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ANTIBACTERIAL ACTIVITY OF *RHUS CORIARIA*. L EXTRACTS GROWING IN PALESTINE

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تأثيرمستخلصات حبيبات السماق الناضجة المزروعة في فلسطين كمضادات حيوية

الملخــص: تـم دراسة تأثير ثلاثة أنواع من مستخلص حبيبات السماق الناضجة (المائي، الكحول الاثيلي و الكحول الميثيلي) كمضادات حيوية على خمسة أنواع من البكتيريا السريرية بالاضافة الى البكتيريا العصوية باسيلاس ساتلاس كسلالة مرجعية باستخدام طريقة انتشار القرص وطريقة التركيز المانع الأدنى. الأنواع البكتـيرية المسـتخدمة هي البكتيريا العنقودية المقاومة للميثيسلين، بكتيريا سيودوموناس ايروجينوزا كثيرة المقاومة للدواء، بكتيريا اشيريشيا كولي O157، بكتيريا بروتيس فلجارس، بكتيريا كلبسيلا نيومونيا.

أظهرت ألنتائج أن ألاختلاف في قطر المنطقة المانعة يعتمد على نوع البكتيريا ونوع المستخلص. تراوح معـدل قطر المنطقة المانعة من صفر- 19ملم، 15 - 25ملم و 15- 22ملم للمستخلص المائي ، الكحول ألايثيلـي والكحول الميثيلي على التوالي. كما وأظهرت النتائج أن التركيز المانع ألأدنى لمستخلص السماق الايثيلي كان 1.25 ملغم/ مل ضد البكتيريا العنقودية المقاومة للميثيسلين، بكتيريا اشريشيا كولي، بكتيريا سيودوموناس ايروجينوزا، بكتيريا بروتيس فلجارس و 0.156 ملغم/ مل ضد البكتيريا العصوية باسيلاس ساتلاس.

بشـكل عـام تشير النتائج ان تأثير مستخلص بذور نبات السماق كمضاد حيوي كان أكثر فاعلية ضد أنواع البكتيريا الموجبة لصبغة جرام.

Abstract: Disk diffusion and micro-dilution techniques were used to determine the antibacterial activity of water, ethanolic and methanolic extracts of *Rhus coriaria* ripe berries against five clinical bacterial strains (Methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Pseudomonas aeruginosa*, enterohemorrhagic *Escherichia coli* O157 (EHEC), *Proteus vulgaris* and *Klebsiella pneumoniae*). *Bacillus subtilis* ATCC6633 was used as a reference strain. The zone of inhibition varies depending on bacterial species and type of extract. The average diameter of inhibition zones ranges from 0 to 19 mm, 15 to 25 mm and 15 to 22 mm for water, ethanolic and methanolic extract, respectively. The MIC value of ethanolic extract was 1.25 mg/ml against MRSA, EHEC, *P. aeruginosa* and *Proteus vulgaris*, while 0.156 mg/ml against *B. subtilis*. In general these results showed that the antibacterial activity of *R. coriaria* ripe berries extracts was more effective against Gram-positive bacteria than Gram-negative.

Running title: antibacterial activity of *Rhus coriaria*

Keywords: *Rhus coriaria*, sumac, antibacterial activity, Palestine

Introduction

Plants are able to produce different compounds that be used to protect themselves against different types of pathogens [1]. Interest in medicinal plants has revived as a consequence of current problems associated with the use of antibiotics [2]. Spices are mainly used in foods mainly because they give desirable flavors and aromas; in addition it shows antimicrobial activity [3].

Medicinal plants are important elements of indigenous medical systems in Palestine as well as in other developing countries. *R. coriaria* (Anacardiaceae) commonly known as sumac (also spelled as sumach), is a wild bush that grows in all Mediterranean areas, including Palestine. Phytochemicals in *R. coriaria* are being used as antibacterial, antidiarrheic, antidysenteric, antihepatoxic, antiseptic, antispasmodic, antiviral, astringent, candidicide, hepatoprotective, hepatotonic, protisticide, analgesic, antigastric, anti-inflammatory, antioxidant, antiulcer, fungicide, cyclooxygenase-inhibitor and lipoxygenase inhibitor due to their contents of ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid [4].

The acidic tasty of *R. coriaria* fruits is made into a condiment and sour drink in the Middle East dishes. Its sour taste is derived form the citric and malic acids found in its juice. In Palestine, *R. coriaria* is a well-known spice, popular and has been utilized extensively in many different meals, such as in zater (dukka) which is a blend of sumac, thyme and citric acid with seasame seeds; almusakhan which is composed from fragmented chicken, small fragments of onions and sumac, as well as in salads and others.

Due to lack information about the effect of *R. coriaria* seeds on bacterial activity [5-7] this study was conducted in order to study the antimicrobial effect of water, methanolic and ethanolic extracts of *R. coriaria* against five clinical species of bacteria.

Materials and Methods:

Extract preparation*:* *R. coriaria* fruits were collected from plants grown in mountains in North of Palestine. The taxonomic identity of the plants was confirmed by us as described by Flora Palaestina [8]. Air-dried and powdered of *R. coriaria* ripe berries (30 g) were extracted with water, 80% methanol and 80% ethanol; the extracts were filtered through Watman No. 2 filter paper under suction. Extracts were concentrated to dryness in vacuum and weighed.

Test Bacteria: Five bacterial strains isolated from clinical material from patients were used in the study: Methicillin-resistant *Staphylococcus aureus*

(MRSA), multi-drug resistant *Pseudomonas aeruginosa*, enterohemorrhagic *Escherichia coli* O157 (EHEC), *Proteus vulgaris* and *Klebsiella pneumoniae*. A reference strain [*Bacillus subtilis* ATCC6633] was also tested.

Antimicrobial activity: The antimicrobial activity was determined by the well diffusion method [(9)]. Wells of (6 mm diameter) were made in Mueller Hinton agar. Plates were seeded with a 24 h old culture of the bacterial strains. Plant extracts were added to the wells, a concentration of 5 mg/well. Triplicates of each concentration for each bacteria species were prepared. The inoculated plates were incubated at 37 ˚C for 24 h. The diameter of the inhibition zones were measured for each plate and the average reading of the three replicates for each antibacterial species are shown in Table 1. The standard tetracycline disk (30 µg) was used as a control.

Minimum inhibitory concentration (MIC) was determined by the micro-dilution method [(10)]. The reconstituted ethanol extract was diluted in Mueller Hinton broth to give a final concentration of 0.0195, 0.0391, 0.078, 0.156, 0.313, 0.625, 1.25, 2.5, 5, 10 mg/ml. The bacterial inoculum size was adjusted to the turbidity of the 0.5 #1McFarland standard so as to deliver a final inoculum of approximately 10^5 colony-forming units (CFU/ml). Using a micropipette, 50µl of the standard microbial broth culture were introduced into the wells. The test plates were incubated at 37 ˚C for 18 h. The endpoint (MIC) is taken as the lowest concentration of drug at which the microorganism tested does not show visible growth.

Results:

The antibacterial activity of *R. coriaria* seed extracts are shown in Table 1. The inhibition zones vary depending on bacterial species and type of extract. The average diameter of inhibition zones ranges from 0 to 19 mm, 15 to 25 mm and 15 to 22 mm for *R. coriaria* seeds water, ethanolic and methanolic extract, respectively. The largest diameter of inhibition zone was observed from ethanolic and methanolic extracts on the growth of MRSA and *B. subtilis*. No effects were detected for water extract on *P. vulgaris*, *P. aeruginosa* and *K. pneumoniae*. The MIC of ethanolic extract was 1.25 mg/ml against MRSA, EHEC, *P. aeruginosa* and *Proteu*s *vulgaris*, while 0.156 mg/ml against *B. subtilis*.

Table 1. The antibacterial activity of water, ethanol and methanol extracts of the dried ripe berries of *Rhus coriaria* species

Name of organisms	Radius of Inhibition in mm (mean of three tests)			Antibiotic Tetracycline (30 µg)
	Water	Ethanol	Methanol	
MRSA	19	25	22	30
Bacillus subtilis	16	23	22	14
EHEC	10	16	16	38
Proteus vulgaris	0	16	15	27
P. aeruginosa	0	15	15	12
K. pneumoniae	0	15	15	30

Abbreviations: MRSA -Methicillin-resistant *Staphylococcus aureus;* EHEC- Enterohemorrhagic *Escherichia coli* O157.

Discussion

Traditional medicine is an important source of products for developing countries in treating common infectious bacteria. The emergence of multi-drug resistant infectious bacteria, high cost of synthetic compounds as well as undesirable side effects of certain drugs insist on pharmaceutical companies to look for new therapeutic agents from other alternative sources including medicinal plants. In recent years, different reports, from different countries were published showing the antimicrobial activities of medicinal plants [6, 11-24]. The result of the present study indicates the existence of antimicrobial activity in the crude extracts of *R. coriaria* seeds. Water extract was more active against gram-positive than gram-negative bacteria. Similar observations were reported elsewhere [7, 25].

Ethanolic and methanolic extract displayed broad spectrum of activity, since Gram positive bacteria including Methicillin-resistant *S. aureus* and Gram negative bacteria including *P. aeruginosa*, enterohemorrhagic *E. coli* O157, *P. vulgaris* and *K. pneumoniae* were inhibited with the *R. coriaria* seed extracts. These results in agreement with those previously published [6, 11, 20]. *B. subtilis* was clearly found to be the most sensitive among them demonstrating a MIC 0.156 mg/ml. The rest of the species found to be more resistant with MIC of 1.25 mg/ml. Increasing the concentration of extracts will lead to increase inactivation of cells in all strains tested [8-7]. The antimicrobial activity demonstrated by sumac extract against the microorganisms tested in this study is comparable with those of other spices reported in the literature which inhibited the growth of Gram-positive organisms [20, 26-28]. Traditional using of this spice may help in protecting from several bacterial diseases spontaneously and may aid in control of bacterial growth in foods.

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