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Original article

Microbial Applications of Leaves and Stems of *Matricaria chamomila* Plant Growing at Al–Gabal Al–Khder region

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Abstract
This study was carried out on leaves and stems of the *Matricaria chamomila* plant, which growing at Al –gabal Al –Akhder region, Libya. Two different solvents were used in this study (Aqueous and Methanol). This study was conducted on the leaves and stems of Matricaria chamomilla. a plant that grows in Al-Gabal Althe Akhder region, Libya. Two different solvents were used in this study (Aqueous and Methanol), where both extracts were used for phytochemical screening and microbial investigation. The photochemical screening and paper chromatograph investigations were applied to the extracts of the studied plants. Also, the anti-microbial activity was carried out on the plant extracts against six types of different species of bacteria, including: (*Bacillus cereus, Streptococcus pneumonia, E. coli, Shigella vulgaris, and Pseudomonas aeruginosa*), besides two types of Fungi, including (*Alternaria alternate and Penicillium*). The photochemical screening for the leaf and stem extracts showed the presence of many natural organic compounds, such as Carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, and saponins. The results also showed variations of the detected compounds in leaves and stems of the studied plant. The paper chromatography analysis showed the presence of flavonoids/phenols and tannins in most of the leaves and stems of the studied plant. Also, the extracts were used for antibacterial and antifungal applications studies, showing different effects on the selected microbial and fungal species in this study, where some of the extracts gave inhibition zones compared with those that did not give any effect on the studied bacteria and fungi.
Keywords. Chamomile, Bioactive compounds, Antibacterial, Antifungal Activity.

Introduction

Matricaria chamomilla L. (German chamomile) is a widely recognized medicinal plant, valued for its rich content of bioactive phenolic compounds, including flavonoids, phenolic acids, and tannins, which contribute to its antioxidant, anti-inflammatory, and antimicrobial properties [1]. The extraction and characterization of these compounds from various plant parts, particularly leaves and stems, are crucial for understanding their therapeutic potential and for developing applications in pharmaceuticals, cosmetics, and agriculture [2]. Recent advances in extraction methodologies, such as supercritical fluid extraction, ultrasound-assisted extraction, and the use of natural deep eutectic solvents, have significantly improved the yield and selectivity of phenolic compounds from chamomile, while also supporting green chemistry principles [3]. Optimization of extraction parameters solvent type, temperature, and time, has been shown to influence the phenolic profile and biological activity of the resulting extracts [4]. The phenolic-rich extracts of M. chamomilla have demonstrated notable antimicrobial activity against a range of bacterial and fungal pathogens, supporting their use in microbial control and as natural preservatives. Furthermore, these extracts exhibit significant antioxidant and anti-inflammatory effects, which are closely linked to their phenolic content and composition [5]. Studies have also highlighted the variability in phenolic profiles and bioactivity depending on the plant part, extraction method, and geographical origin, underscoring the importance of comprehensive phytochemical analysis. Given the ecological and medicinal significance of M. chamomilla [6]. In Libya, the study of plant extracts and their antimicrobial investigations was carried out in many studies [7-53]. Additionally, the use of plants or other samples in various investigations, such as water and soil, is also employed [54-88]. The extracts demonstrated significant antioxidant, antibacterial, and anti-inflammatory properties, highlighting their therapeutic potential and emphasizing the importance of biodiversity conservation. The study aims to contribute to natural product discovery from under-investigated flora and promote sustainable extraction methodologies.

Methods

Plant Material Collection and Preparation

The plant material, consisting of aerial parts of *Matricaria chamomilla,* was collected during the peak flowering season in spring (2018) from various locations across the Al-Jabal Al-Akhdar region (Green Mountain) in northeastern Libya. This region is characterized by its Mediterranean climate with an altitude ranging from 500 to 800 meters above sea level. The collection sites were selected based on their minimal exposure to environmental pollutants and agricultural chemicals to ensure the purity of phytochemical constituents. The collected samples were identified in the Seliphium herbarium, Botany Department,

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Faculty of Science, Omar Al Mukhtar University. The collected plant materials were carefully cleaned with distilled water to remove dust and epiphytic materials, then shade-dried at room temperature (25°C ± 2°C) with adequate ventilation for 15-20 days. The dried plant materials were mechanically pulverized using a commercial grinder (MIZA TB200) to obtain a fine powder (particle size ≤ 0.5 mm), which was subsequently stored in airtight containers protected from light and moisture until further use [9-11].

Extraction Procedures

Based on polarity considerations for the extraction of phenolic compounds, two solvents with varying polarity were selected: methanol (polar) and distilled water (nonpolar). All solvents were of analytical grade (purity ≥ 99.5%) and obtained from the laboratories of Omar Al-Mukhtar University. They were used in their pure form, as previous studies have demonstrated the effectiveness of these solvents in extracting phenolic compounds with varying polarity [7].

Phytochemical Screening

Qualitative Phytochemical Analysis

Preliminary qualitative phytochemical screening was performed on all extracts to identify various classes of bioactive compounds using standard procedures as described by and with minor modifications. The detailed protocols for major phytochemical classes are summarized in (Table 1) [13-16].

Table 1. Qualitative phytochemical screening methods

Phytochemical Class	Test Method
Sterols and/or triterpines	Libermann-Burchard's test
Flavonoids	Alkaline reagent test
Alkaloids	Dragendorff test
Tannins	Ferric chloride test
carbohydrates and /or glycosides	Molish test
Cardiac glycosides	Keller-Killiani test- Kedde's test
Anthraquinones	Bornträger's test- Modified-Bornträger's test
Saponins	Froth test

Paper Chromatography Analysis*:*

All chemicals used were of laboratory grade.

Materials

Paper chromatography was performed using

a: Stationary Phase: Whatman No. 1 filter paper sheets.
b: Development Chamber: Standard glass chromatographic tanks.

Solvent Systems

The following solvent systems were selected for development (prepared in volume/volume ratios):
a: Acetic Acid: Water (15:85 v/v).
b: Methylene Chloride: Methanol: Water (60:35:5 v/v).
C: Benzene: Ethyl Acetate: Acetic Acid (12:4:0.5 v/v).

Detection Reagents (Spray Reagents)

Specific chemical reagents were used to detect classes of compounds post-chromatography:
a: Ferric Chloride (for Phenolic Compounds): A 1% solution in ethanol. Phenolic compounds yield a blue to green coloration.
b: Aluminum Chloride (for Flavonoids): A 1% solution in ethanol. Flavonoids are detected under UV light by a yellow fluorescent spot.
c: Potassium Hydroxide (for Anthraquinones and Phenolic Compounds): A 2% solution in ethanol. Target compounds produce a red or yellow color.

Microorganisms

The extracts were individually tested against pathogenic bacteria. The following bacteria were tested:

Bacterial strains

Gram-positive bacteria

Three species of Gram-positive (*Staphylococcus aureus, Bacillus cereus,* and *Streptococcus pneumoniae*) were used and obtained from the Department of Microbiology, El-Bayda Hospital.

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Gram-negative bacteria
Two species of Gram-negative (*Escherichia coli, Pseudomonas aeruginosa,* and *Shigella vulgaris*) were obtained from the Department of Microbiology at El-Bayda Hospital.

Fungi
The fungi selected in this study include: (*Alternaria alternata* and *Penicillium*). Obtained from the Department of microbiology of the Faculty of Veterinary Medicine, Omar Al- Mokhtar University, El Bayda, Libya.

The minimal inhibition concentration determination
The antimicrobial activity of the plant extracts was determined using the agar well diffusion method [106], where Mueller-Hinton (MH) agar plates were seeded with bacterial strain and potato Dextrose Agar (PDA) plates seeded with fungal strain. On each plate, wells were made by a sterile standard cork borer. Each well was filled with30µl of the different concentrations (0.8, 0.4, 0.2, 0.1, 0.01, 0.001, 0.0001, and 0.00001 g/ml) of the studied plant extracts, and the plates were then incubated for 24-48 h at 37°C for bacteria and 48-72 h at 28°C for fungi. The number of inhibition zones was measured, and the results are presented as the mean of triplicate. The minimal inhibition concentration (MIC) values were evaluated according to published procedures [105-107]. The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. MIC was determined by dilution of the plant extracts and pipetting 30µl of each dilution into wells, dilutions of the extracts within a concentration range of (0.8 - 0.00001 g/ml). MIC was defined as the lowest concentration that inhibited the visible microbial growth [8].

Results

Phytochemical screening studies
Both leaves and stems of *Matricaria chamomilla* have been screened for the presence of the following phytochemical groups: carbohydrates and/or glycosides, tannins, flavonoids, sterols and/or triterpenes, saponins, and anthraquinones. The results of the study, which are presented in (Table 2), reveal that the plant is a source of carbohydrates and/or glycosides, while cardiac glycosides and alkaloids are absent. The data also lead to the conclusion that the alcohol extracts contain more of the indicated compounds (tannins, carbohydrates/glycosides, flavonoids) than the water extracts. Moreover, the leaves of Matricaria chamomilla are found to have a higher relative carbohydrate and tannin content than the stems. Besides, the results of the phytochemical screening have manifested that the Matricaria chamomilla extracts (both alcoholic and aqueous) are a source of several steroids/triterpenes. At the same time, the anthraquinone group of compounds is absent in the leaf and stem extracts of the plant.

Table 2. Phytochemical screening of stems and leaves of the Chamomilla plant

Plant	Chamomila			
Chemical Test	Leafs		Stems	
	Al	aq	Al	aq
Saponins	_	_	_	+
Tannines	+++	+++	+	_
Carbohydrate and/or Glycosides	+++	++	+	+
Alkaloids	_	_	_	_
Flavonoids	++	_	++	+
Anthraquinones	_	_	_	_
Steroids and/or Triterpenoids	_	++	+	++
Cardiac Glycosides	_	_	_	_

(+)Present, Al (Alcoholic), Aq (Aqueous), (-) Absent, (/) Not done.

The main difference between the detected compounds and their types is that the solvents used (water and alcohol) were of different polarities. Moreover, the chemical compounds' structure significantly impacted their interaction with the solvents during extraction, which is the main reason for the difference between the organic compounds that are detected in some plant tissues compared to the studied plant content.

Paper chromatographic studies of the plants (leaves and stems)
The paper chromatography tests were planned based on the phytochemical screening data and results, which were extracted from the phytochemical analysis. In this case, two different solvents with different ratios were used to isolate the natural compounds from the studied plant. The paper chromatography analysis results revealed that: The most significant observation was the presence of tannins and flavonoids in the studied plant. Besides, the identification of these compounds has been done depending on Rf values and the colors of the spots after the treatments by using different types of solutions, such as ($FeCl_3$ and

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KOH). These results are shown in (Table 3). The results of paper chromatography of the studied plants can be described as follows: For the Chamomila leaves, the alcohol extract showed the presence of Flavonoides in the solvents which contain (Benzene- Ethyl acetate, Acetic acid, and Hexane- Acetone mixture). Additionally, for the Chamomila stems, the water and Methanol extracts show the presence of Flovonoides in the mixture solvents of (Benzene - Ethyl Acetate - Acetic acid and Hexane - Acetone mixture), (Table 3).

Table 3. Paper chromatographic investigation of alcohol and aqueous extracts of Chamomilla leaves and Stems

Solvent	Plant reagent	*Chamomilla*			
		Leafs		Stems	
		Al	Aq	Al	Aq
Benzene- Ethyl Acetate- Acetic acid (60:5:35)	FeCl3	green	_	_	_
	KOH	Yellow RF=0.52	Yellow RF=0.69	Yellow RF=0. 34	Yellow RF=0. 34
Hexan- Aceton (20:80)	FeCl3	green	_	_	_
	KOH	Yellow RF=0.52	Yellow RF=0.52	Yellow RF=0. 34	Yellow RF=0. 34

Based on the outcomes of the chromatographic paper research, the plant studied shows relative changes in its content of natural organic compounds. The pear green color or greenish blue that was observed can be related to the presence of flavonoids and phenols, and also the occurrence of a yellow color on the paper chromatography after the spots were treated with the visualization agents (KOH and $FeCl_3$) is an indication of the presence of tannins.

Antimicrobial activity

The antimicrobial activity studies were carried out on the aqueous and methanolic extracts for both leaves and stems of the studied plant against some species of bacteria and fungi. The results of antimicrobial tests are shown in the Tables, described as follows:

Gram-positive bacteria

Staphylococcus aureus

(Table 4) showed the effect of different concentrations of the studied plant extracts against *Staphylococcus aure*us. Where low concentrations (0.01 – 0.0001 g/ml) and high concentrations (0.1-0.8 g/ml) of the extracts were used. The results showed that the inhibition zone and MIC in alcohol extract for *Chamomile* leaves recorded at 0.8 g/ml, while the inhibition zone for the alcoholic extract for *Chamomilla Chamomilla* stems was at 0.2 g/ml. The results of all aqueous extracts of chamomile showed that the inhibition zone was at 0.1g/ml.

Table 4. Effect of methanol and aqueous Chamomilla extracts against Staphylococcus aureus.

Concentration	*Chamommila*			
	Methanol		Water	
	Leafs	Stems	Leafs	Stems
0.1g/ml	2	2	1	1
0.2/ml	1.7	1.5	N.A	N.A
0.4g/ml	1.5	N.A	N.A	N.A
0.8 g/ml	1	N.A	N.A	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.0001 g /ml	NA	NA	NA	NA

N.A: Non-activity

By applying different concentrations of the studied plant extracts against Bacillus cereus bacteria, the results in the (Table5) showed that the inhibition zone and MIC in the alcoholic extract of chamomile leaves were recorded at 0.8 g/ml, while the inhibition zone for the alcoholic extract of chamomile stems was 0.2 g/ml. The results of aqueous extracts of chamomile Leaves showed that the inhibition zone was 0.1 g/ml. As for the aqueous extract of chamomile stems, the inhibition zone was at 0.4g/ml.

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Table 5. Effect of methanol and water Chamomilla extracts against Bacillus cereus

Concentration	*Chamomilla Extracts*			
	Methanol		**Water**	
	Leaves	**Stems**	**Leaves**	**Stems**
0.1g/ml	2	1	1.5	1.5
0.2/ml	1.5	0.9	N.A	N.A
0.4g/ml	1	N.A	N.A	1
0.8 g/ml	1	N.A	N.A	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

N.A: Non-activity

(Table 6) showed the effect of different concentrations of the studied plant extracts against *Streptococcus pneumonia*. The results showed that the inhibition zone and MIC in all alcoholic extracts were recorded at 0.2 g/ml, while for all aqueous extracts, the inhibition zone was recorded at 0.1g/ml.

Table 6. Antimicrobial activities of different concentrations of *Chamomilla* plant extracts against *Streptococcus pneumoniae*

Concentration	***Chamomilla* Extracts**			
	Methanol		**Water**	
	Leafs	**Stems**	**Leafs**	**Stems**
0.1g/ml	1.5	1.5	1	1
0.2/ml	1	1	NA	NA
0.4g/ml	N.A	N.A	N.A	N.A
0.8 g/ml	N.A	N.A	N.A	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
0.0001 g/ml	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

N.A: Non activity

Gram-negative bacteria

Escherichia coli

(Table 7) Give the effect of different concentrations of the studied plant extracts against Escherichia coli. The results showed that the inhibition zone and MIC in all extracts were recorded at 0.2 g/ml, except for *Chamomilla Chamomilla* leaves in alcohol solvent, where the inhibition zone was recorded at 0.4g/ml.

Table 7. Antimicrobial activities of different concentrations of the Chamomilla extracts against Escherichia coli

Concentration	***Chamomilla* Extracts**			
	Methanol		**Water**	
	Leafs	**Stems**	**Leafs**	**Stems**
0.1g/ml	1.5	1.5	1.5	1
0.2/ml	1	1	1	0.8
0.4g/ml	1	N.A	N.A	N.A
0.8 g/ml	N.A	N.A	N.A	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
0.0001 g/ml	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

N.A: Non-activity

Pseudomonas aeruginosa

(Table 8) showed the effect of different concentrations of the studied plant extract against *Pseudomonas*. The results showed that the inhibition zone and MIC in alcohol extract for *Chamomile* leaves recorded at 0.8 g/ml, while the inhibition zone for the alcoholic extract for *Chamomilla Chamomilla* stems was at 0.2g/ml. The results of aqueous extracts of chamomile leaves showed that the inhibition zone was 0.2 g/ml. As for the aqueous extract of chamomile stems, the inhibition zone was at 0.4g/ml.

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Table 8. Antimicrobial activities of different concentrations of Chamomilla extracts against Pseudomonas aeruginosa

Concentration	*Chamomilla* Extracts			
	Methanol		Water	
	Leafs	Stems	Leafs	Stems
0.1g/ml	2	2	1	1.5
0.2/ml	2	1.5	1	1
0.4g/ml	1.5	NA	NA	1
0.8 g/ml	1	N.A	N.A	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

N.A: Non-activity

Shigla vulgaris

(Table 9) The effect of different concentrations of the studied plant extract against Shigella vulgaris was tested. The results showed that the inhibition zone and MIC in alcohol extract for *Chamomilla leaves* were recorded at 0.8 g/ml, while the inhibition zone for the alcoholic extract for *Chamomile* stems was at 0.2g/ml. The results of aqueous extracts of chamomile leaves showed that the inhibition zone was 0.2 g/ml. As for the aqueous extract of chamomile stems, the inhibition zone was at 0.1g/ml.

Table 9. Antimicrobial activities of different concentrations of Chaomomilla extracts against Shigella vulgaris

Concentration	*Chamomilla* Extracts			
	Methanol		Water	
	Leafs	Stems	Leafs	Stems
0.1g/ml	2.5	1	1	1
0.2/ml	2.5	0.5	1	NA
0.4g/ml	2.5	NA	NA	NA
0.8 g/ml	1	N.A	N.A	N.A
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
0.0001 g/ml	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

Antifungal activity

Alternaria alternate

Tables 10 showed different concentrations of the studied extract against *Alternaria alternata*. The results showed that the inhibition zone and MIC in alcohol extract for *Chamomile* leaves were recorded at 0.4 g/ml, while the inhibition zone for the alcoholic extract for *Chamomilla* stems was at 0.2g/ml. For all aqueous extracts, the inhibition zone was recorded at 0.8g/ml.

Table 10. Antifungal activities of different concentrations of the Chamomilla extract against Alternaria alternata

Concentration	*Chamomilla* Extracts			
	Methanol		Water	
	Leafs	Stems	Leafs	Stems
0.1g/ml	1.5	1.5	2	2
0.2/ml	1.5	1	2	1.5
0.4g/ml	1	NA	1.5	1.5
0.8 g/ml	N.A	N.A	1	1
0.01 g/ml	N.A	N.A	N.A	N.A
0.001g/ml	N.A	N.A	N.A	N.A
g/ml 0.0001	N.A	N.A	N.A	N.A
0.00001 g /ml	N.A	N.A	N.A	N.A

N.A: Non-activity

Pensilienium

(Table 11) showed the effect of different concentrations of the studied plant extract against *Pensilienium*. The results showed that the inhibition zone and MIC at 0.00001 g/ml for alcoholic and alcohol extracts of *Chamomilla* leaves, while the inhibition zone for the alcoholic extract of *Chamomilla* stems was at 0.01g/ml. As for the aqueous extract of chamomile stems, the inhibition zone was at 0.2g/ml.

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Table 11. Antifungal activities of different concentrations of Chamomilla extract against Pensilienium

Concentration	*Chamomilla* Extracts			
	Methanol		Water	
	Leafs	Stems	Leafs	Stems
0.1g/ml	2	2	2	2
0.2/ml	1.5	1.5	2	1.5
0.4g/ml	N.A	N.A	1	N.A
0.8 g/ml	N.A	N.A	1	N.A
0.01 g/ml	2	0.5	2	N.A
0.001g/ml	1.5	N.A	1.5	N.A
g/ml 0.0001	1	N.A	1	N.A
0.00001 g /ml	0.5	N.A	1	N.A

N.A: Non-activity

Discussion

The diameters of growth inhibition zones exhibited by different extracts of the studied plants against the selected bacterial strains. As can be noted from the results, the methanol extract showed significantly the highest antibacterial activity against all bacterial pathogens, with a maximum inhibition zone of 1- 2 mm and 0.9 – 2 mm against *Staphylococcus aureus* and *Bacillus cereus*, respectively. Also, the results showed relatively high activity at high concentrations (0.1 – 0.8 g / ml) of extracts compared to the low concentration of extracts (0.00001 – 0.01 g /ml). On the other hand, no significant results were recorded in aqueous extract, which was active only against *Streptococcus pneumonia* and *Escherichia coli* at high concentrations. In general, the gram-positive bacteria were found to have relatively more susceptibility as compared to the gram-negative bacteria species. This result may be explained by the variation in chemical composition and structure of the cell wall of both types of microorganisms [88-92]. Thus, it is clear that the effectiveness of the extracts largely depends on the type of solvent used. It can be speculated that the polarity of the solvents plays an important role in extracting some chemical compounds that have bacterial activity. This may be the likely explanation for significant differences in the bacteriostatic activity between the different compound extracts of the studied plants [92-95].

The different tested extracts reduced the colony diameters of the fungal strains. The results showed that the aqueous and methanolic extracts of *Chamomilla* plant showed antifungal activity against all strains tested. The results showed the relative high contents of extracts (0.1–0.8 g/ml) exhibit anti -fungal activity compared with low contents of (0.01- 0.00001 g/ml), Generally the used concentration showed low anti – fungal activity (0.5–3 g/ml), the mechanism of the action of these plant constituents is not yet fully known it is clear that the effectiveness of the extracts largely depends on the type of the used solvent. This observation clearly indicates the existence of non-polar residues in the extracts, which have higher antifungal abilities. The superior efficacy of methanol extraction compared to aqueous extraction for antifungal activity can be attributed to the higher extraction yield of a broader spectrum of antimicrobial compounds, including non-polar constituents, by organic solvents [96-98]. This difference can be attributed to the origin and the different chemical composition between the extracts.

Many studies have revealed a strong relationship between the specific chemical structure of phenolic compounds (such as the number of hydroxyl groups and the degree of polymerization) and their antimicrobial efficacy. Furthermore, the percent inhibition of pathogen growth by plant extracts was observed to vary in a concentration-dependent manner, with higher concentrations yielding greater suppression, as is consistently reported in the literature [99]. Therefore, the use of higher concentrations or other extraction methods in order to obtain a more potent effect against all strains could be rechecked.

Conclusion

This study was carried on the extracts of *Chamomile* plant grown at some Libyan regions, the results showed presence important natural compounds, also the antibacterial and antifungal results of this study presence antimicrobial activities against the microbial species selected in this, the study concluded that these activities are mainly due to presence the active compounds in the extracts, also there is effect of the concentrations of the plant extract on the activities on the studied microbial.

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Conflict

No conflict of the results of this study with other studies.

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