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Evaluation of Antibacterial Activities of Some Egyptian Medicinal Plant Extracts

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Abstract: The antibacterial activity of hexane, ethanol and aqueous extracts of each of 5 different medicinal plants namely; galangal, cabbage, eucalyptus, elecampane and basil were assayed against the growth of seven pathogenic bacteria representing two Gram-positive bacteria and five Gram-negative bacteria by disc diffusion method. The results revealed that basil hexane extract had broad-spectrum activity against all tested bacteria followed by galangal, eucalyptus, elecampane, and cabbage. Both basil ethanol extract and eucalyptus aqueous extracts had prominent broad-spectrum activity against all tested bacteria and basil hexane extracts and galangal, cabbage, elecampane and basil ethanol extracts. Galangal, eucalyptus, elecampane and basil hexane extracts were chosen to undergo the minimum inhibitory concentration (MIC) determination and qualitative phytochemical screening. Both basil hexane extract and ethanol extract, compared to the other extracts, exhibited the best antibacterial activity and lowest MIC in concentration. Hence, they were chosen as the most potent antibacterial extracts for determining their chemical components using GC/MS analysis.

Keywords: Medicinal plants, antibacterial activity, hexane extracts, ethanol extracts, MIC, phytochemical screening, GC/MS analysis.

1 Introduction

The World Health Organization defined a medicinal plant as any herbal preparation produced by subjecting plant materials to extraction, fractionation, purification, concentration or other physical or biological process which may be produced for immediate consumption or as a basis for herbal products [88]. Phytochemicals often referred to as "secondary metabolities" chemical compounds formed during the plant normal metabolic processes, they were first described at the beginning of the 19th century [18]. The most important of these bioactive compounds of plants are alkaloids, flavanoids, quinones, phenolic compounds, saponins, tannins, coumarins, glycosides, gums, polysaccharides, terpenes and other chemical compounds [3, 25, 45, 63, 78].

Antibacterial activity is the ability of a substance to inhibit or kill bacterial cells [74]. The effectiveness of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections [72]. The multidrug resistant strains of many microorganisms have revealed exploration of alternative antimicrobial agent [79]. In general, bacteria have the

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genetic ability to transmit and acquire resistance to drugs [53].

Medicinal aromatic plants and their essences are rich in antibacterial compounds which could be an alternate way to combat bacterial diseases even against some bacteria which are becoming resistant to certain synthetic medicines [41, 75]. It is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections [15]. A number of reports concerning the antibacterial screening of medicinal plant extracts have been recorded in the literatures [77]. Past two decades, antibacterial properties of various plants and plant parts like root, stem, leaves, seeds and flowers have been well documented for some of the medicinal plants [2, 68, 69].

Some spices are reported to have bactericidal or bacteriostatic activities [64]. It has been proved that various plants extracts possess bacteriostatic and bactericidal effects [37] and most of these plants contain many active compounds. Consequently, they are multipurpose drugs at the same time [56]. Among these compounds are simple phenols which their activity comes from the presence of hydroxyl groups. These groups are capable to bind with the



No.	Arabic name	Latin name	English name	Plant part used	Family name
1	خلنجان	Alpinia galangal Will.	Galangal	Rhizomes	Zingiberaceae
2	الكرنب	Brassica oleracea var. capitata L.	Cabbage	Seeds	Brassicaceae
3	الكافور	Eucalyptus globules Labill.	Eucalyptus	Leaves	Myrtaceae
4	عرق جناح	Inula helenium L.	Elecampane	Rhizomes	Asteraceae
5	الريحان	Ocimum basilicum L.	Basil	Seeds	Lamiaceae

 Table (1): Different investigated species of medicinal plants collected during this study, Arabic name, Latin name, English name, Plant part used and Family name of each kind used.

enzymes and inhibit their action. Other compounds isolated from different plants such as flavonoids, tannins, quinones and coumarins had been extracted and found to be of inhibitory effect on numerous bacteria strains as well as fungi and yeast [40, 45, 51]. Alkaloids had been demonstrated to have high antibacterial activity against Gram-positive and Gram-negative bacteria [36].

Herbs are frequently used as food ingredients. Herbs are bactericidal and have broad-spectrum activity against Gram-positive and Gram-negative bacteria [11, 24, 30]. A detailed study on antibacterial compounds was done evaluating extracts from 120 plant species from 28 different families [76]. It was documented that 81 extracts obtained from 58 plants were active against S. aureus, and five extracts from four other plants inhibited the growth of Pseudomonas aeruginosa. Another studies in Egypt on herbal extracts and compounds were done investigating their antibacterial activity [4, 5, 89]. Extracts of Alpinia galanga also showed synergistic antimicrobial activity in combinations of Rosmarinus officinalisor Eucalyptus staigerana against Staphylococcus aureus [80], Listeria monocytogenes, Escherichia coli, Salmonella typhimurium and *Clostridium perfringens*[87].

On the other hand, the antibacterial activities of essential oils have been recognized for many years [31]; these have led to the development of synthetic alternatives in the last century [29]. The antibacterial properties of essential oils are in part associated with their lipophilic character, leading to accumulation in membranes and to subsequent membrane-associated events such as energy depletion [19]. They pass through the bacterial cell wall and cytoplasmic membrane, disrupt the structure of the different layers of polysaccharides, fatty acids and phospholipids and permeabilize them [10].

Nychas [60] Reported antimicrobial activity of essential oils from oregano, thyme, sage, rosemary, clove, coriander, garlic and onion against both bacteria and moulds. Numerous studies are available in the literature that highlights importance of medicinal plants. It is estimated that phytochemical, pharmacological and antibacterial activity of plant extracts present a unique challenge to strike new source of traditional medication [34].

2 Materials and Methods

2.1 Collection of Medicinal Plant Samples

Five species of medicinal plants belonging to five taxonomic botanical families were collected from local retail markets of drugs, spices and herbs "Attarin" in Egypt (Table, 1).

2.2 Preparation of Plant Extracts:

Sequential extraction method was employed to extract the plant powders using n-hexane, ethanol 95% and sterile distilled water [67].

2.3 Antibacterial Activities of Hexane, Ethanol and Aqueous Extracts of Different Kinds of Medicinal Plants:

a) Agar disc diffusion method:

The antibacterial activities of different plant extracts were carried out by agar disc diffusion method [55]. Disc diameter was 5 mm. Chloramphenicol was used as positive control, while n-hexane, ethanol, sterile distilled water and Dimethylsulfoxide (DMSO) were used as negative control. Nutrient agar plates previously inoculated with 24 h old broth cultures of the bacterial strains were used for antibacterial activity.

b) Organisms:

Seven strains of pathogenic bacteria (three Grampositive bacteria *Bacillus cereus* Frankland and Frankland and *Staphylococcus aureus* Rosenbach and five Gramnegative bacteria *Escherichia coli* (Migula) Castellani and Ghalmers, *Klebsiella pneumonia* (Schroeter) Trevisan, *Proteus vulgaris* Hauser, *Pseudomonas aeruginosa* (Schroeter) Migula and *Salmonella typhi* (Kayser) were used as test organisms. Pure cultures of local isolates of these organisms were obtained from "Microbiology Department, Faculty of Medicine, El-Minia University, Egypt".



 Table (2): Antibacterial activities of different extracts (hexane, ethanol and aqueous) of various tested medicinal plant kinds against Gram-positive and Gram-negative bacteria. Inhibition zone diameter in millimeter (mm).

Medicinal	B. cereus		S. aureus		E. coli		K. pneumonia		P. vulgaris		P. aeruginosa		S. typhi								
plant	A	В	С	Α	В	С	А	В	С	А	В	С	Α	В	С	Α	В	С	А	В	С
Galangal	16	13	11	15	17	12	14	15	13	11	11	7	13	12	7	10	10	6	10	11	7
Cabbage	10	14	9	11	13	11	11	12	10	9	9	7	8	10	7	7	7	6	7	8	NI
Eucalyptus	15	14	17	14	14	17	14	12	13	10	NI	10	12	12	11	10	10	9	11	10	11
Elecampane	14	14	NI	14	14	NI	13	12	NI	9	8	NI	11	11	NI	9	9	NI	9	9	NI
Basil	18	16	9	18	21	10	15	15	8	15	14	NI	17	14	NI	12	11	NI	13	11	NI
Chloramph.		16			20			18			8			9			6			6	
Hexane	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Ethanol	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
Water	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI
DMSO	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI	NI

A = Essential oil; B = Ethanol extract; C = Aqueous extract; NI = No Inhibition; Chloramph. = Chloramphenicol

2.4 Determination of Minimum Inhibitory Concentration (MIC):

The diameter of the inhibition zone around the disc, measured in millimeter, is used as positive bioactivity. MIC was determined according to [43] and employed by Ayoola *et al.* [7, 8].

2.5 Qualitative Phytochemical Analysis:

Phytochemical screening for the presence of glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids and coumarins was undertaken using standard qualitative methods as described by [27, 28, 32, 70, 83, 86].

2.6 Gas Chromatography-Mass Spectrometry (GC/MS) Analysis:

The chromatographic procedure was carried out at Special Unit for Scientific Services and Technology, City for Scientific Research and Technology Application, Universities and Research Center District, New Borg El-Arab, Alexanderia, Egypt. The essential oils and ethanol extract of basil were analyzed using GC/MS according to [12].

3 Results

Hexane, ethanol and aqueous extracts were tested against the growth of seven pathogenic bacteria representing two Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) and five Gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa*, and *Salmonella typhi*). The data recorded in Table (2) revealed that hexane extracts followed by ethanol extracts of the medicinal plants tested had the highest activities against the pathogenic bacteria followed by aqueous extracts.

Gram-positive bacteria were more sensitive to different extracts than Gram-negative bacteria. *B. cereus*

and *S. aureus* were sensitive to all extracts except aqueous extract of elecampane.

Moreover, all extracts of galangal, cabbage and eucalyptus had antibacterial effect against *K. pneumonia*, *P. vulgaris*, *P. aeruginosa* and *S. typhi* except aqueous extract of cabbage and ethanol extract of eucalyptus were not effective against *S. typhi* and *K. pneumonia*, respectively. *E. coli* was affected by all extracts except aqueous extract of elecampane. Both hexane and ethanol extracts of elecampane and basil were effective against *K. pneumonia*, *P. vulgaris*, *P. aeruginosa* and *Salmonella typhi* but their aqueous extracts were not.

Based on the results in Table (3), the MICs of galangal hexane extract against *B. cereus*, *S. aureus*, *E. coli*, *K. pneumonia*, *P. vulgaris*, *P. aeruginosa* and *S. typhi* were 6.04, 4.04, 3.64, 14.77, 6.07, 6.27 and 19.07 mg/ml, respectively. While the MICs of eucalyptus hexane extract were 76.6, 123.02, 36.98, 61.09, 177.41, 500 and 198.6 mg/ml, respectively. Also, the MICs for elecampane hexane extract against the preceding bacteria were 21. 61.09, 6.68, 10, 500, 61.73, 125.89 and 21.08 mg/ml, respectively. Basil hexane extract had the lowest MICs in quantity and highest in activity on tested bacteria as following: 0.86, 0.12, 2.72, 2.73, 13.1, 6.31 and 27.98 mg/ml, respectively.

Regarding to the ethanol extracts, Table (3) exhibits the MICs of galangal against previously mentioned bacterial species as following: 10, 19.95, 7.21, 88.61, 39.53, 62.08 and 500 mg/ml, respectively. Cabbage MICs were 48.75, >500, 52.3, 62.08, 62.23, 62.08 and 62.08 mg/ml, respectively, and elecampane MICs were 6.23, 3.5, 2.63, 62.08, 62.08, 500 and 500mg/ml, respectively. Basil MICs were 13.18, 5.17, 18.87, 53.55, 87.7, 15.49 and 85.86mg/ml, respectively.

The data stated in Table (3) revealed that aqueous extract of galangal showed the MICs 62.23, 500, 84.62, >500, 158.49, 500 and >500 mg/ml, respectively, while MICs for eucalyptus were 24.15, 10.96, 36.3, 500, 31.47, 500 and 500 mg/ml, respectively, against pathogenic bacteria tested.



Table (3): MICs (mg/ml) of hexane, ethanol and aqueous extracts of investigated medicinal plant species with wide
spectrum highly antibacterial activities against tested bacteria.

Type of extract	Medicinal plant	B. cereus	S. aureus	E. coli	K. pneumonia	P. vulgaris	P. aeruginosa	S. typhi
	Galangal	6.04	4.04	3.64	14.77	6.07	6.27	19.07
n-hexane	Eucalyptus	76.6	123.02	36.98	61.09	177.41	500	198.6
	Elecampane	21.81	6.68	10	500	61.73	125.89	21.08
	Basil	0.86	0.12	2.72	2.73	13.1	6.31	27.98
	Galangal	10	19.95	7.21	88.61	39.53	62.08	500
Ethanol 95%	Cabbage	48.75	>500	52.3	62.08	62.23	62.08	62.08
Ethanol 95%	Elecampane	6.23	3.5	2.63	62.08	62.08	500	500
	Basil	13.18	5.17	18.87	53.55	87.7	15.49	85.86
A 200000	Galangal	62.23	500	84.62	>500	158.49	500	>500
Aqueous	Eucalyptus	24.15	10.96	36.3	500	31.47	500	500

Table (4): Phytochemical screening of the bioactive compounds in wide spectrum highly active antibacterial extracts.

Type of extract	Medicinal plant	Flavonoids	Alkaloids	Terpenoids	Glycosides	Coumarins	Tannins	Saponins
	Galangal	+	+	+	+	+	-	-
n-hexane	Eucalyptus	+	+	+	+	+	+	-
II-IIexaile	Elecampane	+	+	+	-	+	-	-
	Basil	+	+	+	+	+	-	-
	Galangal	+	+	+	+	+	+	-
Ethanol	Cabbage	+	+	+	-	+	-	-
95%	Elecampane	+	+	+	-	+	-	-
	Basil	+	+	+	-	+	-	-
A 2000000	Galangal	+	-	+	+	+	-	-
Aqueous	Eucalyptus	+	-	+	+	-	+	-

Table (5): GC/MS analysis of basil hexane extract.

No.	Component Name	Retention time	Percentage %
1	Toluene	4.544	7.26
2	Decane	11.340	8.54
3	Decane, 4-methyl	12.156	2.05
4	Undecane, 2-methyl	13.668	3.29
5	Decane, 3-methyl	13.919	1.47
6	Nonane, 2-methyl	14.998	27.38
7	Tridecane, 2-methyl	17.317	4.16
8	Tetradecane, 1-iodo	17.569	1.86
9	Tridecane, 4,8-dimethyl	18.635	27.57
10	Undecane, 2,6-dimethyl	19.115	3.36
11	Octane, 2,7-dimethyl	25.404	3.65
12	Hexadecane	28.597	7.18
13	Gamolenic acid (γ-Linolenic acid)	45.579	2.19

galangal ethanol extract. Cabbage, elecampane and basil contained flavonoids, alkaloids, coumarins and terpenoids in their ethanol extracts. Moreover, both aqueous extracts of galangal and eucalyptus included flavonoids, terpenoids and glycosides, while coumarins and tannins were detected in them, respectively.

Hexane extract and ethanol extracts of basil were chosen as the most potent antibacterial. Hence, gas chromatography coupled with mass spectrometry (GC/MS) was used to determine the active antibacterial ingredients. Thirteen compounds were identified by GC/MS analysis of hexane extract of basil and eight compounds were identified in ethanol extract of basil (Tables, 5 & 6).

Table (6):	GC/MS a	analysis	of ba	asil eth	anol	extract.

From the results outlined in Table (4), we can	1
conclude that all tested phytochemicals were found in the	2
hexane extracts of galangal and basil except tannins and	3
saponins, while eucalyptus hexane extract have all	4
phytochemicals tested with the exception of saponins.	5
Elecampane hexane extract contained flavonoids and	6
alkaloids in addition to terpenoids.	7

On the other hand, terpenoids were detected in all tested ethanol extracts. Only saponins was absent in

No.	Component Name	Retention time	Percentage %
1	Cyclohexanone	7.967	73.89
2	2-Methylene cyclopentanol	10.990	6.9
3	Tetradecane, 1-iodo	28.605	8.05
4	4-Tridecen-6-yne	29.901	1.55
5	Cyclobutanone, 2-ethyl	32.302	1.4
6	Isoamyl nitrite	41.268	1.44
7	Cyclodecene	45.397	1.86
8	9,12,15-Octadecatrienoic acid (α-Linolenic acid)	45.579	4.9

4 Discussion

The object of the present experiment was to examine the antibacterial activity of hexane, ethanol and aqueous extracts of five different medicinal plant species assayed on the growth of seven pathogenic bacteria; representing two Gram-positive bacteria and five Gram-negative bacteria by disc diffusion method. Minimum inhibitory concentration (MIC) and qualitative phytochemical screening were carried out for the wide spectrum highly active antibacterial extracts. The most potent antibacterial extracts were chosen for determining their chemical components using GC/MS analysis.

The results obtained during this investigation elucidated clearly that generally, Gram-positive bacteria were more sensitive towards different medicinal plant extracts tested than Gram-negative bacteria. These observations are likely to be the result of the differences in cell wall structure between Gram-positive and Gram-negative bacteria, with Gram-negative outer membrane acting as a barrier to many environmental substances including antibiotics [16]. This conclusion is in full agreement to that previously recorded by several researchers. [58, 62, 89] reported that Gram-negative bacteria were not susceptible to plant extracts when compared to Gram-positive bacteria , and this has been attributed to the external lipopolysaccharide (LPS) wall that surrounds the peptidoglycan cell wall of the former [47].

The results obtained during this study revealed that hexane extracts were the most active antibacterial agents followed by ethanol extracts, while aqueous extracts occupied the third place. All hexane extracts assayed (galangal, cabbage, eucalyptus, elecampane and basil) had highly broader spectrum antibacterial activity against all tested pathogenic bacteria tested. Ethanol extracts of galangal, cabbage, elecampane and basil showed highly broader spectrum antibacterial activity against all bacterial species tested, followed by eucalyptus ethanol extract had highly activity against 6 species out of 7 pathogenic bacteria tested. On the other hand, aqueous extract of both galangal and eucalyptus showed highly broader spectrum antibacterial activity against all bacterial species tested, followed by cabbage aqueous extracts had highly activity against 6 species out of 7 pathogenic bacteria tested.

Generally, essential oils of spices possess strong antibacterial properties against food-borne pathogens and contain high concentrations of phenolic compounds [16, 22, 57]. [54] Reported that essential oils had a stronger activity and broader spectrum than those of solvent extracts. The basis of varying degree of sensitivity of test organisms of bacteria may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytocompounds present in the essential oil.

Bacillus group was particularly more sensitive among the Gram-positives. These results were established from the

work of many researchers using different types of medicinal plants [52, 89, 92]. The cell wall structure of Gram-negative bacteria is constituted essentially with LPS. This constituent avoids the accumulation of the essential oils on the cell membrane [13, 38, 81, 82].

Eucalyptus globules essential oilshowed antimicrobial effects only on Gram-positive bacteria [42], while [17] demonstrated that the essential oil of *E. globules* were most effective against *S. aureus, Klebsiella* sp. and *E. coli*. The antibacterial activity of the n-hexane, acetone, ethanol and aqueous extracts from *E. globulus* against *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* showed highly positive activity of the extracts [73].

Petroleum ether, benzene, chloroform, ethyl acetate, methanol and distilled water extracts of *Alpinia galanga* were found to be active against all the bacteria and fungi tested and greater than the activity of the standards [50, 84]. [65] Proved that galangal ethanol extract had less or no activity against tested bacteria except *S. aureus*.

Essential oil, aqueous and chloroform extracts of elecampane exhibited good antibacterial activity against both Gram-positive and Gram-negative bacteria tested except essential oil had no effect on *S. paratyphi* and *K. pneumonia* [89]. [90] Reported that aqueous seed-extracts of five Brassicaceae plants were highly active against bacteria and dermatophytes than essential oils and chloroform extracts. [33] Reported that water extract from cabbage had the highest antimicrobial activity, especially against Gram-negative bacteria.

According to obtained results in the present investigation, basil hexane and ethanol extract had highly antibacterial activity against tested bacteria, while aqueous extract was low. Essential oil of basil obtained from its leaves, has demonstrated the ability to inhibit several species of pathogenic bacteria that have become resistant to commonly used antibiotic drugs [23, 66]. Effect of various extraction solvents (n-hexane, petroleum ether, acetone, ethyl acetate, chloroform, ethanol and methanol) used for *Ocimum basilicum* L. on their *in vitro* antimicrobial properties were already studied [1, 14, 39, 71]. All extracts were different in terms of their antimicrobial activities and the hexane extract showed a stronger and broader spectrum of antimicrobial activity.

[48] Demonstrated thatethanol extract from basil is potentially good source of antibacterial agents against the pathogens*K. pneumonia,S. aureus, S. typhi,P. vulgaris* and *P. aeruginosa.* Crude ethanolic extract exhibited antibacterial activities more than the aqueous extract [35]. [85] Tested the antibacterial activity of ethanol and aqueous extracts of 25 species of well-known herbs and spices against seven food borne bacteria. They classified their findings about basil as strong antibacterial source plants. The aqueous extract of basil did not promote activity against Gram-negative bacteria exclusively [6].



Qualitative analysis was carried out for screening the presence of major pytochemical constituents such as glycosides, alkaloids, tannins, flavonoids, saponins, terpenoids and coumarins in the highly active antibacterial extracts. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties [20, 46].

Galangal is rich in phenolic compounds such as flavonoids and phenolic acids [49]. [73] Reported the presence of flavonoids, glycosides, tannins, terpenoids and saponins in the ethanol extract of eucalyptus. [21] Detected tannins, phenols and resins in eucalyptus ethanol extract, while [26] indicated the presence of alkaloids, flavonoids, reducing sugars, tannins, steroids and saponins, and absence of cardiac glycosides and terpenoids in eucalyptus methanol extract.

Phytochemical screening of oil extracted from basil leavesshowed the presence of alkaloids, terpenoids, steroids, flavonoids, tannins, and saponins, and absence of cardiac glycosides and anthraquinones [9]. Alkaloids, flavonoids, cardiac glycosides, tannins, terpenoids, steroids, reducing sugars, phenolic compounds, amino acids, anthraquinones and saponins were different in terms of their presence when a broad phytochemical analysis of the hexane, petroleum ether, ethyl acetate, chloroform, ethanol and methanol extracts of basil was carried out by [14].

[59] In their quantified study on fatty acids composition of ten seed oils of basil accessions using GLC, they reported that all basil seed oils contained linolenic acid (49-75%), palmitic acid (5-13%), stearic acid (2-3%), oleic acid (6-10%) and linoleic (12-32%). [44] Concluded that linolenic acid has a strong antibacterial activity against B. cereus and S. aureus, and that linolenic acid combined with monoglyceride showed stronger antibacterial activity than using linolenic acid alone. [91] Found that linolenic acid inhibited bacterial enoyl-acyl carrier protein reductase (FabI), an essential component of bacterial fatty acid synthesis, which has served as a promising target for antibacterial drugs. [61] Investigated the antibacterial activities of liposomal linolenic acid against antibioticresistant Helicobacter pylori. They reported that liposomal linolenic acid was effective in killing both spiral and coccoid forms of the bacteria via disrupting bacterial membranes.

5. Conclusion

Medicinal plants can be used as alternative antimicrobial drugs such as basil. The medicinal value of these plants lies in some bioactive natural chemicals (phytochemicals) as possible sources of non-phytotoxic and easily biodegradable alternative antibiotics.

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M. Youssef et al.: Evaluation of antibacterial activities ...

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