



Antibacterial Activity of Some Medicinal Plants against Some Bacteria Species and food spoilage bacteria

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ABSTRACT

In the current study, some plants were selected to screen their potential antibacterial activity. For evaluating antibacterial activity, both aqueous and organic solvents (methanol and acetone) were used. The tested plants were *Cymbopogon schoenanthus*, *Cotula cinerea*, and *Francoeuria crispa*. Agar well diffusion method was used to test the antibacterial activity against two Gram negative (*Brucella spp.*, *Proteus spp.*) and three Gram positive (*Staphylococcus albus*, *Streptococcus spp.*, *Bacillus subtilis*) bacterial species. All extracts were effective antibacterial agents, being capable of inhibiting the growth of each examined bacteria. Based on the findings of this study, methanol, acetone and aqueous extracts of the leaves of *C. schoenanthus*, *C. cinerea* and *F. crispa* showed various antibacterial efficiency towards various bacterial species. Among plant extracts, the strongest antibacterial activity was shown by acetone extract of *F. crispa* against *Streptococcus spp.*, with inhibition zone 27 ± 1 mm, *Bacillus subtilis* with inhibition zone of 26.27 ± 4 mm, followed by methanol against *Streptococcus spp.*, and *Brucella spp.* with inhibition zone of 24 ± 1 mm, 22 ± 1 mm respectively. *Francoeuria crispa* extracts exhibited the greatest antibacterial activity towards each of the evaluated bacteria, followed by *Cymbopogon schoenanthus* and *Cotula cinerea*. In comparison, methanol and acetone extracts showed pronounced inhibitory activity against both gram positive and gram negative bacteria. These findings demonstrate that certain plants may have antibacterial properties. This study suggests the possibility of applying *Cymbopogon schoenanthus*, *Cotula cinerea*, and *Francoeuria crispa* leaves as natural sources of antibacterial and may provide clues to clarify potential candidates for the future development of new chemotherapeutic drugs for the treatment of some infectious diseases.

الفعالية المضادة لبعض النباتات الطبية ضد بعض البكتيريا الممرضة والبكتيريا الملوثة للغذاء

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الكلمات المفتاحية:

الطب الشعبي الليبي
الفعالية المضادة للبكتيريا
المستخلصات النباتية الطبية
نبات الأدر
نبات شاي الجبل
نبات شاي الجمل

الملخص

في الدراسة الحالية ، تم اختيار بعض النباتات لفحص نشاطها المحتمل المضاد للبكتيريا. لتقييم النشاط المضاد للبكتيريا ، تم استخدام مذيبات مختلفة (الماء ، الميثانول ، الأسيتون). و قد كانت النباتات المختبرة هي الأدر و شاي الجبل و شاي الجبل. حيث تم استخدام طريقة انتشار الأجار لاختبار الفعالية المضادة للبكتيريا ضد نوعين من البكتيريا سالبة الجرام (*Proteus spp.*, *Brucella spp.*) وثلاثة أنواع من البكتيريا موجبة الجرام (*Bacillus subtilis*, *Streptococcus spp.*, *Staphylococcus albus*). و وجد أن جميع المستخلصات تمتلك فعالية مضادة للجراثيم ، حيث كانت قادرة على تثبيط نمو كل البكتيريا التي تم فحصها. بناءً على نتائج الدراسة ، أظهر الميثانول والأسيتون والمستخلصات المائية لأوراق لنباتات الأدر و

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شيحة الجمل و شاي الجبل كفاءة مختلفة ضد الأنواع البكتيرية المختبرة. و من بين المستخلصات النباتية، أظهر مستخلص الأسيثون لنبات شاي الجبل أقوى نشاط ضد بكتيريا *Streptococcus spp* ، بقطر منطقة تثبيط 27 ± 1 مم، *Bacillus subtilis* مع منطقة تثبيط 26.27 ± 4 مم ، يليه الميثانول ضد *Streptococcus spp* ، و *Brucella spp* بقطر منطقة تثبيط 24 ± 1 مم ، 22 ± 1 مم على التوالي. و قد أظهرت مستخلصات شاي الجبل أكبر نشاط مضاد للجراثيم تجاه كل من البكتيريا التي تم اختبارها ، تليها الأدر وشيحة الجمل. في المقابل ، أظهرت مستخلصات الميثانول والأسيثون نشاطاً مثبطاً واضحاً ضد كل من البكتيريا موجبة الجرام وسالبة الجرام ، بينما وجدت المستخلصات المائية ضعيفة ضد الكائنات الحية الدقيقة المختبرة. هذه النتائج توضح أن بعض النباتات قد يكون لها خصائص مضادة للجراثيم. تقترح هذه الدراسة إمكانية تطبيق أوراق هذه النباتات كمصادر طبيعية مضادة للبكتيريا و في التطوير المستقبلي لتصنيع مضادات حيوية جديدة لعلاج بعض الأمراض المعدية.

Introduction

Medicinal plants have been widely used in traditional medicine for centuries to treat many health-related conditions [1] and according to the World Health Organization (WHO), approximately 80% of people in the world currently depend on traditional medicine to meet their Primary health care needs [2] Undoubtedly, there is currently a clear and great interest in aromatic and medicinal plants as an important source of raw materials for the medical treatment of many diseases and as an alternative to the use of chemicals. This is mainly due to its high biological activity, being a safe, cheap and powerful natural antioxidant [3] Several researchers have reported that secondary metabolites are known to have many therapeutic activities against many diseases in human categories; therefore, traditional herbal medicines can be used to treat many different illnesses [4], [5] Other studies have also shown that medicinal plants can be used as antimicrobial agents for human pathogens [6], [7].

Few of the Libyan medicinal plants have been scientifically investigated for their antimicrobial activities. A previous study examined extracts from different parts of eight plant species commonly used in Libyan folk medicine and found that the majority of tested samples were able to inhibit the growth of bacteria [8]. The total alcoholic extracts prepared from the aerial parts of four Libyan plants showed a significant antibacterial activity against some tested Gram positive and Gram negative bacteria [9] In addition, other previous results revealed that crude methanolic extracts of the twenty three Libyan folk medicinal plants were particularly effective antibacterial agents, being capable of inhibiting the growth of all tested bacteria [10] All of plants assayed in this study are commonly used as medicinal plants in different localities of Libya and other parts of the world. Their medicinal properties are described in **Table 1**.

Table 1. Plants and their medicinal properties

Plant name (abbreviation) (Family)	Local name (in Arabic)	Traditional Medicinal Uses	Ref.
<i>Cymbopogon schoenanthus</i> L (<i>C. schoenanthus</i>) (Poaceae)	Edkar (أدكر)	Antispasmodic (especially children) menstrual pain and anti-rheumatic	[11]
<i>Cotula cinerea</i> L (<i>C. cinerea</i>) (Asteraceae)	Shehat algamal (شيحة الجمل)	Antiseptic, antipyretic, analgesic, anti-inflammatory, anti-rheumatic	[12]
<i>Francoeuria crispa</i> L (<i>F. crispa</i>) (Asteraceae)	Shay eljebel (شاي الجبل)	Anti-inflammatory, reduce influenza, relief cold symptoms.	[13, 14]

This is the first study to evaluate Libyan plants; *Cymbopogon schoenanthus* L (*C. schoenanthus*), *Cotula cinerea* L (*C. cinerea*) and *Francoeuria crispa* L (*F. crispa*). However, the literature survey revealed that limited works were undertaken on antibacterial activity of aromatic plants growing in Libya. Thus, in the relation to the data mentioned above, the aim of the current study is to evaluate the antibacterial potential of methanol, acetone and aqueous crude extract of leaves of these plants in order to validate some of their traditionally claimed therapeutic properties.

Materials and Methods

Plant collection and extracts preparation

Cymbopogon schoenanthus L, *Cotula cinerea* L and *Francoeuria crispa* L. were collected from Wadi Burjoj (Murzuq) in southwestern Libya, and identified according to the taxonomic characters at the Department of Botany, Faculty of Sciences Sebha University A voucher specimen of the plant was deposited at the herbarium of the department.

Extracts were prepared using the soaked method as previously described by [15]. A 100 g of the powder was mixed with 400 ml of 99.8% (v/v) methanol, acetone and water, and incubated at room temperature for 48 hours. The plant extracts were filtered with Whatman filter paper no. 1, and concentrated with a rotary vacuum evaporator at 40°C. Briefly, the crude extract was dissolved in 100% dimethylsulfoxide (DMSO) at a concentration of 100 mg/mL (10%), and the solution was then further diluted by (1:10 v/v) in distilled water to obtain 1 % stock solution. The final concentration of DMSO was 10% in the stock solution.

Bacterial strains

The antibacterial activity of *C. schoenanthus*, *Cotula cinerea*, and *F. crispa* extracts was tested using four pathogenic bacterial strains and one food spoilage bacteria. The pathogens included *Streptococcus spp.*, *Brucella spp.*, *Proteus spp.*, and *Staphylococcus albus*. Besides that, the food spoilage bacteria *Bacillus subtilis*.

Inoculums preparation

All bacterial species were obtained from the Microbiology Laboratory, Department of Botany, Faculty of Science, and Sebha University. Bacterial cultures were prepared as follows: all bacteria were prepared as recommended by CLSI standards: M02-A11. In brief, stock culture of bacteria was grown on MHA at 37°C for 12-24 hours [15]. Two to three colonies of bacteria were transferred into 1 mL of MHB by using sterile cotton swab and mixed with vortex for 15 minutes. After vortex, the bacterial suspension was grown at 37°C for 12-24 hours, then 10 µL of the suspension was transferred into 10 mL of MHB. The turbidity of inoculums were standardized to 10^5 - 10^8 CFU/mL before test by using standard broth microdilution method [15], [16]. Inoculum quantification was performed by plating 25 µL of bacteria suspension on MHA.

Antibacterial activity of plants extract (well diffusion method)

The antibacterial assay was performed by agar well diffusion method. The *C. schoenanthus*, *C. cinerea* and *F. crispa* extracts were tested for antibacterial activity using the well diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2003). The *Brucella spp*, *Proteus spp*, *Staphylococcus albus*, *Streptococcus spp.* and *Bacillus subtilis* were streaked on MHA plates. With a sterile cotton swab a well was prepared in the plates with the help of a cork-borer (0.85cm). Fifty microliter of the tested extracts was introduced into the well of the 10 mg/mL (w/v) 1%. Finally, the plates were incubated at 37°C for 24 hours. Presence of a clear zone indicated bacterial growth inhibition, and its diameter was measured in mm.

Statistical Analysis

Data were computed and analyzed by using Statistical Package for Social Sciences (SPSS) software (version 22) to check frequency, mean, and standard deviation. All samples were tested in triplicate.

Results and discussion

Table 2. Antibacterial activities of *C. schoenanthus*, *C. cinerea* and *F. crispa* extracts against *Brucella spp.*, *Proteus spp.*, *Staphylococcus albus*, *Streptococcus spp* and *Bacillus subtilis*

Plant	Extract mg/ml	<i>Bacillus subtilis</i>	<i>Brucella spp</i>	<i>Proteus spp</i>	<i>Staphylococcus albus</i>	<i>Streptococcus spp</i>
<i>C. schoenanthus</i> L	Methanol	8±0	7±0	13.67±1.15	7±0	24±1
	Acetone	19.33±0.58	7±0	7±0	7±0	20±0
	Water	7±0	7±0	7±0	7±0	7±0
<i>C. cinerea</i> L	Methanol	12.67±2.08	7±0	13.67±1.15	7±0	14±2.65
	Acetone	18.33±1.15	7±0	7±0	7±0	20±0
	Water	7±0	7±0	7±0	7±0	7±0
<i>F. crispa</i> L.	Methanol	18±2.65	22±1	7±0	7±0	24±1
	Acetone	26.27±4	7±0	7±0	7±0	27±1
	water	10.67±1.15	7±0	13±1	7±0	10.67±1.15

Values represent the mean and ± standard deviation (n=3), diameter of inhibition zones in mm

Antibacterial activity of *C. schoenanthus* L

As can clearly be seen from **Figure 1**, methanol, acetone and water extracts of *C. schoenanthus* exhibited different ranges of activity against tested bacteria. The methanol extract of *C. schoenanthus* being strongly active against *streptococcus spp* (24.0±1.0 mm), Fig (5.D), while it has lowest effect on *Brucella spp* (7.0±0 mm). Meanwhile, acetone extract of *C. schoenanthus* was being strongly active against *streptococcus spp* (20.0±0 mm), while it has a weak activity against *Brucella spp*, *Proteus* and *Staphylococcus albus* (7.0±0 mm). On the other hand, the aqueous extracts of *C. schoenanthus* showed a low activity with diameters of about 7.0 mm against all strains. Our findings are in line with those of [17] who reported that the aqueous and proanthocyanidin extracts of *C. schoenanthus* L exhibited antibacterial activity against *S. sobrinus*, a bacteria responsible for dental caries.

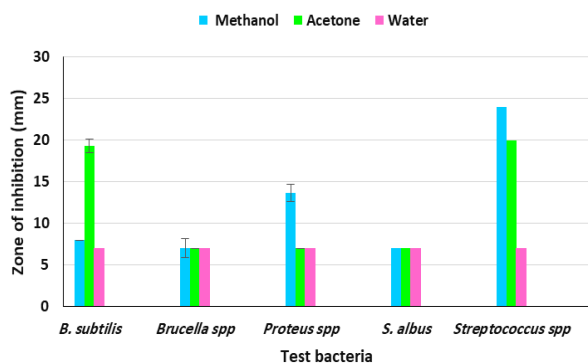


Fig. 1: Antibacterial activity of *C. schoenanthus*

Antibacterial activity of *C. cinerea*

The methanol, acetone, and aqueous extracts of *C. cinerea* L showed varying degrees of action against the tested bacteria, as shown in Figure 2. *Streptococcus spp.* are highly susceptible to the effects of the *C. cinerea* acetone extract (20.0 mm), while *Brucella spp.*, *Proteus*, and *Staphylococcus albus* are least susceptible (7.0 mm). *Streptococcus spp.*, *Proteus*, and *B. subtilis* all responded favorably to the methanol extract of *C. cinerea* (14.0±2.65; 13.67±1.15; 12.67±2.08), whereas *Brucella spp.* and *Staphylococcus albus* responded poorly (7.00 mm). The *C. cinerea* aqueous extracts, on the other hand, had modest activity against all strains, with diameters of about 7.0 millimeters.

Antibacterial activity of plants extracts

Extracts from *C. schoenanthus* *Cotula cinerea*, and *F. crispa* were tested for their ability to inhibit the growth of *Brucella spp.* *Proteus spp* *Staphylococcus albus* *Streptococcus spp* and *Bacillus subtilis*.

The results were listed in Table 2.

The antibacterial activity of *C. cinerea* is well studied by [18], [19] and a recent study has reported on the antibacterial activity of its extracts against *Enterococcus faecalis* [20].

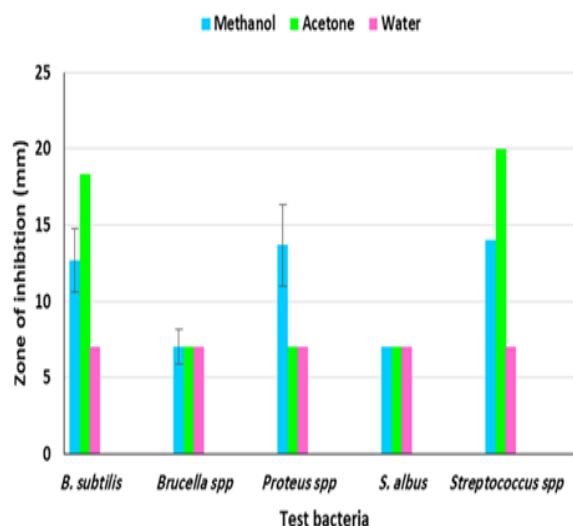


Fig. 2: Antibacterial activity of *C. cinerea* L.

Antibacterial activity of *F. crispa*

Figure 3 shows the antibacterial action of *F. crispa* extracts in methanol, acetone, and water against the tested bacteria. The acetone extract of *F. crispa* being strongly active against *Streptococcus spp* (27±1.0mm) Fig (5.C), while it has lowest effect on *Brucella spp*, *Proteus* and *Staphylococcus albus* (7.0±0 mm). Meanwhile, methanol extract of *F. crispa* was being strongly active against *Streptococcus spp* (24±1.0 mm) followed by *Brucella spp* (22±1.0mm) Fig (5.B) and *Bacillus subtilis* (18±2.65 mm) Fig (5.A), while it has a weak activity against *Proteus* and *Staphylococcus albus* (7.0±0 mm). On the other hand, the aqueous extract of *F. crispa* showed a good activity against *Proteus spp.* with diameters of about 13±1.0 mm followed by *Streptococcus spp.* and *B. subtilis* (10.67±1.15mm).

Our findings are agreed with a previous study which revealed that the methanolic, ethanolic and ethyl acetate extracts of *F. crispa* L leaves have inhibited the growth of different species of Gram-negative and Gram-positive bacteria [21].

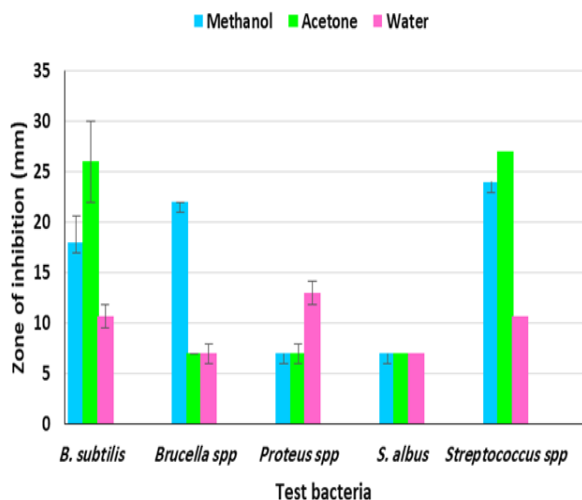


Fig. 3: Antibacterial activity of *F. crispa*

The Comparison of antibacterial activity of plant extracts in vitro

In this study, the methanol, acetone and aqueous extracts of *C. schoenanthus*, *C. cinerea* and *F. crispa* were tested for antibacterial activity against *Bacillus subtilis*, *Brucella spp*, *Proteus spp*, *Staphylococcus albus* and *Streptococcus spp*. The zone of inhibition was measured to evaluate antimicrobial activity. Well diffusion method was used to measure the zone of inhibition against tested bacteria. It is observed from present results that the extracts of *C. schoenanthus*, *C. cinerea* and *F. crispa* showed antibacterial activity against several types of bacteria. However, *Streptococcus spp* was found most susceptible among the tested strains followed by *Bacillus subtilis*, *Proteus*, *Brucella spp*, *Staphylococcus albus* respectively (Figure 4).

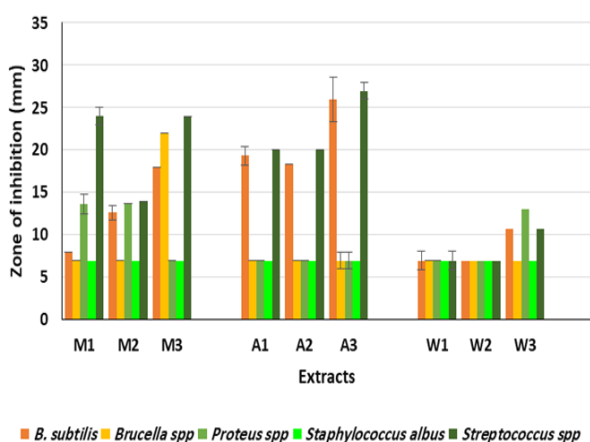


Fig. 4: Comparison of antibacterial activity of plants extracts

M1: Methanol extract of *C. schoenanthus* L; M2: Methanol extract of *C. cinerea* L; M3: Methanol extract of *F. crispa* L.; A1: Acetone extract of *C. schoenanthus* L; A2: Acetone extract of *C. cinerea* L; A3: Acetone extract of *F. crispa* L. W1: Aqueous extract of *C. schoenanthus* L; W2: Aqueous extract of *C. cinerea* L; W3: Aqueous extract of *F. crispa* L.

This study indicated that methanol, acetone and aqueous leaves extracts of *C. schoenanthus*, *C. cinerea* and *F. crispa* showed various antimicrobial effects against a variety of bacterial strains. It is not surprising that there are differences in the antibacterial effects of plant groups, due to the phytochemical differences between species [22].

The *F. crispa* extracts tested exhibited the greatest antibacterial activity towards the most susceptible bacteria, *Streptococcus spp* and *B. subtilis*. These extracts also displayed good antibacterial activity towards Gram-negative bacteria, particularly *Brucella spp* and *Proteus spp*. This is revealed by previous research which proves that plant extracts have a good capacity against Gram-negative microorganisms [23]. The antibacterial activity of extracts of *C.*

schoenanthus L, *Cotula cinerea* L and *Francoeuria crispa* L have been reported in several studies [13], [24], and [25].

Aqueous extracts showed the least antibacterial activity against all the tested strains. However, the maximum activity of aqueous extract was against *Proteus spp* followed by *Bacillus subtilis* and *Streptococcus spp*, while other tested bacteria showed less activity. Moreover, methanol extracts showed some antibacterial activity against *Streptococcus spp* followed by *Brucella*, *B. subtilis*, *Proteus spp*, and *Staphylococcus albus*, respectively. In addition, the acetone extracts showed activity against all the bacterial strains. Maximum activity was against *Streptococcus spp* followed by *B. subtilis*. Minimum activity was against *Brucella*, *Proteus spp*, and *Staphylococcus albus*.

The aqueous extract appears to have less antibacterial activity than acetone and methanol extracts. Interestingly, the traditional method of treating a bacterial infection is done by giving a decoction of the plant or part of the plant prepared by boiling it in water. However, according to our results, an organic solvent is preferable; so it can be more useful.

It was also observed that acetone and methanol extract showed highest inhibitory activity. This could be due to their high polarity and they allow extracting all the phenolic compounds [22]. In addition, this may be due to the type of solvent used for the extraction, as methanol is the most efficient solvent for extracting bioactive compounds, especially antimicrobial components from medicinal plants, compared to different solvents and water [26].

It is found from present results that active antibacterial bioactive compounds could be extracted by methanol and acetone extracts. Moreover, it is also found that many factors are known to influence antibacterial activity. Therefore, we believe that antibacterial activity may vary depending on the plant extract, the solvent used for the extract, and the organism being tested. Differences in susceptibility between Gram-positive and Gram-negative bacteria may be due to structural differences in the cell wall between these classes of bacteria. The outer membrane of the cell wall of Gram-negative bacteria appears to act as a barrier to many substances, including antimicrobial agents [27].

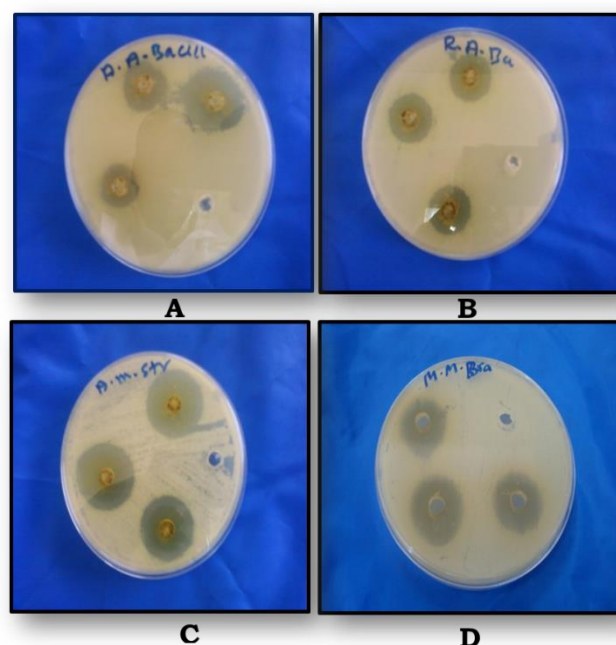


Fig. 5: Susceptibility of plant extracts on bacteria strains

A= Acetone extracts of *F. crispa* L against *B. subtilis*

B= Methanol extracts of *F. crispa* L against *Brucella spp*

C= Acetone extracts of *F. crispa* L against *Streptococcus spp*

D= Methanol extract of *C. schoenanthus* L against *Streptococcus spp*

Conclusion

In this study the selected plant extracts exhibit the potency of broad spectrum antibiotics which are active against both gram-positive and gram negative bacteria that were under study. These results support the use of these plants as traditional medicine and demonstrated the potential of these plants to exert beneficial antibacterial effect and could be used as antimicrobial agents in the search of new chemotherapeutic drugs for the treatment of infectious diseases. Further studies are needed to isolate active compounds responsible for antibacterial activity and to investigate in depth their modes of action.

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