Klebsiella sp.	Antibiotic type					
				20	10	0	6
		Extracts conc.	5%	13*	11	0	0
			10%	7*	9	0	7
			20%	11*	11	0	6
			40%	15*	11	0	0
6	Proteus sp.	Antibiotic type					
				8	4	0	0
		Extracts conc.	5%	13	9	0	0
			10%	13	9	0	0
			20%	13	9	0	0
			40%	13	9	0	0

The results(table5) revealed a significant increase ($P < 0.05$) in the sensitivity of S. aureus to AML antibiotic at 40% concentration compared to the antibiotic alone. This bacterium also showed increased sensitivity ($P < 0.05$) to C30 antibiotic at all concentrations except 40%. Salmonella sensitivity increased significantly ($P < 0.05$) to TE antibiotic at 20% concentration compared to the control (antibiotic alone). Similarly, Pseudomonas and E. coli exhibited significant increases ($P < 0.05$) in sensitivity to TE antibiotic at all concentrations compared to the control (antibiotic alone).

Interestingly, Klebsiella showed an inverse effect on TM antibiotic, where its resistance increased with increasing concentration ($P < 0.05$) compared the control (antibiotic alone).. Proteus displayed no sensitivity to any of the alcohol extract-impregnated antibiotics at all concentrations

DISCUSSION

Chamomile is an herb used for many medicinal purposes and has multiple biological activities, as its extracts have displayed antioxidant activity and anti-growth activity in some bacterial species (Mailänder et al., 2022)

In this study, the ethanolic chamomile extract showed antibiotic activity oppose a Gram-positive isolate, while the Gram-negative species resisted it. This is consistent with the findings of Rizwana et al., as the negative isolates showed resistance to the three extracts they used (ethanol, methanol, and chloroform), while the positive isolates were sensitive and showed a large zone of inhibition. This effect is due to phenolic compounds, flavonoids, and their derivatives with antioxidant and antimicrobial properties. They have an antimicrobial effect by affecting cell membrane permeability, DNA metabolism, inhibiting adhesion, and forming cell membranes. They also work to inhibit hydrolytic enzymes and transport proteins in the cell envelope. As for resistance to Gram-negative species, this may be due to the structure of its cell wall, as it has a complex and solid membrane rich in polysaccharides, and this wall may work to obstruct the passage of the active inhibitory substances in the chamomile extract (Rizwana et al., 2016, Ahmad et al., 2020) .

On the other hand, the results of the current study do not agree with what some researchers have achieved, which is the observation of the inhibitory effect of chamomile extracts on gram negative and positive bacteria (Sah et al., 2022). Moreover, the outcomes obtained by Alidoust showed that aqueous and alcoholic extracts of chamomile in concentration have an inhibitory effect and that the ethanolic extract can prevent the growth of Klebsiella pneumonia bacteria in low concentrations (Azizi Alidoust et al., 2020). According to Azari et al., the chamomile leaf extract presented antibacterial activity

against P. aeruginosa isolates, While the flower extract showed better activity against methicillin-unaffected Staphylococcus aureus (MRSA) isolates(Ahani Azari and Danesh, 2021) . The antibacterial effect of the ethanolic extract was against S. Aureus, which is consistent with the outcomes of some studies. This inhibition was the highest concentration used in this study (Ismail et al., 2013, Azizi Alidoust et al., 2020) .These results agreed with those of Ismail et al., who recorded an inhibition diameter of 15 mm. Additionally, they reported that the aqueous extract had no inhibitory effect on any of the species included in this study, including Gram-positive and Gram-negative ones.

Chamomile leaf extracts were effective with ceftriaxone against S. aureus bacteria and with tetracycline against Salmonella, Pseudomonas, and E. coli bacteria. The extracts were reported to be effective with nitrofurantoin against E. coli bacteria, and these bacterial species are the causative agents of human diseases. This was demonstrated through the results recorded in This study is demonstrate resistance to some antibiotics used to treat infections caused by them, which indicates that these extracts contain compounds that can support the action of these antibiotics, which helps in reducing the therapeutic dose of these antibiotics.

In the same way, a study by Sah et al. showed that chamomile essential oil and its extracts in hexane, diethyl ether, and dichloromethane combined with antibiotics such as ampicillin, cefuroxime, tetracycline, fluconazole, and nystatin were effective against Staphylococcus aureus and Escherichia coli. The effect was more pronounced with tetracycline. These results may be due to the use of different parts of the chamomile extract in the study, which suggests that multiple components play together and exhibit antimicrobial properties. This study also showed that applying chamomile and tetracycline ointments to treat wounds that had been infected with the bacteria Pseudomonas aeruginosa showed a decrease in wound healing time. (5.3 days) compared to the antibiotic group (6.3 days)(Sah et al., 2022).

Preceding in vitro studies have shown a synergistic effect of combinations of pure compounds derived from different plants and antibiotics, and this synergism between plant-derived compounds and antibiotics allows antibiotics to be used synergistically with those derivatives when their efficiency as single agents is reduced.

CONCLUSIONS AND RECOMMENDATIONS

The results, it was shown that the plant has an inhibitory effect on some bacterial species, as well as a synergistic effect. It was also shown from the recorded data that it has an effect at low concentrations. However, more extensive studies must be conducted to learn more about the effect of the anti-flower plant against pathogenic bacterial species.

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