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Efficacy of Aqueous and Ethanol Extracts of Some Palestinian Medicinal Plants for Potential Antibacterial Activity

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Abstract: Nine medicinal plants growing in Palestine were screened *in* vitro for potential antibacterial activity against 6 bacterial strains by well diffusion and micro-dilution techniques. Both aqueous and organic solvents were used. The dried extracts of Sacropoterium spinosum (Rosaceae) (seed), Ruta chalepensis L (Rutaceae) (leaf), Cassia senna (Ligumenosa) (leaf), Lawsonia inermis (Lythraceae) (leaf), Psidium guajava (Myrtaceae) (Leaf), Carataegus azerullus (Rosaceae) (Leaf), Ranunclus asiaticus(Ranunculaceae) (Flowers), Calendula officinalis (Composita) (Flowers),and Salvia syriaca (Labiatae) (leaf) were screened. The bacterial strains tested were; Methicillin-resistant Staphylococcus aureus (MRSA); three strains (1, 2 & 3), multidrug resistant Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumonia. The average diameter of inhibition zones ranged from 9 to 30 mm and 11 to 28 mm for aqueous and ethanol extract, respectively. Methicillin-resistant Staphylococcus aureus (MRSA) was the most inhibited microorganism. Sacropoterium spinosum extract was the most active against Methicillin-resistant Staphylococcus aureus (MRSA) and multidrug resistant Pseudomonas aeruginosa. The MIC value of ethanol extract was 0.781 mg/ml against MRSA while 0.390 mg/ml against Pseudomonas aeruginosa. The combination effect of ethanol extracts of (Sacropoterium spinosum with Lawsonia Inermis) on bacterial species tested exhibited a higher effect than that of any individual extract.

Such results lead to an interesting promise for further investigation to design potentially active antibacterial augmentative agents of natural sources.

Key words: Antibacterial activities, plant extracts, methicillin resistant Staphylococcus aureus, multi-drug resistant Pseudomonas aeruginosa, Palestine.

تأثير المستخلصين (المائي والكحول الايثيلي) لبعض نباتات فلسطين كمضادات حيوية

ملخص: تم في المختبر دراسة تأثير تسعة أنواع من مستخلصات النباتات الطبية التي تنمو في فلسطين (المستخلص المائي ومستخلص الكحول الايثيلي) كمضادات حيوية محتملة ضد 6 سلالات

من البكتيريا بطريقة انتشار الحفرة وطريقة التركيز المانع الأدنى. أما خلاصة الأجزاء الجافة للنباتات التي تمت دراستها فكانت للنباتات التالية: : البلان (بذور النتش)، الفيجن (الاوراق)، سنامكة (الاوراق)، الحناء (الاوراق)، الجوافة (الاوراق)، الزعرور (الاوراق)، الأقحوان (الزهور)، شقائق النعمان (الزهور)، ونبات الخويخة (الاوراق). وأما السلالات البكتيرية التي جرى اختبارها فكانت: البكتيريا العنقودية المقاومة للميثيسيللين (ثلاثة سلالات – 1، 2، 3)، بكتيريا سيدومونا س ايروجينوزا المتعددة المقاومة للدواء، بروتيس فلجارس، وكلبسيلا نيومونيا. أظهرت النتائج تراوحا في معدل قطر المنطقة المانعة وكان يتراوح من (9- 30 ملم) و(11- 28 ملم) لكلا المستخلصين (المائي والكحول الايثيلي) على التوالي. وقد كانت البكتيريا العنقودية المقاومة للميثيسيللين هي الأكثر حساسية. وكان مستخلص نبات البلان هو الأكثر تأثيرا على البكتيريا العنقودية المقاومة للميثيسيللين وبكتيريا سيدومونا س ايروجينوزا المتعددة المقاومة للدواء. وظهر من النتائج أيضا أن التركيز المانع الأدنى لمستخلص الكحول الايثيلي كان (78، 0 ملغم/مل) ضد البكتيريا العنقودية المقاومة للميثيسيللين، و(39 ،0 ملغم/مل) ضد بكتيريا سيدوموناس ايروجينوزا. كما أن تأثير المجموعات المستخلصة بواسطة الايثانول للنباتين (ساكروبوتيريوم سبينوسوم مع لاوسونيا ينيرميس) على سلالات الجراثيم المستخدمة كان الأعلى من أي تأثير لأي مستخلص فردي.

إن مثل هذه النتائج تؤدي إلى المزيد من الاهتمام لتحقيق الوعد في البحث من أجل تكوين مضادات حيوية معززة ونشطة من مصادر طبيعية.

Introduction:

Since long time, medicinal plants and herbs were used intensively in folkloric medicine for treatment of various diseases. The scientific experiments which have been carried out on antimicrobial properties of plant components were first documented in the late 19^{th} century (1). Today they are the basic source of knowledge of modern medicine.

In recent reports, multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease (2,3). For this reason, the antibacterial effects of plant extracts have been studied in different parts of the world by a very large number of researchers (4-6).

Nowadays, there is a widespread interest in drugs derived from plants. This interest primarily stems from the belief that medicinal plants are safe and dependable, compared with synthetic drugs that are costly and have adverse effects. More than 50% of all modern clinical drugs are of natural product origin (7). Natural products play an important role in drug development programs in the pharmaceutical industries (8). Therefore, there has been a great shift from the prescription of antibiotics to the use of medicinal plants.

In the present study, 9 different medicinal plants were evaluated for their antibacterial properties as well as evaluation of efficacy of some extracts' combination on 2 different species of Gram-positive and Gram-negative bacteria.

Materials & methods:

Plant collection and Extract preparation:

The plant parts used in this study were collected from the mountains and hills of northern Palestine. Dr. Firas. D. Sawalha, Department of Plant Production, Faculty of Agriculture, An-Najah National University, Nablus, Palestine, identified these plants. The fresh plant parts have been washed under running tap water, air dried, then homogenized to fine powder and stored in airtight bottles.

Thirty grams of the air dried fine powder were extracted with hot distilled water and 90% ethanol. The extracts were filtered through Watman No. 2 filter paper by suction with vacuum pump. After filtration of total extracts, the extracts were evaporated to dryness in vacuo and weighed, then stored at 4 ºC for further studies.

Microorganisms:

Six strains of bacteria were tested in this study; they were:

Methicillin-resistant Staphylococcus aureus (MRSA); three strains 1, 2 and 3, *multidrug resistant Pseudomonas aeruginosa, Proteus vulgaris and Klebsiella pneumonia.*

Antibacterial activity tests:

Determination of antibacterial activity was done by the well diffusion method (9). Petri plates of Mueller Hinton agar media were prepared. A 24 h culture of different bacterial strains was seeded into Petri plates. The inoculum size was adjusted in order to deliver a final inoculum of approximately 10^8 colony-forming units(CFU)/ml. Triplicates of each concentration for each bacteria species were prepared. Wells of (6 mm diameter) were punched in the culture media using sterile cork borers. The test plant extracts were introduced into the wells (50 µl) in a concentration of 5mg /well and all inoculated plates were incubated at 37 ºC for 24 h. Antibacterial activity was determined by measuring the diameter of the inhibition zone formed around the well and the mean values are presented in (Table 1). A standard disk of tetracycline (30 µg), was used into plates as a positive control and a negative control was also incloded. The Antibacterial activity was also determined for combined plant extracts of aqueous and ethanol, in a mixing ratio of (1:1) on two different bacterial strains as presented in (Table 2).

The micro dilution technique was performed to determine the MIC (Minimum Inhibitory Concentration) using serially diluted (2-fold) plant extracts. (10). The tested ethanol extracts of plants were as the following: *Sacropoterium spinosum, Lawsonia inermis* and *Psidium guajava*. The MIC values are presented in (Table 3). Bacterial inoculums were adjusted to the turbidity of the 0.5 McFaraland standard, so as to contain approximately 10^5 Colony-Forming Units (CFU) /ml. 50µl of the standard microbial broth culture were introduced into the wells using a micropipette. The test plates were incubated at 37 ºC for 18 h.

Results:

Both single and combined extracts of medicinal plants were screened in this study. The single aqueous and ethanol extracts of 9 screened medicinal plants belonging to 8 families were tested against 3 Gram positive bacterial strains and 3 of Gram-negative. The antibacterial activities of single aqueous and ethanol extracts of screened medicinal plants in this study are presented in (Table 1). The inhibitory properties (inhibition zone diameter in mm) of aqueous and ethanol extracts on different bacteria varies, depending on bacterial species and type of extract.

The largest zone of inhibition (30mm) was observed from the single aqueous extract of *Sacropoterium spinosum* against *MRSA strain 2,* while (27mm) from ethanol extract of *Sacropoterium spinosum* against *MRSA strain 2*.

The inhibition zones diameter obtained from the screened plant extracts ranges from 9 to 30 mm and 11 to 28 mm for aqueous and ethanol extract, respectively.

Furthermore, the antibacterial activities of combined aqueous and ethanol extracts of *Sacropoterium spinosum, Lawsonia inermis* and *Psidium guajava*, (being the most active against tested microorganisms) were screened against *Methicillin-resistant Staphylococcus aureus (MRSA) strain 2*, and *multidrug resistant Pseudomonas aeruginosa*. Their antibacterial activities are presented in (Table 2).

The combined ethanol/ethanol extracts of (*Sacropoterium spinosum and Lawsonia inermis*) against *MRSA stain 2* showed the largest inhibition zone (28mm) thus produce a synergistic effect on *MRSA strain 2*. Synergistic effects also have been seen against *P. aeruginosa* from the Ethanol/ Ethanol extract combination of (*Sacropoterium spinosum and Lawsonia inermis*) and from the Aq/ Aq combination of (*Sacropoterium spinosum and Psidium guajava*). In general the inhibition zone diameter of the combined aqueous and ethanol extracts of the screened plants ranges from 14-28 mm.

The MIC (Minimum Inhibitory Concentration) of the ethanol extracts of *Sacropoterium spinosum*, *Lawsonia inermis* and *Psidium guajava* against two bacterial strains is presented in (Table 3). The MIC fells in the range of

0.781- 3.125 mg/ml for *MRSA strain 3*, and 0.390- 3.125 mg/ml for *P. aeuroginosa*. The ethanol extract of *Sacropoterium spinosum* showed the most potent inhibition property against *P. aeruginosa* having MIC value of 0.390 mg/ml.

Table 1. Inhibitory properties (inhibition zone diameter in mm) of single aqueous and ethanol extracts of screened medicinal plants on different bacteria.

Test plants	Bacterial Species											
	S.aureus* *Strain 1		***S.aureus* *Strain 2***		***S.aureus* *Strain 3***		***P. aeruginosa***		***Proteus***		***Klebsiella***	
	Aq	**E**	**Aq**	**E**	**Aq**	**E**	**Aq**	**E**	**Aq**	**E**	**Aq**	**E**
Sacropoterium spinosum	21	20	30	27	20	20	25	21	6	17	16	17
Ruta chalepensis	6	6	6	6	6	6	6	6	6	6	6	6
Cassia Senna	6	6	6	6	6	6	14	6	6	6	6	11
Lawsonia inermis	17	21	17	25	15	20	24	22	15	15	15	18
Psidium guajava	13	15	20	23	15	15	6	17	6	6	9	15
Carataegus azerullus	6	12	9	18	6	14	6	6	6	6	6	15
Ranunculus asiaticus	-	-	-	-	6	6	6	6	6	6	6	6
Calendula officinalis	-	-	-	-	6	12	6	6	6	6	6	6
Salvia syriaca	-	-	-	-	6	6	6	6	6	6	6	6
Tetracycline (30 µg)	25		32		22		13		25		15	

Aq: Aqueous extract; E: Ethanol extract Includes diameter of well (6 mm).

Table 2. Inhibitory properties (inhibition zone diameter in mm) of combined plant extracts of aqueous and ethanol extracts on two bacterial strains.

Test plants	Combined extracts	Bacterial species	
		S. aureus starin 2	*P. aeruginosa*
Sacropoterium spinosum/Lawsonia inermis	Aq/Aq	27	24
Sacropoterium spinosum/Lawsonia inermis	E/E	28	27
Sacropoterium spinosum/ Psidium guajava	Aq/Aq	25	27
Sacropoterium spinosum/ Psidium guajava	E/E	27	20
Lawsonia inermis/ Psidium guajava	Aq/Aq	20	14
Lawsonia inermis/ Psidium guajava	E/E	22	20
Tetracycline(30 µg)		32	13

Combined plant extracts: 50 µl (1:1 mixing ratio).
Aq: Aqueous extract; E: Ethanol extract
Includes diameter of well (6mm).

Table 3. Antibacterial activity (MIC in mg/ml) of the ethanol extracts on two bacterial strains.

Test plants	Bacterial Species	
	S. aureus strain 3	*P. aeruginosa*
Sacropoterium spinosum	0.781	0.390
Lawsonia inermis	3.125	0.781
Psidium guajava	1.563	3.125

Discussion:

Nowadays, plenty of reports have been published from various countries, assuring the antimicrobial activities of single or combined extracts of medicinal plants (11-24).

In our present study, out of nine plant extracts screened, three plant extracts are almost having antibacterial activity against both Gram - positive and Gram - negative bacteria. These plant extracts were of *Sacropoterium spinosum*, *Lawsonia inermis* and *Psidium guajava*. The three plant extracts were more active against Gram – positive than against Gram – negative bacteria. The widest inhibition zone was noticed of the single aqueous extract of *Sacropoterium spinosum* (30mm / 50 µl), (25mm / 50 µl) of single ethanol extract of *Lawsonia inermis* and (23 mm / 50 µl) of single ethanol extract of *Psidium guajava* against *MRSA strain 2*. These results approve the previous reports, in the sense that plant extracts are more active against Gram-positive bacteria than against Gram-negative bacteria (25,26).

Regarding - our data, we have also noticed that, the plant extracts of *Ruta chalepensis, Ranunculus asiaticus* and *Salvia syriaca* were not having antibacterial activity neither against Gram -positive nor against Gram - negative bacteria.

Furthermore, the widest inhibition zone was noticed from the E / E combined extracts of (*Sacropoterium spinosum and Lawsonia inermis*); it was (28mm / 50 µl) and (27mm / 50 µl) against *MRSA strain 2* and *P. aeuroginosa* respectively. A significant enhancement in the antibacterial activity against *MRSA strain 2* and *P. aeuroginosa* appeared by the ethanol / ethanol combined plant extracts of *Sacropoterium spinosum* and *Lawsonia inermis* than that of any individual extract.

This antibacterial enhancement indicates that there was a synergistic effect of the combined plant extracts against *MRSA strain 2* and *P. aeuroginosa*. Thus, this combination form of *Sacropoterium spinosum and Lawsonia inermis* has a broad spectrum antibacterial activity, and can be a useful drug of plant source in order to treat infections caused by these microorganisms and may partly explain the use of these combinations in Folkloric medicines in Palestine in order to treat different diseases.

Results obtained from this study also indicate that the growth of *P. aeruginosa* was remarkably inhibited by the ethanol single extract of *Sacropoterium spinosum* (MIC 0.390 mg/ml).

Infections caused by *P. aeruginosa* are among the most difficult infections to be treated with currently used antibiotics, especially those with multi-drug resistance (27,28).

Therefore such results are of a significant value that confirm the therapeutic potency of some plants used in traditional medicine and should form a good basis for further phytochemical and pharmacological investigation.

Useful antimicrobial phytochemicals are: Phenolics and Polyphenols (such as simple phenols and phenolic acids, quinones, flavones, flavonoids, and flavonols. Tannins, coumarins); Terpenoids and Essential Oils; Alkaloids; Lectins and Polypeptides; Mixtures; plus other compounds. The mechanisms thought to be responsible for these phytochemicals against microorganisims vary and depend on these compounds. Their mechanism of actions may include enzyme inhibition by the oxidized compounds, and act as a source of stable free radical and often leading to inactivation of the protein and loss of function. They have the ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls and disrupt microbial membranes, some have ability to intercalate with DNA, formation of ion channels in the microbial membrane, competitive inhibition of adhesion of microbial proteins to host polysaccharide receptors(29).

The main conclusion of the present study supports the traditional medicine use of different plant extracts in treating different infections caused by pathogenic bacteria in Palestine, either by using a single or combined extracts, and suggest that a great attention should be paid to medicinal plants which are found to have plenty of pharmacological properties that could be sufficiently better when considering a natural food and feed additives to improve human and animal health.

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