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Antibacterial Activity and Phytochemical Analysis of Some Medicinal Plants from Gaza Strip-Palestine

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Abstract: *The present study was designed to screen in-vitro antibacterial activity of Paronychia argentea Lam., Matricaria aurea , and Verbascum sinuatum L from Gaza strip-Palestine. The dried aerial parts of plant were successively extracted with chloroform, ethanol and aqueous solvents using serial exhaustive cold maceration extraction. All extracts were screened for its antibacterial activity using agar well diffusion method and micro-dilution technique. The microorganisms used for antibacterial activity were Escherichia coli, Klebsiella pneumoniae, Morganella morganii,* Methicillin-sensitive *Staphylococcus aureus and* Methicillin-resistant *Staphylococcus aureus. The average diameter of inhibition zones against the tested bacteria ranged from 9 to 14 mm, 9 to 19 mm and 9 to 20 mm for chloroform, ethanol and aqueous extract, respectively. The extracts showed antibacterial activity were subjected to minimum inhibitory concentration assay; a micro-broth dilution assay was performed on 96-well plates using 2, 3, 5-Triphenyl Tetrazolium Chloride (TTC) as an indicator for bacterial growth, the average minimum inhibitory concentrations (MICs) values ranged from 2.08 to 33.33 mg/ml, 1.04 to 8.33 mg/ml and 0.52 to 4.17 mg/ml for chloroform, ethanol and water extracts, respectively. Phytochemical analysis revealed that Paronychia argentea, Matricaria aurea chloroform extracts appear to contain sterols, while, essential oils were found to be present in all the three plant extracts what might indicate lipophilic character. Tannins, saponins and flavonoids were found to be present in all the three plant extracts. Test for carbohydrates were positive for the three plant aqueous extracts. None of the extracts tested were positive for alkaloids.*

1.1 Introduction

With the increasing incidence of diseases caused by bacteria and other pathogenic microorganisms, as well as the development of drug resistance, there is an urgent need to search for alternatives from plants and other sources to combat these pathogens. Natural drugs could represent an interesting approach to limit the emergence and spread of these organisms, which are currently difficult to treat. Recently, scientific interest in the study of plant materials as sources of new compounds for processing into therapeutic agents has increased considerably **(1)** Nearly all cultures from ancient times to the present day have used plants as a source of medicines. As a result, different remedies tended to develop in different parts of the world **(2).** Numerous studies have been carried out to extract various natural products for screening antimicrobial activity **(3)**. Medicinal plants are important elements of indigenous medical systems in Palestine as well as in other developing countries**(4).** Complementary and alternative medicine (CAM) utilization in Palestine is very common; some of the types of CAM used in Palestine are common elsewhere, whereas other types were unique to this area**(5)**. Current strategies to overcome the global problem of antimicrobial resistance include research in finding new and innovative antimicrobials from plants **(2)**. Medicinal plants are important elements of indigenous medical systems in Palestine as well as in other developing countries**(3**). The Silvery whittle-wart, *Paronychia argentea* Lam. is commonly used in many indigenous Palestinian communities for the treatment of a variety of conditions such as in urinary system and kidney stones dissolving **(6).** In West Bank, Palestine; *Matricaria aurea* is listed as a popular plant of the region and used primarily in the respiratory system treatment**(6).** Infusions from the leaves and flowers of different *Verbascum* spp. are still used for their expectorant and demulcent properties to treat respiratory problems such as irritating coughs with bronchial congestion.

Due to rapid increase of antibiotic resistance in our region, plants which have been used as medicines over hundreds of years, constitute an obvious choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. The present study of *in-vitro* antibacterial evaluation of some local medicinal plants forms a primary platform for further phytochemical and pharmacological studies**.**

1.2 Materials and Methods

1.2.1 Plant material

Medicinal plants including *Paronychia argentea* Lam. (Caryophyllaceae), *Matricaria aurea* (Compositae), and *Verbascum sinuatum* L. (Scrophulariaceae), were utilized in this study. The taxonomic identities of these plants were identified and authenticated at the Pharmacognosy Department , Faculty of Pharmacy, Al- Azhar University, Gaza. Palestine .The plant materials were dried under shade and ground into fine powder using electric blender and stored in airtight bottles. The names, parts used of the examined plant species are given in Table 1.

Table 1: List of medicinal plants used in the antibacterial assay

Botanical Name	Family	Local Arabic Name	Common Name	Part Used
Paronychia argentea	Caryophyllaceae	رجل الحمامة	Silvery whittle-wart	Aerial part
Matricaria aurea	Compositae	بابونج	Golden cotula	Aerial part
Verbascum sinuatum	Scrophulariaceae	عوور	Wavyleaf mullein	Aerial part

1.2.2 Extraction of selected plant material powder by maceration method

The extraction method used in this study was a modification of (**7)** Plant extracts were prepared by cold maceration method. Serial exhaustive extraction, which involves successive extraction with solvents of increasing polarity from a non-polar to a more polar solvent to ensure that a wide polarity range of compounds could be extracted **(8)**. The plant materials were dried under shade and ground into fine powder using electric blender. 100 g of dried powder of *Paronychia argentea* was soaked in 500 ml of chloroform for 48 hours with intermittent shaking. The plant extract was filtered through Whatman No. 1 filter paper. The plant residue was re-extracted with addition of chloroform, and after 24 h it was filtered again. Combined filtrates were concentrated on a rotary evaporator at 40°C for solvent elimination and the extract was kept in sterile bottles under refrigerated conditions until use. The remaining plant residue was dried and soaked in 500 ml of anhydrous ethanol and distilled water successively as described earlier. *Verbascum sinuatum, Matricaria aurea* (100 g) were separately extracted by cold maceration. Chloroform, ethanol and distilled water (0.5L each) were used to extract the samples successively as above and the extracts were collected as described earlier.

3.3 Phytochemical screening methods

The screening procedures were adapted in order to perform the following chemical tests, plants extracts were subjected to phytochemical screening using standard procedures **(9-11)**.

3.3.1 Chloroform extract

Table 2: Chemical examination (chloroform extract)

SR NO.	Plant Constituents	Tests &Reagents
1	Sterols and triterpenes	Liebermann Burchard`s test
2	Volatile oil	The presence of essential oil can be detected by a characteristic pleasant odor
3	Flavone's aglycones	Shibata`s reaction
4	Anthraquinone aglycones	Borntrager`s test
5	Alkaloids	Mayer's reagent Dragendroff`s reagent

3.3.2 Ethanol extract

Table 3: Chemical examination (ethanol extract)

SR NO.	Plant Constituents	Tests &Reagents
1	Alkaloids	Mayer's reagent Dragendroff`s reagent
2	Tannins	Ferric chloride test
3	Reducing compounds	Fehling`s I+ II
4	Flavonoids glycoside	Shibata` s reaction
5	Anthraquinone glycoside	Borntrager`s test
6	Saponins	Foam test

3.3.3 The aqueous extract

Table 4: Chemical examination (water extract)

SR NO.	Plant Constituents	Tests &Reagents
1	Carbohydrates	Molisch`s test
2	Polyuronides	Methylen blue

3.4 Antibacterial assay

Antibacterial assays were carried out in the Microbiology Laboratory within the Faculty of Science, AL-Azhar University, Gaza strip.

3.4.1 Bacterial isolates

Bacterial isolates were kindly provided from Al-Remal clinic, ministry of health, Gaza strip and confirmatory identification was done at Microbiology Laboratory, Faculty of Science, AL-Azhar University, Gaza strip. The selected clinical isolates were Escherichia *coli*, *Morganella morganii*, *Klebsiella pneumoniae*, Methicillin-sensitive *Staphylococcus aureus* and Methicillin-resistant *Staphylococcus aureus.*

3.4.2 Culture media

Mueller Hinton agar medium was prepared, enclosed in a screw cap container and autoclaved at 121°C for 15 min. The medium was later dispensed into sterile agar plates and left to set.

3.4.4 Preparation of inoculum

The bacteria were activated by inoculating a loopful of the isolates in the nutrient broth medium, incubated at 37 ºC on a rotary shaker for overnight. These cells suspensions were diluted with the same broth medium to provide initial cell counts of about 10^8 cfu (colony forming unit)/ml**.**

3.4.5 Screening of the antibacterial effect of plant extracts

A modified cup plate diffusion method was used in this survey **(12**). Culture plates inoculated with the desired tested organisms were used in this test. Wells (7 mm diameter) were punched in the agar, after which drops of water agar (15g/l) were put in holes; the extracts were reconstituted by dissolving 0.2 gm of each in 1 ml of dimethyl sulfoxide (DMSO). To each hole, 50µl of each extract was added and allowed to diffuse at room temperature. DMSO was used as negative control and a standard 30 µg tetracycline disk served as positive controls. After that plates were incubated at 37°C for 24 hr. The experiment was performed 3 times under strict aseptic conditions. Microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values were recorded.

3.4.6 Determination of minimum inhibitory concentration(MIC)

MIC was determined in the plant extracts that showed some efficacy against the tested isolates , extracts were tested against the isolates for their inhibitory activity, using a common broth microdilution method in 96 multiwell microtiter plates, in duplicate, as reported by(**13)** with slight modification.

For susceptibility testing, 50 µl of Mueller-Hinton broth was distributed from the second to the twelfth test wells. Dry extracts from each plant part were initially dissolved in dimethylsulfoxide (DMSO) and then in broth medium, to reach a final concentration of 66 mg/ml; 100 µl of these suspensions were added to the first test well of each microtiter line, and then 50 µl of scalar dilutions were transferred from the first to the twelfth well. Then, a 0.5 McFarland standard suspension of test bacteria was made in Mueller–Hinton broth medium, from which 50 µl of the final inoculum containing approximately $1x10^8$ colony forming units (cfu) was added to the appropriate wells. The final concentrations of the extracts adopted to evaluate the antibacterial activity were 0.0325 to 66.66 mg/ml.

Inoculated plates were incubated at 37°C for 24 h. One hour before the end of incubation 50 µl of a 0, 01% solution of 2, 3, 5- triphenyl tetrazolium chloride (TTC) was added to the wells and the plate was incubated for another hour. Since the colorless tetrazolium salt is reduced to a red colored product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC. The lowest concentration of each extract showing no visible growth was recorded as the minimum inhibitory concentration (MIC). Inoculated and uninoculated wells of plant extract-free broth was included (the first controls of the adequacy of the broth to support the growth of the organism; the second is a check of sterility). chloromphenicol (100 µg/ml) **(14)** was used as positive controls.

Results & Discussion

4.1 Extraction

Table 5 lists the amounts of extracts obtained in the various extraction procedures

Table 5: Extracts and their amount in (g) yield

PLANT NAME	SOLVENT	AMOUNT (IN G)
Paronychia argentea (100 g)	Chloroform	2.9
	Ethanol	2.2
	Distilled water	6.3
Matricaria aurea (100 g)	Chloroform	3.4
	Ethanol	2.7
	Distilled water	6.9
Verbascum sinuatum (100 g)	Chloroform	2.5
	Ethanol	3.2
	Distilled water	5.6

As is apparent from Table 5, water extracts were highest in yield. This may due to the fact that Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure **(10)**. This may be due to the better solubility of the active components in suitable solvent. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and, in addition to their intrinsic bioactivity, by their ability to dissolve or diffuse in the different media used in the assay **(15).**

When choosing an extraction method, maintaining the activity of the extracted compound(s) is the priority. Wet extractions involve solid material in direct contact with a liquid solvent. During the extraction, organic solvents diffuse into the solid material and solubilize compounds with similar polarity. The nature of the solvent used will determine the types of chemicals likely extracted from the plant. Common extraction method is serial exhaustive extraction which involves successive extraction with solvents of increasing polarity from a non-polar to a more polar solvent to ensure that a wide polarity range of compounds could be extracted **(5).**

Fresh or dried plant material can be used as a source for secondary plant components. However, most scientists working on the chemistry of secondary plant components have tended to use dried plant material for several reasons. Differences in water content may affect solubility of subsequent separation by liquid-liquid extraction and the secondary metabolic plant components should be relatively stable, especially if it is to be used as an antimicrobial agent **(16).** Plant extract sterilization by autoclave is not possible due to the risk of possible heat degradation of the active compounds. DMSO extracts could not be filter-sterilized as DMSO dissolves the filter material**(17).** The DMSO dissolved plant extracts showed no microbial contamination by "streak plate" method**.** The relatively high freezing point of DMSO means that at, or just below, room temperature it is a solid, which can limit its diffusion .Therefore, plant extracts dissolved in DMSO, were added to the wells of agar medium and allowed to diffuse at room temperature.

4.2 Chemical tests

Basic chemical tests were performed to identify the presence / absence of certain compound classes in the various extracts. As can be seen from the obtained results *Paronychia argentea*, *Matricaria aurea* chloroform extracts appear to contain sterols and triterpenes, while, essential oils were found to be present in all the three plant extracts what might indicate lipophilic character. Tannins, saponins and flavonoids were found to be present in all the three plant extracts . Test for carbohydrates were positive for the three plant aqueous extracts . None of the extracts tested were positive for alkaloids(Table 6).

Table 6: Phytochemical analysis of screened medicinal plants

Plant	Solvent	St	Vo	Alk	Aa	Fa	T	S	F	A	R	C	P
Paronychia argentea	***$CHCl_3$***	+++	+++	Nil	Nil	Nil							
	EtOH	Nil					+++	+++	++	Nil	+++		
	H_2O											+++	Nil
Matricaria Aurea	***$CHCl_3$***	+++	++++	Nil	Nil	Nil							
	EtOH	Nil					+++	+	+	Nil	+++		
	H_2O											+++	Nil
Verbascum sinuatum	***$CHCl_3$***	Nil	++	Nil	Nil	Nil							
	EtOH	Nil					+++	++++	+++	Nil	+++		
	H_2O											+++	*Nil*

+ faint, ++ clear, +++ very clear, ++++ highly intense

Sterols and Triterpenes St , Volatile oil Vo , Alkaloids Alk , Anthraquinoneaglycones Aa , Flavone's aglycones Fa , Tannins T , Saponins S, Flavonoids F, Anthracyanosides A, Reducing compounds R, carbohydrates C , polyuronides p.

The phytochemical analysis of chloroform extract of *Paronychia argentea* ,*Matricaria aurea*, and *Verbascum sinuatum* revealed the presence of essential oils, where only *Paronychia argentea* and *Matricaria aurea*, showed positive result for the presence of (sterols and triterpenes) (Table 6). Published results in the literature showed that essential oils from various aromatic plants are known to show a wide spectrum of anti-microbial activity against both plant and human pathogenic microorganisms **(18)**. There has been a resurgence of interest in essential oils because they are perceived to be natural alternatives to chemical biocides and, in some applications antibiotics **(19).**

Phytochemical test revealed the presence of tannins, together with other active ingredients in the ethanloic extract of *Paronychia argentea*, *Matricaria aurea* and *Verbascum sinuatum* (Table 6), preliminary phytochemical screening of the crude extract of the plant extracts showed the presence of phenolic compounds (as shown by strong reaction with ferric chloride) but no reaction with Draggendoff's and Mayer's reagents indicating the absence of alkaloids in the plant extract. Alkaloids were not detected at all, this is not surprising as alkaloids readily decompose with time. Tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis **(20).** The phytochemical analysis of ethanol extract suggested that antibacterial activity of plant extracts may due to the presence of phenolic compounds.

The present study carried out on the ethanolic plant extracts revealed the presence of flavonoids in all the three plant extracts were investigated (Table 6). Flavonoids are a group of natural compounds known to have various pharmacological properties such as antioxidative, anti-inflammatory and diuretic. One of the well-known actions of flavonoids is antimicrobial activity **(21).** Furthermore, flavonoids represent novel leads, and future studies may allow the development of a pharmacologically acceptable antimicrobial agent or class of agents**(22).**

Our phytochemical investigation showed the presence of saponins, in all the three plants extracts. Saponins may be responsible for the antimicrobial activity **(23)**.

The result of the photochemical screening (Table 6) showed that *Paronychia argentea*, *Matricaria aurea* and *Verbascum sinuatum* aqueous extracts contain carbohydrates. Carbohydrates among a group of substances which have been found through several *in-vitro* and *in-vivo* studies to be responsible for the antimicrobial activity **(24).** These metabolites present in various parts are known to have varied pharmacological actions in man and animals **(25)**. The observed activity in the present study may be

due to the presence of phytoconstituents in the different plant extracts**.**

4.3 Antibacterial activity

4.3.1 Antibacterial evaluation of medicinal plants by the well diffusion method

The antibacterial effect of the medicinal plants is well documented; plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in-vitro* to have antimicrobial properties **(26).** The results of this study provides evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains . In this study it was observed that extracts of *Paronychia argentea*, *Matricaria aurea* and *Verbascum sinuatum* produce antibacterial activity against Gram-negative and Gram-positive bacteria which were tested. The growth media also seem to play an important role in the determination of the antibacterial activity**.** As reported by **(16)**, Muller-Hinton agar appears to be the best medium to explicate the antibacterial activity and the same was used in the present study. Antibacterial assays were performed in triplicates and the average of the obtained results is listed in Table7.

Table 7: Antibacterial activity of chloroform, ethanol and aqueous extracts of screened medicinal plants

Plant	Solvent	Zones of inhibition (mm) *				
		E. coli	*K. pneumonia*	*M. morganii*	MSSA	MRSA
Paronychia argentea	***$CHCl_3$***	9	11	10	14	N.I
	EtOH	N.I	14	N.I	13	19
	H_2O	N.I	13	N.I	N.I	20
Matricaria aurea	***$CHCl_3$***	9	N.I	10	N.I	9
	EtOH	13	N.I	9	15	13
	H_2O	15	10	9	12	N.I
Verbascum sinuatum	***$CHCl_3$***	10	13	10	13	11
	EtOH	N.I	12	N.I	11	12
	H_2O	N.I	14	N.I	N.I	14
Positive control 30 µg tetracycline disk		13	18	11	20	9
Negative control Dimethyl sulfoxide		N.I	N.I	N.I	N.I	N.I

mm* = Mean of three replicates ,N.I = No inhibition

The antibacterial activity of organic chloroform extract of *Paronychia argentea* is shown in Table 7. The most susceptible bacterium was *Klebsiella pneumoniae* followed by *Morganella morganii* amongst the Gram-negative bacteria, while amongst Gram-positive bacteria, the most susceptible was Methicillin-sensitive *Staphylococcus aureus*, while Methicillin-resistant *Staphylococcus aureus* showed negligible activity. The results shown in (Table 7) indicate that the antibacterial activity was shown by ethanolic extract of *Paronychia argentea* against *Klebsiella pneumoniae* , while it did not show any activity against *Morganella morganii* and *E. coli* amongst the Gram-negative bacteria, The extract showed maximum antibacterial activity against Methicillin- resistant *Staphylococcus aureus* followed by Methicillin-sensitive *Staphylococcus aureus*. Antibacterial activity of the crude aqueous extract of *Paronychia argentea* (Table 7) showed that the extract from plant exhibited an antibacterial effect against some of the tested bacteria .The results showed that the extract possessed maximum antibacterial activity against Methicillin-resistant *Staphylococcus aureus* followed by *Klebsiella pneumoniae*.

Chloroform extract of *Matricaria aurea* (Table 7) showed activity against *Morganella morganii* and *E. coli* amongst the Gram-negative bacteria, while

amongst Gram-positive bacteria, the most susceptible was Methicillin-resistant *Staphylococcus aureus*, while Methicillin-sensitive *Staphylococcus aureus* showed negligible activity.

The ethanolic extract of *Matricaria aurea* (Table 7) exhibited antibacterial activity against *E. coli* followed by *Morganella morganii*, it displayed antibacterial activity against Gram-positive bacteria including both Methicillin- sensitive and resistant *Staphylococcus aureus*. The most susceptible bacterium amongst the Gram-negative bacteria was *E. coli* of the aqueous extract of *Matricaria aurea* (Table 7), it showed antibacterial activity against Methicillin sensitive *Staphylococcus aureus* , but was inactive against Methicillin-resistant *Staphylococcus aureus* .

The results of antibacterial activity revealed that the chloroform extract of *Verbascum sinuatum* (Table 7) showed inhibitory activity against all the tested Gram-negative pathogens, *Klebsiella pneumoniae* was the most sensitive bacterium. The extract also exhibited inhibitory activity against both Methicillin- sensitive and resistant *Staphylococcus aureus. Klebsiella pneumoniae* was the most susceptible bacteria amongst the Gram-negative bacteria to ethanolic plant extract of *Verbascum sinuatum*, while it was inactive against *E. coli* and *Morganella morganii*. The extract also exhibited inhibitory activity against all the tested Gram-positive bacteria. Aqueous extract of *Verbascum sinuatum* (Table 7), showed antibacterial activity against *Klebsiella pneumoniae* but didn't show any activity against *E. coli* and *Morganella morganii*. The extract displayed inhibitory activity against Methicillin-resistant *Staphylococcus aureu*s, while Methicillin- sensitive *Staphylococcus aureus* showed negligible activity.

4.3.2 Determination of minimum inhibitory concentration

Extracts were tested against the isolates for their inhibitory activity, using a common broth microdilution method in 96 microtiter plates, in duplicate and the average of the obtained minimum inhibitory concentrations (MICs) is listed in Table 8. The micro-titer plate or broth microdilution method has provided a potentially useful technique for determining MICs of large numbers of test samples. It is advantages over diffusion techniques include increased sensitivity for small quantities of extract which is important if the antimicrobial is scarce as is the case for many natural products **,** some researchers however, have reported MICs values obtained by the agar diffusion method, although high activity in the disk diffusion assay does not necessarily correlate to low MIC values in the microtiter plate method **(16).**

Table 8: Minimal Inhibitory Concentration (MIC) of plant extracts

Plant	**solvent**	**MIC (mg/ml) ***				
		E. coli	*K. pneumoniae*	*M. morganii*	MSSA	MRSA
Paronychia argentea	***$CHCl_3$***	16.67	33.33	4.17	4.17	N.T
	EtOH	N.T	1.04	N.T	2.08	2.08
	H_2O	N.T	4.17	N.T	N.T	4.17
Matricaria Aurea	***$CHCl_3$***	4.17	N.T	2.08	N.T	4.17
	EtOH	1.04	N.T	2.08	1.04	1.04
	H_2O	0.52	4.17	2.08	1.04	N.T
Verbascum sinuatum	***$CHCl_3$***	4.17	4.17	8.33	2.08	4.17
	EtOH	N.T	2.08	N.T	1.04	1.04
	H_2O	N.T	1.04	N.T	N.T	1.04

(mg/ml)* = Mean of two replicates ,N.T = Not tested, MIC (Minimal inhibitory Concentration)

The MIC of the chloroform extract of *Paronychia argentea* against *E. coli* , *K. pneumoniae* and *Morganella morganii* was 16.67 mg/ml, 33.33 mg /ml and 4.17 mg/ml respectively. The MIC was found out to be 4.17 mg/ml for Methicillin-sensitive *Staphylococcus aureus* (Table 8). The MIC for the ethanol extract of *Paronychia argentea* (Table 8) was found out to be 1.04 mg/ml for *Klebsiella pneumoniae*. While both Methicillin- sensitive *and* resistant *Staphylococcus aureus* required about 2.08

mg/ml of the crude extract for effective activity. The MIC values of *Paronychia argentea* aqueous extract against the tested microorganisms (Table 8) were found out to be 4.17 mg/ml for both *Klebsiella pneumoniae*, Methicillin-resistant *Staphylococcus aureus*.

The MIC of *Matricaria aurea* chloroform extract (Table 8) was 4.17 mg/ml and 2.08 mg/ml against *E. coli* and *Morganella morganii* respectively, while 4.17 mg/ml against Methicillin-resistant *Staphylococcus aureus* .The growth of *E. coli and Morganella morganii* was inhibited by the ethanol extract of *Matricaria aurea* at a minimum inhibitory concentration of 1.04 mg/ml and 2.08 mg/ml respectively, the MIC value was 1.04 mg/ml against both Methicillin- sensitive and resistant *Staphylococcus aureus* (Table 8). Aqueous extract from *Matricaria aurea* (Table 8) showed antibacterial activity against the tested bacteria, and the strongest activity was seen against *E. coli* (***MIC***=0.52mg/ml), the extract exhibited from moderate to no activity against tested Gram-positive bacteria, it was active against Methicillin sensitive *Staphylococcus aureus (**MIC***=1.04).

The MICs of the *Verbascum sinuatum* chloroform extract against the test organisms are shown in Table 8. Gram-negative organisms were inhibited within the MIC values of 4.17 mg/ml for *E. coli and Klebsiella pneumoniae*, while 8.33 mg/ml agains**t** *Morganella morganii* . The MIC was 2.08 mg/ml against the clinical isolates of Methicillin- sensitive and 4.17 mg/ml for Methicillin- resistant *Staphylococcus aureus*. The results of minimum inhibitory concentration (MIC) were shown in Table 8. The results showed that *Verbascum sinuatum* ethanol extract is sensitive against *Klebsiella pneumoniae* (MIC =2.08 mg/ml). The MIC value was (1.04 mg/ml) for both Methicillin- sensitive and resistant *Staphylococcus aureus*. As can be seen in Table 8. MIC assay for *Verbascum sinuatum* aqueous extract revealed that the MIC value for *Klebsiella pneumoniae* and Methicillin -resistant *Staphylococcus aureu*s was 1.04 mg/ml.

A point that needs specific consideration is that medicinal properties in plants are due to the combinations of secondary products. Different plants often have taxonomically distinct combinations of these secondary metabolites resulting in unique medicinal properties in individual plants . Secondary metabolites that are generally produced for defense against predators, pathogens or competitors or for protection/adaptation to environmental stress related to changes in soil conditions, temperature, water status, light levels, UV exposure, and mineral nutrients in their natural habitats; and are responsible for most of the biological activities **(27).** It is not surprising that there are differences in the antimicrobial effects of plant groups, due to phytochemical properties and differences among species. The investigated plants did not show strong antibacterial activity; however, negative results do not mean absence of bioactive constituents nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed. Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects or negating the positive effects of the bioactive agents. With no antibacterial activity, extracts may be active against other bacterial species which were not tested**(10).**

Standard criteria for evaluation of plant antimicrobial activity are lacking and results greatly differ between authors. Sometimes it is difficult to compare results obtained, when dealing with plant extracts, with published results in the literature because several variables influence the results. This variation may be because of the dose used in the study, the method of extraction of medicinal plants, choice of plant extracts ,the method of antimicrobial study, the genetic variation of plant, age of the plant and environmental and climatic conditions under which the plant grew, standardization is required for intra- and inter-laboratory reproducibility as results may be significantly influenced by the method used **(16)**. However, the lack of susceptibility of the microorganisms to the plant extracts could be attributed to the fact that, unlike conventional pharmaceutical products which are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, herbal medicinal products are prepared from materials of plant origin which may be subjected to contamination and deterioration. The storage of extracts may require special condition of humidity or temperature or protection from light. The plant extracts might contain little of the active ingredient. The extracts which were inactive in-vitro may have properties similar to pro-drugs which are administered in an inactive form; their metabolites could be active in-vivo **(2)**.

In conclusion Crude extracts were utilized in this study exhibited antimicrobial activity against both Gram- negative and Gram- positive organisms. The results of present study supports the traditional usage of the studied plants to a certain degree, and suggests that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The obtained results could form a good basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds.

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