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Evaluation of Antimicrobial Activity of Some Medicinal Plant extracts grown on Gaza Strip (Palestine) Against some Bacterial Pathogens

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الملخص

تقييم نشاط التضاد الميكروبي لمستخلصات بعض النباتات الطبية النامية في قطاع غزة (فلسطين) ضد بعض انواع البكتيريا الممرضة

تم تقييم النشاط المضاد للميكروبات لخمسة مستخلصات لنباتات طبية وبعض المضادات الحيوية ضد بعض سلالات البكتيريا الممرضة للإنسان.(Pseudomonas aeruginosa و Staphylococcus aureus وEscherichia coli). ولقد كانت النباتات الطبية المستخدمة هي نبات القطف(الفصيلة الرمرامية)، وزهرة الأقحى (الفصيلة البوراجينية)، وعنب الديب والسجوة الزيتية (الفصيلة الباذنجانية)، والفيوماريا (الفصيلة الفيوماريةة).

ولقد تم دراسة تأثير تلك المستخلصات على البكتيريا منفردة أو مجتمعة مع المضادات الحيوية. وتمت عملية الاستخلاص بمساعدة الميكروويف في وجود 80% كحول أثيلي.

وقد أظهرت النتائج أنه على الرغم من أن المستخلصات النباتية منفردة لم تظهر أي نشاط للتضاد البكتيري، إلا أن الجمع بين المستخلصات والمضادات الحيوية قد أظهر نشاطا متآزرا ضد البكتيريا المقاومة للمضادات الحيوية. وقد لوحظ أن تأثير التآزر عند الجمع بين مستخلصي نبات الفيوماريا وعنب الديب مع المضادات الحيوية المختلفة ضد كل من بكتيريا E. coli وP. aeruginosa كان أكثر قوة من تأثيرهم ضد بكتيريا S. aureus . وأيضا أظهرت النتائج أن مستخلصي نباتي عنب الديب وزهرة الأقحى لهما أكبر نشاط تضادي ضد بكتيريا E. coli وP. aeruginosa عند اضافة المضادات الحيوية لهما.

Abstract

The antimicrobial activity of five medicinal plant extracts and some antibiotics were evaluated against some resistant human pathogenic bacterial strains (*Pseudomonas aeruginosa* , *Staphylococcus aureus* and *Escherichia coli*). The medicinal plants used were *Atreplix sembiccata*

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(Chenopodiaceae*), Echium angustifolium (*Boraginaceae), *Solanum nigrum (*Solanaceae), *Solanum elaeagnifolium* (Solanaceae) and *Fumaria capreolata (*Fumaraceae).

In this study, the synergistic effects of the plant extracts when combined together and with the antibiotics were determined. The extraction was carried out by Microwave-assisted extraction method in the presence of 80% ethanol as a solvent.

The results revealed that, there was no antibacterial activity of the plant extract alone. However, the combination of the plant extractions with the antibiotics showed synergistic antibacterial activity against the antibiotic-resistant bacteria. The synergistic effect of combined plant extracts of *Fumaria capreolata* and *Solanum nigrum* with different antibiotics against *Escherichia coli* and *Pseudomonas aeruginosa* was more potent than *Staphylococcus aureus*. Also, the results showed that *S. nigrum* and *Echium angustifolium* had the highest synergistic activity with various antibiotics against *E. coli* and *P. aeruginosa*.

Keywords: Antimicrobial activity, Atreplix sembiccata, Solanum elaeagnifolium, Solanum nigrum, Fumaria capreolata, Echium angustifolium, Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli, Microwave-assisted, Synergistic activity.

Introduction:

The use of medicinal and aromatic plants, herbs and spices in the region has a long history and forms significant part of a number of cultures. Traditional medicine still plays a vital role in health care systems in spite of the availability of modern medicine (Heywood, 1999). About 60-80% of the world's population still depends on traditional medicines for the treatment of popular illnesses (Zhang 2004; WHO, 2005; Ramzi *et al*., 2008). Because of multi-drug resistance is a world-wide problem and the importance of producing more effective antimicrobial agents, microbiologists, botanists ethnopharmacologists and chemists are examining for phytochemicals for the treatment of infectious diseases (Tanaka *et al.*, 2006). The medicinal plants are playing an important role in modern medicine as a raw material for some significant antibiotics and drugs that made a revolution in controlling various diseases(Sridhar et al., 2011).World Health Organization has specified the significance of the medicinal plants and has been active in

making standards and strategies for ethnobotanical medicine (Sridhar *et al.*, 2011).

The people of Palestine are drawn towards herbal treatments because of their increasing popularity and health benefits. The vast medicinal plant resources of the country have been threatened due to the ongoing conflicts with Israel, also severely affecting the health care method of the country (Handa *et al.*, 2006).

Palestinian flora have an important plant species that concludes herbs, shrubs and trees, which belong to 138 families and 2750 species (Danin, 2004; Sawalha, 2005). Various human activities such as over-collecting of natural plant species, noxious environment and urban expansion effect on the habitat of plants species (ARIJ, 2007). There are reports indicates that 600 plants species are suffering from the above reasons and 90 species of which are very rare and the existence in Gaza Strip and West Bank is threating (ARIJ, 2007). Because of the lack of national program to preserve the flora diversity of Palestine, some investigations have been carried out for maintaining some medicinal plants (Alkowni and Sawalha, 2012).

Atreplix sembiccata, commonly as saltbush, is a plant in the family of *Chenopodiaceae* has been known for its significant medicinal value, three known flavone glycosides 1, 2, and 3 were isolated for the first time from the whole plant. Also, potent estrogenic and antimicrobial activities and four saponins were isolated (Shaker and Mostafa, 2004).

Solanum elaeagnifolium, commonly as tomato weed, of family Solanaceae plants are easily recognized by amplitude of the purple or blue flowers. The plant definitely has adverse toxicity to animals, especially when mature (Burrows *et al*., 1981). The plant is considered an important species among *Solanum* species as a source of different extracts (Heap *et al*., 1997). The plant has been tested, in Tunisia, as a potential source of Potato virus Y (PVY) propagation (Boukhris-Bouhachem *et al*., 2007). The preparation of corticosteroid and contraceptive drugs from the steroidal alkaloid Solasodine extracted from *S. elaeagnifolium* berries (Maiti, 1967). Recent studies have specified other potential uses for *S. elaeagnifolium* as plant extracts have shown moluscicidal and nematicidal activity, as well as cancer-inhibiting activity (European and Mediterranean Plant Protection

Organization, 2007). *Solanum nigrum* is commonly known as blacknight shade and is belongs to Solanaceae family. *S. nigrum* elaborated a wide spectrum of medicinal properties such as antidiuretic, antiseptic, antidysenteric and it has very considerable gastric ulcerogenic activities (Sridhar *et al*., 2011), anticancer and antioxidant (Al-Qirim *et al*., 2008), neuroprotective (Jainu and Devi, 2005).

The species *Fumaria capreolata* belongs to the Fumariaceae family. The protopine, stylopine, fumaricine, isoquinoline alkaloids, fumaritine, fumarophycine, fumarofine and fumariline were specified in *Fumaria capreolata* (Maiza-Benabdesselam *et al*., 2007). It was mentioned that the plant was used as laxative, sedative, tonic, diuretic, anthelmintic, antidyspeptic, blood purifier, cholagogue, and useful of treat diarrhea, abdominal cramps, fever, leprosy and syphilis (Gilani *et al.,* 2005).

Echium angustifolium is a plant species of Boraginaceae family, known mainly that distributed in the Mediterranean region. In traditional medicine, different parts of *Echium* species have been used for demulcent, diuretic rheumatic pain, wound healing, sedative and antioxidants (Ghorbani, 2005; De Natale and Pollio, 2007; Mirdeilami *et al*., 2011). In Turkey, *E. angustifolium* have traditional use due to their wound healing effects and have powerful antioxidant activity against different oxidative systems *in vitro* and analgesic activities in vivo (Tessuro Fujita *et al.,* 1995; Eruygur *et al.,* 2012). Previous studies on Echium species said that they have antibacterial, antiproliferative, antioxidant, anti-inflammatory, anxiolytic, antidepressant, antiviral and cytotoxic properties (Abolhassani, 2004; Moallem *et al*., 2007; Karakaş *et al*., 2012; Farahani *et al.,* 2013).

The aim of this study was to evaluate the antimicrobial activity of microwave-assisted plant extracts of the above mentioned five medicinal plant species against *Pseudomonas aeruginosa, Staphylococcus aureus and Escherichia coli*.

Materials and Methods

Collection of Plant material and identification

Fresh samples of five plant species *Atreplix sembiccata, Solanum elaeagnifolium, Solanum nigrum, Fumaria capreolata and Echium angustifolium* were collected from various positions of the area of wadi Al-

salqa, Gaza Strip, Palestine. Botanical identification of plant material was implemented, the specimens were prepared as herbarium material according to herbarium techniques. Plant voucher specimens were deposited at the Laboratory of Biology Department, Faculty of Science, Al Aqsa University. Fresh plant materials were washed under tap water, dried in shade and then pulverized into fine powder by kitchen blender. Scientific, family and common names and the plant part of the tested plants are given in Table 1.

Table 1: Scientific, family and common names and the plant part of the tested plants

Scientific name	Family	Common Arabic name	Plant part
Atrepilx sembiccata	Chenopodiaceae	قطف	Shoot
Solanum elaeagnifolium	Solanaceae	سجوة زيتية	Shoot
Solanum nigrum	Solanaceae	عنب الديب	Shoot
Fumaria capreolata	Fumariaceae	زر الدجاج المتسلق	Stem, leaves
Echium angustifolium	Boraginacea	زهرة الافعى	Stem, leaves

Microorganisms:

Three bacterial species, gram- positive *Staphylococcus aureus* (methicillin resistant), Gram- negative *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* were used. These microorganisms were acquired from Microbiology Department, El-Shifa Hospital and identified using morphological and biochemical diagnostic test at Medical Technology Department, the Islamic University, Gaza. The bacteria were subcultured overnight at 37 ºC in nutrient agar.

Preparation of Plant Extracts:

Ten grams of dried powder plant material were put into a flask, with 150 ml ethanol 80% (v/v). The flask was exposed for 1 min in a microwave, then it was cooled to room temperature by cooling water. The above steps were repeated 12 times (microwave extractor for 1 min. then cooling) (Hao J. *et al.*, 2002). The extracts were transported into glass watch. Then the

solvent was removed by oven at 45ºC overnight, after that, the extracts sterilized by filtration through a 0.2 membrane filter. The different organic extracts of the plants were dissolved in dimethylsulfoxide (DMSO), 1g/1ml (Essawi and Srour, 2000). The extracts were stored in sterile glass bottles at -20 ºC until use.

Antibacterial Testing:

Antibacterial activity of the crude organic extract of different plants was carried out by the disc diffusion method (Lennette, 1985). The micro-organisms were sub-cultured overnight at 37 ºC in nutrient agar. The bacterial cultures were grown in nutrient broth medium at 37 ºC. After 4 h of growth, each microorganisms were inoculated by streaking the swab over the entire surface of Muller Hinton agar plates. Suspension of bacterial strains with an optical density of 10^6 bacterial cells/ml. Sterile, 6 mm diameter filter paper were impregnated with 10 µl of extract, and positioned on seeded plates. One other sterile blank disk impregnated with DMSO, was used as negative control. After incubated at 37°C for 24 hour, all plates were observed for zones of growth inhibition, and the diameter of these zones was measured in millimeters. Antibiotics; Amikacin, Amoxyclave and chloromphenicol were also used as positive control. The antibiotics and their potency were presented in Table 2.

Table 2: List of the used antibiotics and their potency

Antibiotic	Antibiotic potency (µg)
Amikacin	30
Amoxyclave	30
Minocycline	30
Kanamycin	24
Tetracycline	30
Erythromycin	15
Gentamicin	10
Pinicillin-G	10
Cefruxim	30
Chloromphenicol	30
Clindmicin	10

Determination of Synergistic Effects of Plant Extracts:

This was carried out by using disk diffusion technique (Elbashiti *et al*., 2010; Elkichaoi *et al*., 2015; Jouda *et al*., 2016). The bacterial culture was grown in in nutrient broth medium at 37 ºC. After 4 h of growth, each microorganisms were inoculated by streaking the swab over the entire surface of Muller Hinton agar plates. Suspension of bacterial strains with an optical density of 10^6 bacterial cells/ml. Commercial antibiotic discs were positioned on agar plates that seeded with the tested microorganisms. The antibiotic discs were fertilized with 10 µl of extract. After incubation at 37°C for 24 hour, all plates were observed for zones of growth inhibition, and the diameter of these zones was measured in millimeters and compared with that of the antibiotics alone. Both single and combined extracts of medicinal plants were screened in this Study.

Results:

Determination of Antimicrobial Activity:

The results approved that there was no antibacterial activity in the plant extracts against any of the three bacteria by using Microwave-assisted extraction (MAE) method and 80% (v/v) ethanol as a solvent. Antimicrobial activity as exhibited by the zone of inhibition, were observed by the extracts of *F. caproolata, S. alaeagnifolium, A. sembiccata, E. angustifolium* and, *S. nigrum* tested against the three bacteria (Tables 3).

Determination of Synergistic Effect:

In vitro synerigism between extracts of *S. elaeagnifolium* (shoots), *S. nigrum* (whole plant), *F. capredata* (stem, leaves) and antimicrobial drugs utilized against *E. coli*, *S. aureus* and *P. aeruginosa* strains were estimated by using disc diffusion method .The five plant extracts were tested and showed various degree of synergistic inhibition effect against the three bacterial strains investigated as presented in Tables 3-5**.**

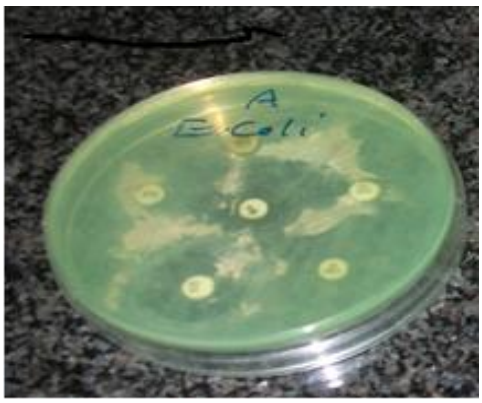
a- Against *Escherichia coli*:

The plant extracts differed from each other in their synergistic ability to inhibit the growth of *E. coli*. Table 3 showed that *S. nigrum* had a synergistic effect with various antibiotics and was able to suppress the *E. coli* growth, however its extract a lone was ineffective. Other plant extracts had various synergistic inhibitory effect with various antibiotics against *E. coli*. The highest significantly synergistic effect was observed with

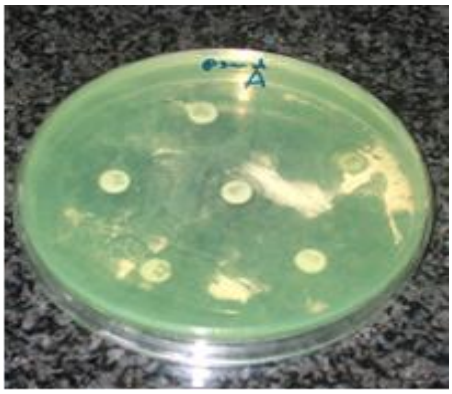
Amoxyclave, with all plant extracts. *F. capreolata*, *A. sembiccata* extracts had a synergistic effect with Amikacin, kanamycin and Minocyclins, however the result shows that no synergistic effect with cefuroxime and Tetracycline. Also *S. alaeagnifolium* and *E. angustifolum* extracts had a synergistic effect with amikacin , kanamycin , Minocycline and tetracycline only (Table 3 & Fig. 1).

Table 3: The Synergistic effect of some plant extracts, extracted by 80% ethanol and microwave-assisted method on *E.coli* (all values in mm)

Antibiotic	Anti. Alone	F. capreolata	S. elaeagnifolium	A. sembiccata	E. angustifolium	S. Nigrum
		Ex +Anti	Ex+Anti	Ex+Anti	Ex+Anti	Ex+Anti
Amikacin	22	25	26	25	26	24
Kanamycin	12	14	7	15	13	12
Minocycline	-	8	11	10	9	10
Cefuroxime	-	-	-	-	-	7
Tetracycline	-	-	8	-	9	7
Amoxyclave	-	14	10	14	12	14



A



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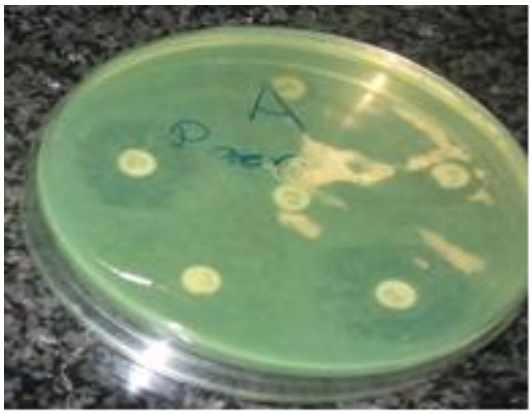

C

Figure1: Synergistic effect assay plate *for Atriplex sembiccata* extract on growth of (A) *E. coli* ,(B) *S. aureus* and (c) *P. aeruginosa* showing wide zone of inhibition**.**

b- Against *staphylococcus aureus*

The synergism rate differed significantly in their synergistic ability to **i**nhibit the growth of *S. aureus*. The result approved that there was no synergistic effect with minocycline, Tetracycline and kanamycin with any plant extracts. *S. elaeagnifolium* extracts had the highest synergic inhibitory effect only with Erythromycin and Clindmicin with inhibition zone of (21,

15 mm) respectively. Also *S. nigrum* and *E. angustifolium* extracts had a synergistic effect with Gentamicin (Table 4).

Table 4: The synergistic effect of the plant extracts, with different antibiotics on *S. aureus* (all values in mm).

Antibiotic	anti. alone	F. capreolata	S. elaeagnifolium	A. sembiccata	E.. angustifolium	S. nigrum
		Ex +Anti	Ex +Anti	Ex+Anti	Ex+Anti	Ex+Anti
Minocycline	**14**	**8**	**10**	**8**	**10**	**10**
Tetracycline	**12**	**11**	**10**	**12**	**10**	**11**
Kanamycin	**15**	**10**	**9**	**11**	**12**	**11**
Erythromycin	**-**	**-**	**21**	**-**	**-**	**7**
Clinamycin	**-**	**-**	**15**	**-**	**-**	**-**
Gentamicin	**22**	**24**	**21**	**23**	**25**	**26**

c- Against *Pseudomonas aeruginosa*

F. caprolata extract had an inhibitory synergistic effect against *P. aeruginosa* more than the antibiotic chloramphenicol. *A. sembiccata* had synergistic effect with Tetracycline (13mm), Amikacin (27mm) for *E. angustifolium* and *S. nigrum* . Even though this bacterium was resistant against the majority of the antibiotics tested (Table 5 and Fig. 2).

Table 5: The synergistic effect of the plant extracts on *P. aeruginosa* (all values in mm).

Antibiotic	anti. alone	F. capreolata	S. alaeagnifolium	A. sembiccata	E. angustifolium	S. nigrum
		Ex +Anti	Ex+Anti	Ex+Anti	Ex+Anti	Ex+Anti
Tetracycline	**10**	**9**	**10**	**13**	**11**	**10**
Amikacin	**26**	**26**	**25**	**24**	**27**	**27**
Chloromphenicol	**14**	**19**	**15**	**13**	**18**	**15**
Erythromycin	**-**	**-**	**-**	**-**	**-**	**7**
Gentamicin	**25**	**21**	**21**	**24**	**25**	**24**
Penicillin-G	**-**	**-**	**-**	**-**	**-**	**-**

(-) no inhibition zone, Anti: antibiotic, Ex: extract.

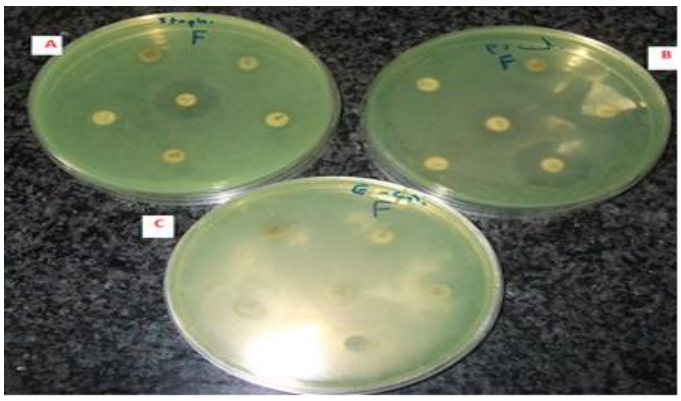

Figure **2:** Synergistic effect assay plate for *Fumaria caprolata* extract on growth of (A) *S. aureus*,(B) *P. aeruginosa* and (c) *E. coli* showing wide zone of inhibition.

The effect of plant extracts combination:

The synergistic effects of combined Microwave-assisted ethanol extracts of *(Fumaria caprolata)*, Ex_1 and (*Solanum nigrum*) Ex_2 with antibiotics were screened against the tested bacteria; Methicillin-resistant *S. aureus* (MRSA) strain, *P. aeruginosa* and *E. coli*.

The synergistic effect of the two combination extracts inhibited the growth of *E. coli* bacteria particularly with antibiotics Minocycline, Amoxyclave and Tetracycline aganist (*E. coli* resistance to these commercial antibiotics), and the diameter of inhibition zones ranged between 10-12 mm. However, the combination of two extracts did not show any antimicrobial effect alone. Also, the synergistic effect against *P. aeruginosa* had mild inhibition with inhibition zones 30 mm, 25 mm with Amikasin and Chloromphenicol antibiotics respectively.

Results also, show that the synergistic effect of the two combination extracts had low inhibition zones against *S. aureus*, the highest synergistic inhibitory effect only with Gentamicin which increased from 22 mm to 26 mm (Table 6).

Table 6: The synergistic effects of combination two plant extracts with the antibiotics on three bacteria species. (-) no inhibition zone, Anti: antibiotic, Ex_1:extract 1 *(Fumaria caprolata)*, Ex_2 *: (Solanum nigrum)*

E .coli			***S.aureus***			***P.aeruginosa***		
Antibiotic	**Anti alone**	**Ex_1+Ex_2 +Anti**	**Antibiotic**	**Anti alone**	**Ex_1+Ex_2 +Anti**	**Antibiotic**	**Anti alone**	**Ex_1+Anti +Ex_2**
Amikacin	22	28	**Minocycline**	14	8	**Tetracycline**	10	12
Kanamycin	12	13	**Tetracycline**	12	10	**Amikacin**	26	30
Minocycline	-	10	**Kanamycin**	15	16	**Chloromphenicol**	14	25
Cefruxim	-	-	**Erythromycin**	-	-	**Erythromycin**	-	-
Tetracycline	-	12	**Clindmicin**	-	-	**Gentamicin**	25	27
Amoxyclave	-	10	**Gentamicin**	22	26	**Pinicillin**	-	-

Discussion:

Assessment of Antimicrobial activity:

In this study the Microwave-assisted ethanolic extracts of the five plants revealed varying levels of antibacterial (enhancement synergistic effect), while the results of antimicrobial activity indicated that the crude extracts of the species studies showed no effect against any of gram positive and gram negative, this results may be due to the extraction method, the type of secondary metabolites and the type and/or the concentration of the extracting solvent (Rinez *et al.*, 2012), or may be due to the little dose of the extracts (10 µL). Another reason for this findings could be that all of the identified active components from these plants active against microorganisms, dissolve in water or methanol solvent not ethanol. This results agree with other observations that no susceptibility of bacteria to the water extracts as determined by diffusion (Abu–Shanab *et al.*, 2004). The microorganism *E. coli*, which is already known to be multi–resistant to drugs, was also resistant to the plant extract tested.

Evaluation of synergistic effect of tested medicinal plants

The plant extracts differed significantly in their synergistic ability to inhibit the growth of *E.coli*. Our results showed that *S. nigrum* end *E. angustifolium* had the highest synergistic effect with various antibiotics and were able to suppress the *E. coli* growth and their extracts alone were ineffective (Table 3). These results agreed with Elbashiti *et al.,* 2011, among the seven plant extracts (i.e. *Cakile maritime* (root and shoot), *Mesembryanthemum crystallinum* (whole plant), *Marrubium vulgare* (stems), *Marrubium vulgare* (leaves), *Atriplex halimus* (leaves) and *Cakile maritime* (root and shoot) added as crude extract of 10 µL /well, the plant extracts differed in their synergistic ability to inhibit the growth of *E.coli* and *M. vulgure* stems and leaves) had the most synergistic effect against *E. coli*. Also the observed resistance of *E. coli* to the prepared extracts of the five studied plants could be due to the cell membrane permeability barrier, membrane accumulation mechanisms or due to other genetic factors (Ashour and El-Astal 2005).

The synergistic capacity against *P. aeruginosa* was promising for the extracts of some plants such as *S. nigrum* and *E. angustifolium* which had the most synergistic inhibitory effect against *P. aeruginosa* which presented synergism with most drugs. *S. nigrum* is well known for its antifungal and antiulcer activity (Sridhar *et al.,* 2011).

This result also showed that *F. caprolata* had a synergistic effect with various antibiotics and was able to suppress the *P. aeruginosa* growth while their extracts alone were ineffective. The highest synergistic effect was observed with Chloramphenicol (19mm). Also *A. sembiccata* had synergistic effect with Tetracycline (13mm).

The synergistic effect of combined extracts of (*Fumaria caprolata*) and (Solan*um nigrum)* against *E. coli* and *P. aeruginosa* (Gram negative organism) was more potent than *S. aureus* (Gram-positive organism).

Conclusions

Our results revealed that:

1. The microwave-assisted extraction method has great potential for plant extraction as a very rapid method compared with the other methods.

2. The use of plant extracts together with antibiotics in treating infectious diseases could be helpful against particularly the resistant pathogens.

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