

## Antimicrobial study of active constituents of *Rhus coriaria*

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### Abstract:

The present study was conducted to investigate the antimicrobial of fruit of *Rhus coriaria* (Sumac). Various constituents (alkaloids, glycosides, phenol and terpenoids) were extracted and separated then submitted for the determination of their antimicrobial activity against *Pseudomonas aurogenosa*, *Staphylococci aureus* and *Candida albicans*. Extracts of *R. coriaria* at 100 mg/ml, exhibited inhibition zones ranged, 0-27, 20-25, 20-24 and 9-23 mm of alkaloids, glycosides, phenols and terpenoids respectively. These results indicate that *R. coriaria* have antibacterial and antifungal activity in vitro.

### تأثير التصاد المايكروبي للمكونات الفعالة لثمار نبات السماق

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### الخلاصة:

أجريت الدراسة الحالية لبحث فعالية المكونات الكيميائية لنبات السماق ضد الأحياء المجهرية. تم استخلاص وعزل المكونات الفعالة القلوانيات والكلايكوسيدات والفينولات والتربينات وللكشف عن فعالية هذه المكونات ضد الأحياء المجهرية. تم اختبار فعاليتها ضد بكتريا الزوائف الزنجارية والمكورات العنقودية وخميرة المبيضات البيضاء واطهرت نتائج الاختبار ان المستخلصات القلوانية والكلايكوسيدية والفينولية والتربينية الخام لنبات السماق بتركيز 100 ملغم \ مل نطاقا تثبيطيا: 24-20، 25-20، 27-0 و 23-9 ملم على التوالي. ومن هذه النتائج تبين ان نبات السماق يمتلك قدرة تصادية للبكتريا والفطريات خارج الجسم الحي.

### Introduction:

Sumac is the common name for a genus *Rhus* spp. that contains over 250 individual species of flowering plants in the family Anacardiaceae.

*Rhus coriaria* ( dyeing and tanning sumac ), which grows wild in the region from the Canary Islands over the Mediterranean region to Iran and

Afghanistan, is commonly used as a spice by grinding the dried fruit with salt, and is also widely used as a medicinal herb in the Mediterranean and Middle East, particularly for wound healing (1).

Phytochemicals in *R. coriaria* are being used as antibacterial, antidiarrheic, antidysenteric, antihepatotoxic, antiseptic, antispasmodic, antiviral, astringent, candidicide, hepatoprotective, hepatotonic, protisticide, analgesic, antigastric, anti-inflammatory, antioxidant, antiulcer, fungicide. (2).

Organic solvent extracts of *R. coriaria* ( petroleum ether , ether , acetone , ethanol and methanol ) exhibited a broad antibacterial spectrum against Gram –positive , Gram – negative , acid – fast and spore – forming bacteria. The most sensitive test organisms were the Gram–positive (*Staphylococci* , *Streptococci* and *Corynebacteria* ) , sporeformers (*Bacillus* species ) and acid – bacteria (*Mycobacterium phlei*).The least sensitive organisms were the gram – negative bacteria(*Salmonella* , *Shigell* , *Brucella* ).(3).

## Material and methods:

### Preparation of extracts

Fruit of Sumac brought from folk market and ground well into a fine powder in a mixer grinder. Twenty five g. of dried plant powder was extracted in a soxhlet apparatus using 250 ml of solvent until the last portion of the extract became colorless. Solvents of all extracts were removed under low vacuum by

using rotary evaporation. Crude extracts were maintained at +4 °C until use. (4)

### Extraction

Terpenoid were extracted with Petroleum ether for 24 hrs. The dried extracts were stored in a refrigerator at 4<sup>0</sup>C (present terpenoids) (5)Harborne, 1984).The dried residue after defatted with petroleum ether retains in a soxhlet and extracted with methanol 90%. For extraction phenol the methanol extract acidify with 2 m H<sub>2</sub>SO<sub>4</sub> (pH< 3) and partition in a separating funnel with CHCl<sub>3</sub>.(5).Glycosides were extracted with n-butanol according to (6).Alkaloids were extracted by sonication 10 g of the powder after suspended in 400 ml of surfactant solution sodium dodecylsulfate (SDS) for 2.5 hrs. in an ultrasonic bath at a constant temperature of 25 °C .(7).

### Isolation and identification of bacteria from patients

Isolates of *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from patients suffering from burns and wound infection attended to AL-Hmza Hospital / AL-Qadisiya governorate .Swabs were taken from patients by using sterile cotton swabs with transport media .The samples were unrigged in nutrient broth at 37°C for 18 hours .Each sample was subcultured on blood agar and MaConky agar then incubated at 37C° for 24 hours, pure culture was obtained after isolation on appropriate media. A single colony was taken from each primary

positive culture on blood agar and on MacConkey agar and it was identified depending on its morphology (colony shape, size, color, borders, and texture) and then it was examined by the microscope after being stained with Gram's stain. The suspected colonies of *Staph. aureus* which were positive for Gram-stain, cocci, catalase and coagulase test, and  $\beta$  hemolysis on blood agar cultured on Mannitol salt agar to observe the change in the color from red to yellow (acid production). The suspected colonies of *P. aeruginosa* which were negative for Gram-stain, bacilli, positive for oxidase test, cultured on (Nutrient agar) at 42 C° to observe pyocyanine blue and fluorescein yellow-green. Motility was observed on semisolid nutrient agar. Then the biochemical tests were performed (8,9).

#### **Isolation and identification of *C. albicans***

Isolate of *C. albicans* was isolated from women suffering from vaginitis infection attended to AL-Hamzh Hospital. Swabs were taken from patients by using sterile cotton swabs with transport media. The samples were cultured on Sabouraud dextrose agar that contains chloramphenicol to prevent bacterial contamination and incubated at 37 C°. The cultures were examined macroscopically and microscopically. Inoculated blood serum for producing germ tubes within two hours at 37 C° (9).

#### **In vitro antibacterial and antifungal activities of *R. coriaria* extracts studies**

Concentration of active constituents (alkaloids; glycosides; phenols and terpenoids) were used 25, 50 and 100 mg/ml of phenols and terpenoids, 25, 50, 100 and 200 mg/ml of alkaloids and glycosides (The concentration 25-100 mg/ml of alkaloids and glycosides gave no results in *P. aeruginosa*. Therefore, higher concentrations (200 mg/ml) were then used and proceeded in order to follow the effectiveness of the plants extracts). Glycosides and phenols dissolved in distilled water (D.W.). Alkaloids dissolved in D.W.:methanol (7:3). Terpenoids dissolved in dimethylsulfoxide (DMSO). (10). Also we use 0.1 from oil of *R. coriaria* directly in the wells (without DMSO). Antibacterial and antifungal activity of crude extracts were determined by the well diffusion method according to the (11). A standard 10 mcg tobramycin disc and Fluconazole (100 mg/ml) was used as a positive control and solvents distilled water for alkaloids; glycosides and phenols and DMSO for terpenoids were used as a negative control. (12). Determination of the minimum inhibitory concentration (MIC) was carried out using the broth dilution method (13). After incubation, the concentration at which no visible growth was seen and recorded as the MBC.

Experiments include two factors with three replication in design completely random design (CRD)

and tested by F-test (p-value and LSD at level (0.05 and 0.001).(14).

### Results and discussion:

The concentration of 25 and 50 mg /ml of all four active constituents ( alkaloids, glycosides, phenols and terpenoids ) of fruits of *R.coriaria* extracts were not effective against three pathogenic ( *P. aurogenosa* ,*Staph.aureus* and *C.albicans* ) while they were effective when increased concentration to 100 and 200 mg /ml ( Table 1 -2). *R.coriaria* possessed a good antibacterial and antifungal activity in 100 mg / ml concentrations. Inhibitory activity of glycosides and phenols extract of *R.coriaria* to a greater extent as compared to tobramycin. And alkaloids; glycosides; phenols and terpenoids extracts of *R.coriaria* to a greater extent as compared to fluconazole (Table 1).Similar result were obtained by other researchers (15,16).

Alkaloids extract did not showed an antibacterial activity against *P. aeruginosa* in 100 mg / ml concentration .However , a slight antibacterial activity was recorded against *P. aeruginosa* in a concentration of 200 mg/ml with inhibitory zone ranged from 7 -16 mm. *Staph.aureus* showed moderated sensitive of alkaloids extracts in both 100 and 200 mg / ml concentration with inhibitory zone ranged from 7 -15 mm. Alkaloids of *R.coriaria* extract was the most effect on *C.albicans* with inhibitory zone 27 mm when

compared with fluconazole 21.66 mm in a concentration of 100 mg /ml.

Glycosides of *R.coriaria* showed inhibition zone 22 ,20 mm against *P. aeruginosa* and *Staph.aureus* respectively . And were most effective against *C.albicans* with inhibition zone 25 mm.

Phenols of *R.coriaria* was effective against *P. aeruginosa* and *Staph.aureus* with inhibition zone 20 and 21 mm respectively . *C.albicans* was most sensitive to Phenols of *R.coriaria* with inhibition zone 29mm.

Activity of phenols and glycosides of *R.coriaria* belonging to *R.coriaria* rich in anthocyanins and hydrolysable tannins, gallic acid ( the main phenolic acid in *R.coriaria* ), anthocyanin fraction contained cyanidin, peonidin, pelargonidin, petunidin, and dolphinidin glucosides and coumarates. Pentagalloyl glucose (17). Traditional using of this spice may help in protecting from several bacterial diseases spontaneously and may aid in control of bacterial growth in foods.

Pre-existing antifungal phenolics are simple phenols, phenolic acids, flavonols and dihydrochalcones. In addition, many flavones and flavanones have been shown to be active against fungal pathogens commonly found during the storage of fruits and vegetables, Tannins are quite potent antibiotics. It is possible that inhibition of extracellular fungal enzymes (cellulase, pectinase, lacca-

se, xylanase, etc.), nutrient deprivation of substrates (metal complexation, protein insolubilization) and action on fungal membranes (inhibition of oxidative phosphorylation) are involved in tannin toxicity (18).

Terpenoids had moderate activity in both *P. aeruginosa* and *Staph.aureus*. While had highest activity against *C.albicans* with inhibition zone 23mm. These results showed contrarily activity compare with results which get by (19) who mentioned the highest activity of leaves *R.lancea* was noticed against *E. coli* (19.2 mm zone of inhibition when use 100 mg/ml of oil while *C. albicans* appeared to be more resistant with activity less than 50% compared to 59.7% for the positive control (Fluconazole).

Inhibition zone of oil of *R.coriaria* when use directly in the wells without DMSO were lesser than with DMSO except oil of *R.coriaria* against *Staph.aureus* (14

mm against *C. albicans* 13mm against *Staph.aureus*, while no effect against *P.aeruginosa*). This is due to the DMSO increase diffusion oil in the media.

All solvents were also used for the test as control but showed no inhibited action against pathogens signifying that it serves as a dilutant.

The statistical analysis showed significant differences after treating the microorganisms with *R.coriaria* extracts ( $P < 0.001$ ) compare with control and antibiotic .

Minimum inhibitory concentration (MIC) of *R.coriaria* extracts were 6.25,3.12,3.12 and 6.25 mg / ml of alkaloids, glycosides, phenols and terpenoids respectively against *P.aeruginosa*.1.56,3.12,6.25 and 3.12 mg / ml of alkaloids, glycosides, phenols and terpenoids respectively against *Staph.aureus*.1.56,1.56,3.12 and 1.56 mg/ml of alkaloids, glycosides, phenols and terpenoids respectively against *C. albicans*.(Table 3).

**Table (1): Antimicrobial effect of *Rhus coriaria* extracts (100 mg / ml) against *C.albicans*, *P.aeruginosa*, *Staph.aureus***

Constituents	Inhibition zones ( mm )		
	<i>P. aeruginosa</i> *	<i>Staph.aureus</i> *	<i>C.albicans</i> **
Alkaloids	0	15	27
Glycosides	22	20	25
Phenols	20	21	24
Terpenoids	9	12	23
Antibiotic	18	20	22
Control	0	0	0

LSD<sub>(0.001)</sub> = 1.53. Antibiotic: \* =Tobramycin (10mcg),\*\* =Fluconazole (100mg/ml).( Tobramycin : Resistant 12mm or less : Sensitive 19 mm or more)

**Table ( 2): Antibacterial and antifungal activity of active constituents of *R.coriaria* at concentration 200 mg / ml against *P.aeruginosa* *Staph.aureus*, and *C. albicans***

Constituents	Inhibition zones ( mm )		
	<i>P. aeruginosa</i> *	<i>Staph.aureus</i> *	<i>C.albicans</i> **
Alkaloids	7	18	29
Glycosides	25	22	26
Antibiotic	18	20	22
Control	0	0	0

LSD<sub>(0.05)</sub> = 4.829. Antibiotic: \* =Tobramycin (10mcg),\*\* =Fluconazole (100mg/ml).

**Table (3): The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of active constituents extracts of *R.coriaria* . (mg / ml ) .**

Microorganisms	Alkaloids		Glycosides		phenols		Terpenoides	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
<i>P.aurogenosa</i>	6.25	12.5	3.12	6.25	3.12	6.25	6.25	12.5
<i>Staph.aureus</i>	1.56	3.12	3.12	6.25	6.25	12.5	3.12	6.25
<i>C.albicans</i>	1.56	3.12	1.56	3.12	3.12	6.25	1.56	3.12

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