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The Antimicrobial Effects of *Boswellia Carterii*, *Glycyrrhiza Glabra* and *Rosmarinus Officinalis* some Pathogenic Microorganisms

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Abstract

Evaluate the antimicrobial activity of *Boswellia carterii* (frankincense), *Glycyrrhiza glabra* (Licorice) and *Rosmarinus officinalis* (Rosemary) against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *klebsiella pneumoniae*. The extracts were prepared using soxhlet apparatus for 8 hours. Evaluation of antimicrobial activity was carried out using disc diffusion method and Minimum inhibition concentration method (MIC) at different concentrations for (200mg/ml - 0.39mg/ml) for all tested bacteria. The results of this study showed that the D.W, ethanol and chloroform extracts of rosemary showed the lowest minimum inhibitory concentration against *S. aureus* and all extracts of licorice against *K. pneumonia*. However, extracts of frankincense showed a broad change in values from (12.5mg/ml) to (50mg/ml). These results illustrate the importance of tested the plant extracts against pathogenic organisms as the first step in the path to further encourage the use of natural extracts as alternative method to chemical treatment.

Keywords:

Antimicrobial activity, Plant extracts, Minimum inhibition concentration method, Disc diffusion method.

1. Introduction:

Medicinal plant has a significant role for people's health. As the medical value resides in being able to produce, chemicals that plays a clear role in the physiological functioning of the human body (Hill, 1952). In Africa and some Asian countries, there has been widespread use of folk medicine, (Akpanabiatu *et al.*, 2005; El Kichoi *et al.*, 2016) Where doctor's traditionalists provide health care for the majority of patients, while developing countries rely on preventive orientation (Agbor *et al.*, 2007). Since ancient Egyptians were familiar with medicinal herbs and are aware of the enormous benefits to them (Manniche, 1999). In the West Bank and the Gaza Strip last three decades showed that there is a

rise in the types of diseases spread by 600 species of which 90 species are rare cases (ARIJ, 2007). Palestinian plants characterized by their ability to resist many types of bacteria. (Adwan & Mhanna, 2008). One of the major causes of health problems for humans and loss of plant and animal is the infection by bacteria and fungi. *Glycyrrhiza glabra*, *Boswellia carteri* and *Rosmarinus officinalis* are useful medicinal plants (Abeyasinghe & Weeraddana, 2011). Active components of licorice is the most study where it is characterized as a sweet taste, and for more than 4,000 years there has been use of the roots of licorice types of plants (Aoki, 2005).

The genus of what? Consists of 30 species of liquorice, (Fukai *et al.*, 2003) where the use of these species in the medical treatment of many health problems and numerous injuries. (Cherng *et al.*, 2006). Rosemary, *Rosmarinus officinalis* L. (Lamiaceae) is one of the perennial trees that spread in the world, which is widely used spice. (Al-Sereiti, *et al.*, 1999; Bai *et al.*, 2010; Harach *et al.*, 2010); in addition, their ability to treat variety of health problems, including respiratory disorders, and it is antispasmodic and renal colic, (Takaki *et al.*, 2008), and be used for oral health and colds. In addition, on the other level, there was a great use of the resin from the *Boswellia carteri* and *Boswellia serrata* against rheumatoid arthritis. (Erenmemisoglu *et al.*, 1997; Sotelo-Felix *et al.*, 2002 Koga *et al.*, 2006; Dearlove *et al.*, 2008; Gutierrez *et al.*, 2010; Harach *et al.*, 2010; Nabekura *et al.*, 2010).

The relationship of the study between plants and people (Ethnobotany) are employed in choosing the plants for the development of pharmaceutical industries (Saranraj & Sivasakthi, 2014). One of the important sources of the natural product is plant kingdom due to both medicinal and economical values (Kharjul, *et al.*, 2012). The first step to development of pharmacological agents to treat diseases is the *in vitro* antibacterial activity assay. The search for new antimicrobial agents is of great concern today due to the increasing development of drug resistance to human pathogens and the appearance of unwanted effects of certain antifungal agents (Höfling, *et al.*, 2010).

2. Materials and methods:

2.1 Chemicals:

Nutrient agar (NA), Nutrient Broth (NB), 80%Ethanol, Chloroform, Distilled water (D.W) and Dimethyl Sulphoxide (DMSO).

2.2 Plant collection:

Boswellia carterii (frankincense), *Glycyrrhiza glabra* (Licorice) and *Rosmarinus officinalis* (Rosemary) plants were collected from several areas of the Gaza Strip and used in this study.

2.3 Preparation of extraction:

The plant parts used for our research roots and leafs. Then all the dried parts cut into small pieces.

- 40 grams of these pieces extracted in a Soxhlet extractor using 200 ml of 80%ethanol, chloroform, and water for 3-8 hours.

- The resulting extracts were evaporated using oven at temperature 55°C for 3 days.
- Then all extracts were dissolved in DMSO.
- One gram of each extract was dissolved in 5 ml of DMSO. Thus, 200 mg / ml of stock was obtained as a standard concentration of extracts.
- Then extracts were sterilized using 0.45 µm syringe filters and all samples were maintained at 4°C until the usage time (Abeysinghe & Weeraddana, 2011).

2.4 Antimicrobial susceptibility test:

2.4.1 Microorganisms:

Microorganisms, which have been used in this study, are the bacteria (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *klebsiella pneumonia* and *Bacillus subtilis*) which were isolated from clinical samples delivered from El-Shifa Hospital. *S. aureus*, *E.coli* isolates were identified in microbiology laboratories of Islamic university-Gaza, by Stafsistem 18 R Kit for *S. aureus*, Enterosystem 18R Kit for *E. coli*.

2.4.2 Antimicrobial test:

2.4.2.1 Agar disc-diffusion method:

Agar disc-diffusion method was followed to determine the antimicrobial activity. A suspension of inoculum of pathogenic microorganisms was introduced to Nutrient agar (cooled to 40-45°C) swirl gently to mix well. After solidification, sterile filter paper discs approximately 6mm in diameter were impregnated with stock extracts of concentration (200 mg/ml) and placed on the surface agar plate. Incubation period of 24h at 37°C for bacteria. The antimicrobial activity was evaluated by measuring zones of inhibition of microbial growth surrounding the plant extracts (Sharma, 2011).

2.4.2.2 Antibiotic sensitivity test:

Antibiotic sensitivity test was performed using disc diffusion method. The selected antibiotics Table 1 were inoculated on the surface of plate and the plates were incubated at 37°C for 24 h. Then the zones of inhibition were measured in millimeter by using ruler (Elbashiti *et al.*, 2011).

Table 1 The antibiotics and their potencies

Antibiotics	<i>B. subtilis</i>	<i>S. aureus</i>	<i>K. pneumonia</i>
CN30	16	16	-
CXM30	-	19	-
GEN10	20	20	20
OXI	-	20	-
NA30	20	20	17
RIF5	15	30	-
CAZ30	-	-	-

(-): Non inhibition zone.

2.4.2.3 Minimum Inhibitory concentration:

ANTIBACTERIAL ASSAY: The activity of extracts against microorganism was also determined by the broth minimum inhibitory concentration method (96- well plates). Extracts were diluted a number of times through a sterile diluent (NB) after were diluted the obtained concentration ranges were from (200mg/ml - 0.39mg/ml). Then, 10 µl of inoculum was added and incubation to overnight growth microorganisms to each well except a positive control. Extract with media was used as a positive control and inoculum with media was used as a negative control. The test plates were incubated at 37°C for 24 h. After 18 h 50 µl of a 0.4% solution of 2, 3, 5 triphenyl tetrazolium chloride (TTC) as indicator was added to the wells and the plate was incubated for another hour. Since the colorless tetrazolium salt is reduced to red colored product by biological active bacteria, the inhibition of growth can be detected when the solution in the well remains clear after incubation with TTC (Shanab et al., 2004).

3. Results:

3.1 Bioactivity of plant extract by disc diffusion method:

Table 2 showed the results of the antimicrobial activity by disc diffusion method against tested microorganisms. For *S. aureus*, the Distilled water extract of *R. officinalis* showed the highest effect with an 18.5 mm zone of inhibition followed by chloroform extract of *R. officinalis* with an 18mm zone of inhibition. *G. glabra* extracted by ethanol and chloroform showed moderate activity. No antimicrobial activity was observed by *B. carterii* extracted by D.W, ethanol, and chloroform. For *B. subtilis*, the distilled water extract of *R. officinalis* was showed the highest effect with a 15.5 mm zone of inhibition followed by the chloroform extract of *R. officinalis* with a 15 mm zone of inhibition. The ethanolic extract of *R. officinalis* and *G. glabra* also showed a good activity with a zone of inhibition (14.5mm). For *P. aeruginosa*, *E.coli* and *K. pneumonia*,

extracted by D.W, ethanol and chloroform for *R. officinalis*, *G. glabra*, *B. carterii* observed no antimicrobial activity.

Table 2 Antimicrobial activity of plant extracts by disc diffusion method

MO	Solvents	Disc diffusion method "mm"		
		B	G	R
<i>S. aureus</i>	D.W	-	10	18.5
	E	-	13.5	15
	Ch	-	13	18
<i>B. subtilis</i>	D.W	8	8	15.5
	E	7.5	14.5	14.5
	Ch	7.5	11.5	15

(-): no clear zone, B: *Boswellia carterii*, G: *Glycyrrhiza glabra*, R: *Rosmarinus officinalis*, D.W: Distilled water, M: 80% Ethanol, Ch: Chloroform.

3.2 Bioactivity of plant extract by micro-dilution method:

MIC values of all tested plant extracts against tested microorganisms are summarized in Table 3. After calculation the mean of MIC value for all microorganisms which effected by plant extract, and the best results for *P. aeruginosa*, *B. subtilis*, *K. pneumonia*, *S. aureus* and *E. coli* respectively and a shown in Figure 1.

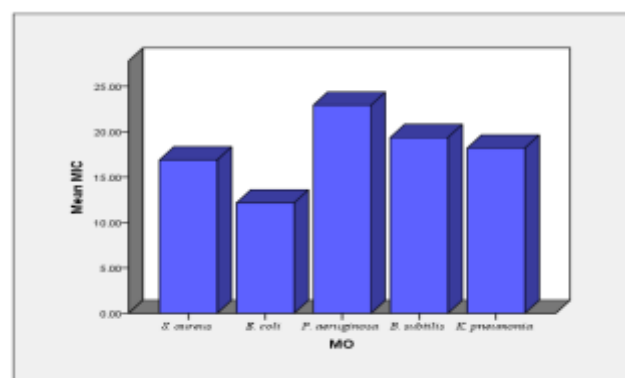


Figure 1 Show the mean value of MIC for all MO

For *S. aureus* the MIC of the chloroform and ethanol extract of rosemary and licorice showed very strong activity with the best MIC (0.39mg/ml). Also D.W extract of rosemary showed the same result. Followed by D.W extract of licorice and ethanol extract of frankincense with MIC (25mg/ml). MIC of the D.W and chloroform of frankincense showed moderate activity with MIC (50mg/ml). For *E. coli* the MIC of the D.W and ethanol extract of rosemary showed very strong activity with the best MIC (1.56mg/ml).

Followed by chloroform extract of rosemary and licorice with MIC (3.125mg/ml).

MIC of the ethanol and D.W extract of licorice showed good activity with MIC (12.5mg/ml) and the MIC value of frankincense extracted by D.W, ethanol and chloroform also showed with MIC (25mg/ml). The MIC value of chloroform extracts of licorice and rosemary showed good activity with MIC (3.125mg/ml).

For *P. aeruginosa* the MIC of D.W and ethanol extract of licorice showed good activity with MIC (3.125mg/ml) and MIC value of D.W extract for rosemary and chloroform extract for licorice were (6.25mg/ml). In addition, the MIC of ethanol extract for rosemary and D.W extract for frankincense showed good results with MIC (12.5mg/ml) and (25mg/ml) respectively. However, MIC value of ethanol and chloroform extracts for frankincense and chloroform extract for rosemary showed low activity with MIC (50mg/ml). For *B. subtilis* the best activity of extracts were ethanol and chloroform extract of licorice with MIC (0.39mg/ml).

Then D.W extract for licorice and ethanol extract for rosemary with MIC (1.56mg/ml) and (3.125mg/ml) respectively. Moreover, for D.W extract for frankincense and licorice MIC value showed moderate activity with MIC (12.5mg/ml) and (6.25mg/ml) respectively.

Nevertheless, the MIC showed low activity of ethanol and chloroform extract for frankincense with MIC (25mg/ml) and very low activity of chloroform extract for rosemary with MIC (100mg/ml). Finally, for *K. pneumonia* the best activity for MIC value of D.W, ethanol and chloroform extracts of licorice with MIC (0.39mg/ml) and showed moderate results of D.W extract for frankincense and rosemary, and ethanol extract for rosemary with MIC (2.15mg/ml).

However, ethanol and chloroform extracts for frankincense and chloroform extract for rosemary the MIC showed low activity with (50mg/ml) and (25mg/ml) respectively.

Table 3 Antimicrobial activity of plant extracts by MIC method

MO	Solvent	MIC "mg/ml"
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		B	G	R
<i>S. aureus</i>	D.W	50	25	0.39
	E	25	0.39	0.39
	Ch	50	0.39	0.39
<i>E. coli</i>	D.W	25	12.5	1.56
	E	25	12.5	1.56
	Ch	25	3.125	3.125
<i>P. aeruginosa</i>	D.W	25	3.125	6.25
	E	50	3.125	12.5
	Ch	50	6.25	50
<i>B. subtilis</i>	D.W	12.5	1.56	6.25
	E	25	0.39	3.125
	Ch	25	0.39	100
<i>K. pneumonia</i>	D.W	12.5	0.39	12.5
	E	50	0.39	12.5
	Ch	50	0.39	25

B: *B. carterii*, G: *G. glabra*, R: *R. officinalis*, D.W: Distilled water, M: 80% Ethanol, Ch: Chloroform

4. Discussion:

The current study aimed to detect the antibacterial effect of *B. carterii*, *G. glabra* and *R. officinalis* extracts against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and *B. subtilis* "which have multi-resistance characteristic against antibiotics". Susceptibility of each plant extracts were tested by serial microdilution method (MIC) and agar disc diffusion method.

All extracts showed a low value in MIC and these results obtained are not in agreement with the previous study (Adwan & Mhanna), which explained this result that crude extracts have many different phytochemicals which might inhibit bacteria by different mechanisms (Adwan & Mhanna, 2008). Secondary metabolites in plant such as carotenoids, flavonoids, vitamin, alkaloids and pigments which have biological significance and may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modifications and decrease intracellular drug accumulation (Abeyasinghe & Weeraddana, 2011). Our experiments showed that all ethanol, chloroform and distilled water of licorice were active against the tested microorganism.

In this study, crude extracts appear to be more potent inhibitor against Gram-positive bacteria than Gram-negative bacteria, which could be due to the difference in the structure of the bacterial cell wall.

Our results were consistent with previous in vitro study which reported the plant antibiotic substances effect appear to be an inhibitor to Gram-positive than Gram-negative bacteria (Elbashiti, et al., 2011). In conclusion, this study revealed that the licorice contain potential antimicrobial components that may be of great use for

the development of pharmaceutical industries as a therapy against various diseases such as skin and teeth infections. The results of the study may support the development of new antimicrobial drug from tested plant extracts. In vitro, our results revealed that crude extracts proved their effectiveness with acceptable degree in control of growth some pathogenic microorganism however in vivo experiments are needed to confirm these results.

5. Conclusion:

In conclusion, the results of present study were confirmed that the use of plant extract maybe substantial solution for many problem of multi drug resistance bacteria such as used the licorice root and rosmary leaf against many pathogenic bacteria like *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumonia* and *B. subtilis*, which confirmed by these study.

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التأثير المضاد للبكتيريا لكل من شجرة اللبان، عرق السوس وإكليل الجبل ضد بعض أنواع من الكائنات الدقيقة الممرضة

كلمات مفتاحية:

النشاط الضد ميكروبي.
المستخلصات النباتية.
طريقة حساب أقل تركيز مثبط.
طريقة الإنتشار في القرص.

صممت هذه الدراسة لعمل فحوصات مخبرية لقياس مدى تأثير بعض المستخلصات وعلى رأسها اللبان، عرق السوس وإكليل الجبل على بعض أنواع البكتيريا الممرضة المقاومة للمضادات الحيوية. تم إستخلاص المواد الفعالة من الأجزاء الهوائية المجففة للنباتات بإستخدام جهاز السوكسلت لمدة 8 ساعات. تم فحص تأثير هذه المستخلصات على البكتيريا المذكورة بإستخدام تقنية الإنتشار في القرص، بينما تم تحديد أقل تركيز مثبط بإستخدام تقنية التخفيف الجزئي الدقيق مع ضبط تركيز من 0.39 ملغرام لكل ملل إلى 200 ملغرام لكل ملل، لجميع أنواع البكتيريا. أظهرت النتائج أن المستخلصات المانية، الإيثانولية، والزيوت العطرية لنبات إكليل الجبل أقل أدنى تركيز ضد بكتيريا المكورات العنقودية الذهبية، ونبات عرق السوس ضد بكتيريا الكلبسيلا الرئوية. حيث أن نتائج هذه الدراسة توفر معلومات أولية حول إمكانية إستخدام هذه المستخلصات النباتية والزيوت العطرية في علاج الأمراض والالتهابات التي تسببها هذه البكتيريا المقاومة لعدة مضادات حيوية مما يشجع على استخدام المستخلصات الطبيعية كطريقة بديلة للعلاج الكيميائي.