

## SCREENING IN VITRO AND IN VIVO THE ANTIBACTERIAL ACTIVITY OF *RHUS CORIARIA* EXTRACT AGAINST *S. AUREUS*

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### ABSTRACT

The problem of antibiotic resistance has become increased in recent years. The present study investigated the antibacterial activity of aqueous extract of *Rhus coriaria* against *Staphylococcus aureus* in vitro and in vivo. The results showed that *S. aureus* was multi-drug resistant where it was resistant to nine antimicrobials (AMC, CFM, CTR, DA, OB, MY, ME, NIT, and V) among seventeen used antimicrobials. The aqueous extract of *R. coriaria* showed a strong effect in inhibition the growth of tested isolate in vitro and in vivo. The MIC was determined where it was 0.025% and was used as curing agent for curing bacterial plasmids. The phytochemical screening showed that *R. coriaria* contains tannin, phenol, anthraquinon and saponin.

**Keywords:** *S. aureus*, Antibacterial activity, Anti-plasmid, *Rhus coriaria* extract, Phytochemicals.

### 1. INTRODUCTION

Traditional medicine is an important source of products for developing countries in treating common infectious bacteria. The emergence of multiple 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 (1). Recently there has been a renewed interest in improving health and fitness through the use of more natural products. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value (2).

Plants are able to produce different compounds that be used to protect themselves against different types of pathogens (3). Naturally occurring compounds in spices such as, sulphur compounds, terpenes and terpene derivatives, phenols, esters, aldehydes, alcohols and glycosides have shown antimicrobial functions, The main factors that determine the antimicrobial activity are the type and composition of the spice, amount used, type of microorganism, composition of the food, pH value, temperature of the environment, and proteins, lipids, salts, and phenolic substances present in the food environment (4).

*Rhus coriaria* (anacardiaceae) commonly known as *sumac* (also spelled as *sumach*) is a wild bush that grows in all Mediterranean areas. Phytochemicals in *Rhus coriaria* are being used as antibacterial, antidiarrhoea, antidysenteric, antihepatotoxic, antiseptic, antispasmodic and antiviral due to their contents of ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid (3,5).

The objectives of this study were: (i) to investigate the antibacterial activity (against pathogenic bacteria) of the Sumac extract in vitro, (ii) inhibition the bacterial number in vivo, (iii) curing the plasmids that mostly responsible for antibiotic resistance and (iv) to determine the chemical composition of it.

### 2. METHODS AND MATERIALS

#### Bacterial strains:

The bacterial strain used in this study was *Staphylococcus aureus*, which obtained from Microbiology Lab/ Department of Biology- College of Education.

#### Plant Extraction

##### Collection and preparation of plant sample

The plant (Sumac) was purchased from market in Erbil city-Iraq. It was converted into powder by using mortar (household flourmill), then the ground plant was separated from its stone form and stored in polyethylene bags in the refrigerator at 4°C for further processing.

##### Extracts preparation

300 ml of sterilized distilled water was added to 30 g of ground dried plant, heated below the boiling point and stirred for 2 ½ - 3 h. The extract was filtered by muslin cloth, then by filter paper (Whatman No. 1) and then stored in the refrigerator at 5 °C for using (6).

### **Phytochemical screening of Extract**

The methods described by (7, 8, 9), were used to test for the presence of saponins, tannins, alkaloids, flavonoids and in the test samples.

#### **Saponins**

Each of the plant extracts (0.5g) was separately stirred in a test tube, foaming which persisted on warming was taken as an evidence for the presence of saponins (7).

#### **Tannins**

Extract of each sample (0.5g) was separately stirred with 10ml of distilled water and then filtered. To the filtrate was added two drops of 5% Iron (III) Chloride (FeCl<sub>3</sub>) reagent. Blue – black or blue – green colouration or precipitate was taken as an indication of the presence of tannins (7).

#### **Alkaloids**

Extract of each plant sample (0.5g) was separately stirred with 1% hydrochloric acid (HCl) on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with two drop of Mayer's reagent. The two solutions were mixed and made up to 100ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extracts (7).

#### **Phenols**

To 2ml of test solution, added alcohol and then few drops of neutral ferric chloride solution was added. The test result was observed (8).

#### **Quinones**

To the test substance, sodium hydroxide was added. Blue green or red colour indicates the presence of Quinone (8).

#### **Coumarin**

To the test sample 10% of sodium hydroxide and chloroform were added. Formation of yellow colour indicates the presence of Coumerin (8).

#### **Flavonoids**

4ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red color was observed for flavonoids and orange color for flavones (9).

#### **Phlobatanins**

The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate shows the presence of phlobatanins (9).

#### **Anthroquinone**

About 0.5 g of the extracts was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl<sub>3</sub> was added to the filtrate. Few drops of 10% NH<sub>3</sub> were added to the mixture and heat. Formation of rose-pink colour, indicates the presence of authraquinones (9).

**Sensitivity to antimicrobial agents:** The isolate was tested against 16 different antimicrobial agents by using the Kirby-Bauer standardized single disc method as mentioned in table2 (10).

### **Screening of Antibacterial Activity**

The antimicrobial assay was performed using the standard procedure as described (11) with some modification. The previously prepared inoculums were adjusted to 0.5 McFarland standards, which are equal to  $1 \times 10^8$  CFU/ml and then 0.1 ml was transferred to Mueller Hinton agar (MHA) plates and spread with cotton swabs. One hundred microliters of extract were poured on wells with 8mm diameter made by cork borer in MHA. Inoculated plates were incubated at 37°C overnight. After a 24-hour incubation period, the inhibition zone diameters (mm) were measured. The experiment was performed in triplicates and the interpretation of antibacterial properties was conducted according to (12). Inhibition zones > 15 mm were categorized as strong activity, from 10-15 mm as moderate activity and <10 mm as weak activity.

### Measurement of minimal inhibition concentration (MIC) using agar dilution technique

According to NCCLS agar dilution method (13), the MIC of Sumac extract was tested with some modification. Briefly a series dilution of each extract ranging from (0.025%, 0.05%, 1% (v: v) to 10% (v: v)) was prepared in nutrient agar. After solidification of media the plates were inoculated with bacterial suspension. Inoculated plates were incubated at 37 °C for 24 h. Minimum inhibitory concentrations (MICs) were determined after 24 h., as lowest concentration of extract inhibiting the visible growth of each organism on the agar plate. The presence of one or two colonies was disregarded. All experiments were applied in triplicates.

### Isolation of plasmid DNA content for plasmid profile (fermentas)

#### Quantum Prep plasmid miniprep kit

1. An overnight culture was transferred to a micro test tube centrifuged at 8000 round per minute (rpm) for (30 second). Supernatant was removed.
2. 250 µl of re-suspension solution vortexed until the cell pellet is completely re-suspended.
3. 250 µl lyses solution and 8 µl of stock lysozyme solution (50 mg/ml) were added and mixed by inverting 10 times.
4. 300 µl of neutralization solution was added. Mixed by inverting 10 times then centrifuged for 5mint.
5. A spin column inserted into a 2 ml wash tube.
6. The supernatant to transferred to a spin column, centrifuged at 8000 rpm for 1 mint to pull fluid though the column.
7. The spin column was removed, the filtrate discarded, and the column replaced. 500 µl wash buffer added, centrifuge for 2 minutes followed by a 2 minutes spin to remove all wash buffers.
8. The spin column removed to a clean 1.5 ml tube, 100 µl sterilized H<sub>2</sub>O was added, and then centrifuged for one minute.

The above steps were done for the same bacteria after treating it with SMIC (sub MIC is the concentration less than MIC, in this study was 0.020%) of *R. coriaria* extract for 24 hrs.

### Agarose electrophoresis technique (14)

#### Preparation of 1% agarose gel:

The gel (1%) was prepared by dissolving 1 gm of agarose powder in 100 ml of 0.5 X TBE (Tris base ethidium bromide) buffer, boiled until all agarose was dissolved and left to cool at 50 °C, 8 µl of ethidium bromide was added, the gel was poured in to the glass plate that contained appropriate comb, the gel was left to solidify and the comb was removed gently, the gel was soaked in a gel tank containing TBE buffer should cover the surface of the gel.

#### Sample loading

Ten µl of plasmid DNA samples were mixed with 5 µl of loading buffer, and the mixture was slowly loaded in to the wells on the gel; also a molecular weight marker was loaded as control.

#### Running the electrophoresis

The electrophoresis apparatus was joined to power supply, turned on and the samples electrophoresed at 75 volt/cm for 1 hour. The gel was visualized by UV-transilluminator, and then photographed.

### Antibacterial activity of plant extract in vivo

The experiment was done by using 30 Swedish mice (*Mus musculus*), their age ranged between (8-12) weeks with weight ranged between (22-25) g. These were maintained in the animal house of Biology Department/ College of Education, where they were put in standard cages with food and water and at optimum conditions included temperature, light and ventilation. The mice were grouped into six groups of five animals each. The six groups were injected subcutaneously:

Group I (Control): injected with 0.1 ml normal saline.

Group II (Control II): injected with 0.1 ml of plant extract.

Group III: injected with 0.1 ml of bacterial suspension ( $1 \times 10^8$  CFU/ml).

Group IV: injected with 0.1 ml of bacterial suspension ( $1 \times 10^8$  CFU/ml).

Group V: injected with 0.1 ml of bacterial suspension ( $1 \times 10^8$  CFU/ml).

Group VI: injected with 0.1 ml of bacterial suspension ( $1 \times 10^8$  CFU/ml).

Second to five day the Groups IV, V and VI were injected with plant extract (the dose given was depended on MIC), antibiotic (Gentamicin (Menarini company-Italy), the dose depended to the weight of the body 40mg/kg/day) and interaction (antibiotic + plant extract each/each with half amount) respectively. After five days the blood was taken from the tested mice for bacterial count (a loopful), then was diluted in 0.5 ml normal saline, then 0.1 of it inoculated on nutrient agar and manitol salt agar for identification the inoculated bacteria.

### 3. RESULTS

*Staphylococcus aureus* is the leading cause of bacterial infections involving the bloodstream, lower respiratory tract, skin and soft tissue in many developed countries (15). Table 1 shows that *S. aureus* was sensitive to (AK, CE, DO, G, NV, PRL, TE and TOB), and resistant to other tested antimicrobials, this leads to decide that this isolate is a multi drug resistant.

Table 1: Resistance of *S. aureus* under Study to antimicrobials

Antimicrobial: µg/disc	Company	Zone of inhibition/ mm
Amikacin (AK)30	Al-Raze/Baghdad	33
Amoxicillin-Calvulanic acid (AMC) 30	BBL	-
Cefixime (CFM) 5	Bioanalyse	-
Ceftriaxon (CTR) 30	Al-Raze/Baghdad	-
Cephadrine (CE) 30	Bioanalyse	20
Clindamycine (DA)	Bioanalyse	-
Cloxacillin (OB) 5	Oxoid	-
Doxycillin (DO) 30	Oxoid	22
Gentamycine (G) 10	Al-Raze/Baghdad	35
Lincomycine (MY) 15	Bioanalyse	-
Methicillin (ME)10	Bioanalyse	-
Nitrofurantion (NIT) 300	Al-Raze/Baghdad	-
NovaBiocin (NV)30	Bioanalyse	20
Piperacillin (PRL)100	Bioanalyse	15
Tetracyclin (TE)30	Al-Raze/Baghdad	33
Tobramycin (TOB)	Al-Raze/Baghdad	35
Vancomycin (V)	Bioanalyse	-

-: No inhibition growth (resistance)

Table 2 and fig. 1 show the activity of aqueous extract of sumac as antibacterial agent against *S. aureus*, and it appeared that this extract had a strong effect for inhibition bacterial growth especially at the concentration of 100% where the inhibition zone was 24 mm. The MIC was determined as the lowest concentration of extract inhibiting the visible growth of each organism on the agar plates, and in this study the MIC was 0.025%. Table 3 shows that *R. coriaria* contains tannin, phenol, anthraquinon and saponin.

Table 2: Antibacterial activity of *Rhus coriaria* against selected bacteria.

Scientific Name	Concentration					MIC (V:V) %
	*Zone of inhibition/mm <i>Rhus coriaria</i>					
	100%	75%	50%	25%	12.5%	
<i>S. aureus</i>	24	20	19	17	15	0.025%

Table 3: Phytochemical screening of Aqueous Extract of *Rhus coriaria*

Plant Extract	Tanin	Phlobatanin	Phenol	Quinone	Antraquinone	Coumerin	Alkaloid	Flavonoid	Saponin
Rhus coriaria	+	-	+	-	+	-	-	-	+

+: Positive  
 -: Negative



Figure 1: Antibacterial activity of *R. coriaria* aqueous extract against *S. aureus*

Fig. 2 illustrates that the aqueous extract of *R. coriaria* cured three plasmids (Lane 3) from *S. aureus* isolate at concentration of SMIC (0.020%), where it contained four plasmids (Lane 2) before treating it with plant extract.

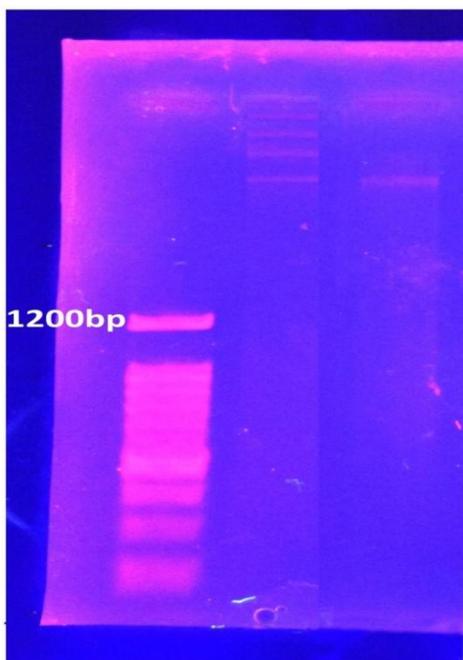


Figure 2: Plasmid profile of *S.aureus* isolate.  
 Lane 1: DNA marker 1200bp. Lane 2: *S.aureus* isolate before treatment with *R. coriaria* extract.

Lane 3: *S.aureus* isolate after treatment with *R. coriaria* extract at SMIC.

Table 4, illustrates the clinical symptoms that noted on infected mice with *S. aureus*, and treated mice with the Sumac aqueous extract, in addition to the number of bacteria in mice blood samples. It appeared from (Table 4) the number of bacteria was decreased in group VI, where the infected mice of this group were treated with combination of aqueous extract of sumac and antibiotic (Gentamicin), followed by group V (treated with antibiotic alone), followed by Group IV that treated with aqueous extract of sumac. Fig 3 and Fig 4 show the effect of sumac extract and number of bacteria in blood samples taken from mice groups respectively.

Table 4: Clinical symptoms of infected mice, mice treated with *R. coriaria* extract, and number of bacteria in mice blood samples

Group	No. of bacteria (drop of blood/ml)	Clinical symptoms			
		Diaharia	Dehydration	Weight decrease	Others
Group I (Control)	-	Not observed	Not observed	Not observed	-
Group II (Control II)	3.33	Not observed	Not observed	Not observed	A large wound in place of injection
Group III	402	Observed and moderate	Not observed	Observed	Less activity, Depression
Group IV	135	Observed and moderate	Observed	Observed	Less activity, death in second and third day. Depression
Group V	94	Not observed	Not observed	observed	-
Group VI	-	Not observed	Not observed	Not observed	-

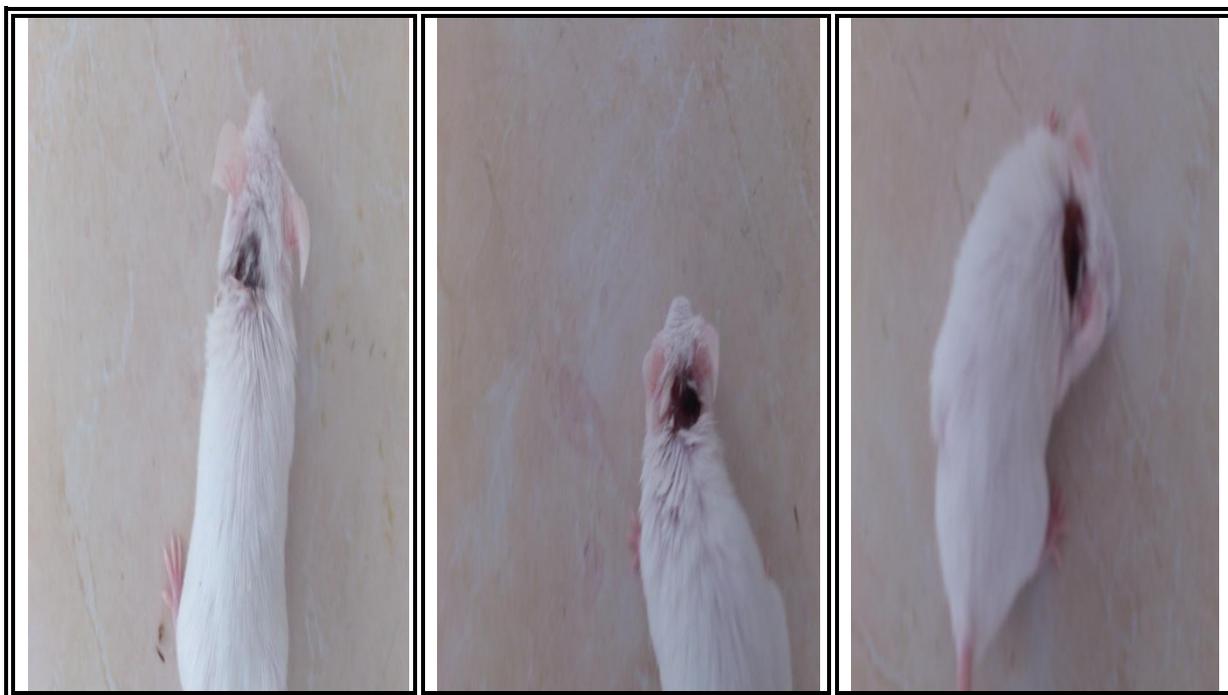


Figure 3: Mice injected subcutaneously with aqueous extract of *Rhus coriaria*

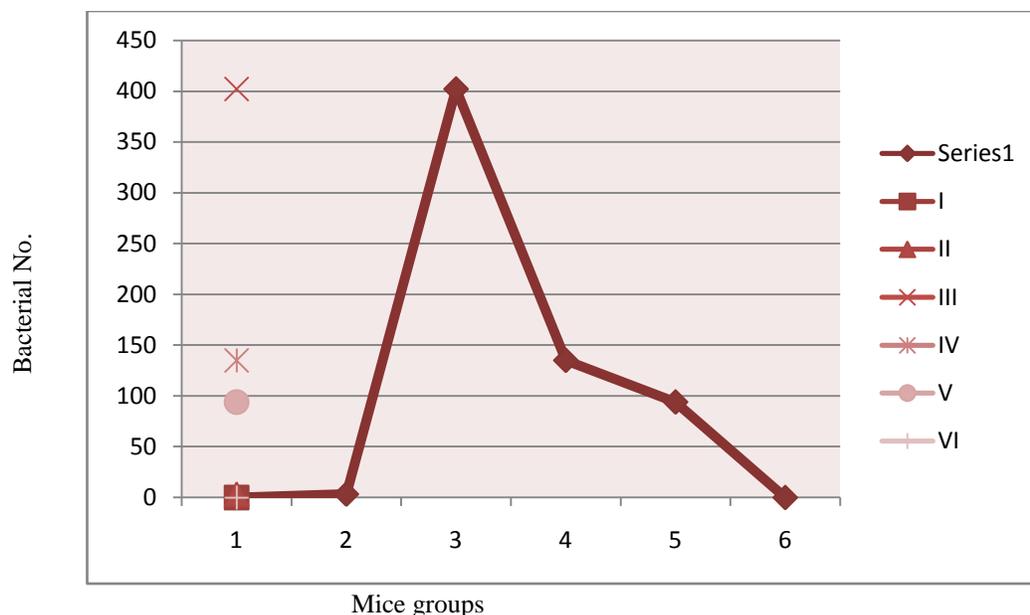


Figure 4: Number of bacteria in tested mice

#### 4. DISCUSSION

It appeared from the results that this bacterium considered a multi drug resistant bacterium, and its resistance may return to several reasons, one of those is having penicillinase enzyme (Penicillin was initially highly effective against staphylococcal infections, but penicillinase-producing *S. aureus* emerged in the mid-1940s, and its prevalence increased dramatically within a few years and known as methicillin-resistant *S. aureus* (MRSA)), which leads to be resistant to  $\beta$ -lactam antibiotics (16). The resistance of bacteria back to presence of antibiotic resistance genes on its chromosome or plasmids or by getting antibiotic resistance gene through several processes as mutation, clonal evolution and horizontal gene transfer, or this resistance was entirely attributable to widespread use of penicillin or other antibiotics, which selected for bacteria containing resistance genes. These evolutionary processes enhance the pathogenic and antimicrobial-resistant properties of *S. aureus* strains (16).

Parallel to increasing the resistance of microorganisms to the currently used antibiotics and the high cost of production of synthetic compounds, pharmaceutical companies are now looking for alternatives. Medicinal plants could be one approach because most of them are safe with little side effects if any, cost less and affect a wide range of antibiotic resistant microorganisms. The use of plants as source of remedies for the treatment of many diseases dated back to prehistory and people of all continents have this old tradition (17). The results of present study showed that the aqueous extract of *R. coriaria* inhibited the growth of tested isolate strongly; this may be due to presence of the phytochemical groups as mentioned in Table 3; Badi *et al.* (18) reported that the activity of this plant may be attributed to different content of compounds founds in this plant like ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tanic acid and each of these groups has antibacterial effects against bacteria due to their toxicity and affection on bacterial enzymes (19). This result was in accordance with the results of Abu-Shanab *et al.* (3) who also reported that *R. coriaria* has strong antibacterial activity. Similar observations were also shown by (20). On the other hand this extract showed that it has antiplasmid agent according to the present result, this means that this plant can cure the antibiotic resistance genes that located on plasmid; other researchers also reported in their studies that medicinal plants (cardamom, coriander, bitter fennel, clove, cinnamon and colocynth) have anti-plasmid activity (21), (22) and (23). The effect of this extract against bacteria in vivo gave a good result as bacterial inhibitory agent (in case of comparing group IV with group III). While when it compared with group V and group VI, it had a less efficacy than the two mentioned groups. This may be interpreted that the administrated dose of tested extract was little as affected dose, or may at this dose the immunity system of mice be compromised (may be a toxic for the host), so it appeared from studies that the tannins can cause several disorders in mice and rats such as liver cancer...etc (24) (this is in contrast with the positive results of aqueous extract of tested plant on laboratory animals), or the mice may need to other course of administrating the plant extract. The number of bacteria in group VI was zero, and this result gives a shiny way for using a drug composed of a combination of plant extract and antibiotics as a new generation of drugs to attack the antibiotic resistance of bacteria. On the other hand it must take in consideration that the plant extract may have a side effect for the patient where it observed that the mice of group II affected locally (in place of

injection); therefore it is very necessary to use plant extract has a strong inhibition against bacteria and a little side effect for the host.

## 5. CONCLUSION

It can be concluded that aqueous extract of *R. coriaria* is a strong inhibitor for bacterial growth in vitro and in vivo and it has anti-plasmid property for curing the resistance genes located on resistance plasmids. Further studies must be done to focus on fractionation of the extract in vivo as antibacterial.

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