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Antibacterial and anti-biofilm effects of Sumac (*Rhus coriaria* L) fruits extracts against some multidrug-resistant pathogenic bacteria

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Sally K. Abd. Alaameri* BSc/ Biology
Huda S. A. Al-Hayanni BSc /Biology, MSc &PhD /Microbiology**

Abstract

Background: The prevalence of multidrug-resistant bacteria and their contribution to increased morbidity and mortality due to the difficult-to-treat diseases caused by these bacteria, has demonstrated a need to develop and use alternative antimicrobial agents to control multidrug-resistant bacteria. There has been a growing interest in medicinal plants and herbs and their extracts for the discovery of new natural therapeutic alternatives. Therefore, this current study aimed to know the antibacterial activity of aqueous and alcoholic extracts of the Sumac (*Rhus coriaria* L) fruits against multidrug-resistant clinical bacterial isolates and the effect of these extracts on biofilm production as an important virulence factor.

Materials and Methods: The Sumac (*Rhus coriaria* L) plant was selected for this study, and aqueous and alcoholic extracts were prepared from its fruits. They were tested against four multi-antibiotic resistant bacterial isolates that produce biofilms (Gram-positive and Gram-negative), namely *Staphylococcus aureus, Enterococcus faecalis, Acinetobacter baumannii*, and *Pseudomonas aeruginosa* which were isolated from Iraqi patients with wounds and burns in Medical City hospitals.

Results: The current study proved that the aqueous and alcoholic extract of Sumac (*Rhus coriaria* L) fruit is effective as an antibacterial and anti-biofilm against the studied multidrug-resistant bacterial isolates at all tested concentrations with significant differences. The study also showed that the alcoholic extract is more effective as an anti-bacterial and anti-biofilm than the aqueous extract of the Sumac.

Conclusion: The current study provides valuable results for the use of extracts of medicinal plants and herbs, including sumac extracts, to treat pathogenic bacteria that have become more resistant to antibiotics. These plant extracts also contain natural compounds that can be used without causing any harmful effects on patients.

Keywords: Antibacterial activity**,** Anti-biofilm activity, Multidrug-resistant bacteria**,** *Rhus coriaria* L., Sumac.

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Introduction:

According to the World Health Organization (WHO), antibiotic resistance has seriously threatened global public health. The advent of multidrug-resistant bacteria exacerbates the situation and humanity is condemned to increased morbidity and mortality from microbial diseases (1). Plants have long been recognized to have medicinal qualities. Plant-derived antimicrobials and other drugs are becoming more commonly acknowledged in conventional medicine (2) **.** When conventional antibiotics (microorganism products or their synthetic derivatives) become ineffective, new infections remain unmanageable by this kind of medicine (1). According to (WHO) statistics, 80 percent of people in developing countries feel that medicinal herbs may help with basic health care. Because microbes are becoming increasingly resistant to commercially accessible medications, there is a rising interest in learning more about medicinal plants and their active components (3).

Sumac is the common name for the Rhus genus, which has 91 recognized species names in the Anacardiaceae family and is represented in Iraq by one species, Rhus coriaria L., which grows wild and/or farmed near communities in northern Iraq. The term "Sumac" is derived from the Arabic word "summq," which means "dark crimson," and is now used for the spice product Rhus coriaria, which has been used in spice blends in Asian traditional remedies since ancient times (4). *Rhus coriaria* L (Sumac) contains various biologically active phytochemicals utilized in herbal drugs for antimicrobial, antimalarial, antidiarrheic, antidysenteric, antihepatotoxic, antiseptic, antimutagenic, antispasmodic, antiviral, astringent, candidicide, hepatotonic, neuroprotective, antinociceptive, cardioprotective, antidiabetic (5). It has been recently widely used in the treatment of COVID-19 (6). Due to this plant's medically important secondary metabolites, this work concentrated on the usage of the fruits of the Sumac

**Department of Biology, College of Science for Women, University of Baghdad, Iraq.*
sally.khodair1202a@csw.uobaghdad.edu.iq
hudasa_bio@csw.uobaghdad.edu.iq

plant as an anti-bacterial agent and anti-biofilm as the most important virulence factor. Only a few research have examined the antibacterial activities of *Rhus coriaria* L. aqueous and alcoholic extracts on MDR bacteria and the effects on bacterial biofilm development. In order to evaluate *in vitro* the effects of *Rhus coriaria* L. extracts on four isolates of MDR bacteria obtained from burn injury and wounds from Iraqi patients, the current investigation was carried out.

Materials and Methods

Bacterial isolates: In the present study, four multidrug-resistant and biofilm-producing bacterial isolates were selected, which were isolated from Iraqi patients admitted to Hospitals of Medicine City hospitals for the period from October 2021 to February 2022. Two of which were Gram-positive (GP) (*Enterococcus faecalis* and *Staphylococcus aureus*), and two were Gram-negative (GN) (*Pseudomonas aeruginosa* and *Acinetobacter baumannii*). Antibiotics susceptibility test (AST): Antibiotics susceptibility test was done via disk diffusion method (Kirby-Bauer method) as described by (7, 8) and Clinical Laboratory Standard Institute recommendations (CLSI. 2021), and the results were confirmed by Vitek 2 System. (9). Evaluation of phytochemicals groups in aqueous and alcohol powder fruit sumac extracts: The screening of preliminary qualitative phytochemicals of Sumac fruit aqueous and alcohol extracts was done to evaluate the presence of bioactive components. The presence of flavonoids, alkaloids, glycosides, phenols, terpenoids, steroids, saponins, resins, tannins, and coumarin was determined according to the regular methods outlined in (10). Preparation of Sumac (Rhus coriaria L.) extracts: The *Rhus coriaria* L fruit was bought from the Baghdad local markets. and identified by the Department of Biology/ College of Science for women. Aqueous and ethanol extracts of the Sumac fruit were prepared according to (11, 12). with some modifications: by drenching 50 g each of the dry grind plant materials in 500 ml of solvent (distilled water in aqueous extract) and in 250 ml of solvent (ethanol in alcohol extract) at room temperature for 48 hrs. with shaking. The extracts were filtered after 48 hrs. through cotton wool and then through Whatman No. 1 filter paper to eliminate the plant residue. The extracts were concentrated by using a rotary evaporator with the water bath set at 40˚C to get the crude extracts. The percentage yields of extracts extended from 3-8% w/w. The crude extracts were kept at 4˚C in sterile containers until more use. Antibacterial activity study: The study of antibacterial activity was carried out according to the method (13) with some modifications. Of each of the different concentrations prepared from the Sumac aqueous and alcoholic extracts, 0.1 ml was mixed with 0.1 ml of the bacterial inoculum suspension (approximately 1.5×10^5 CFU / ml). They were incubated together for one hour in the incubator at 37°C, then they were spread onto Mueller Hinton agar plates using a standard micropipette after incubation for 24 hours the number of colonies developing on the surface of the plates was counted, and by comparing with the control the minimum inhibitory concentration (MIC: is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation) was determined. Three plates (replicates) were used for each concentration to reduce the errors that might result from conducting the experiment.

Anti-biofilm activity of Sumac extracts: Anti-biofilm effect of extracts was done according to (14), with some modification: Microtiter plate containing 199µL of Mueller–Hinton broth augmented with 1% glucose was inoculated with 100µL from suspended bacterium and 100 µL of each concentration of MIC were(1.878%), to aqueous extract and (0.47 %) to alcoholic extract for antibacterial effect, Microplates are incubated for 24 h at 37°C. Adhesive cells were rinsed twice with PBS, and the wells were parched at 37°C for less than an hour at 37°C. Crystal violet was then dyed on the specimen and incubated for 15 minutes. The crystal violet-stained microplate wells were rinsed twice with PBS. After air-drying microplate wells, 150µL of 95% ethanol re-solubilizes biofilm color. The microplate reader was measured spectrophotometrically at OD_{580} nm after 5-10 min.

Statistics analysis: SPSS V.16 software was used to analyze the data. ANOVA and LSD were used to calculate the mean, standard error, and significant differences between values (15).

Results:

Antibiotics susceptibility test: According to the antibiotics susceptibility test by the Kirby-Bauer method, chosen bacterial isolates exhibited a multi-drug resistant (MDR) pattern that included resistance to Ampicillin, Tetracycline, Amikacin, Ciprofloxacin, Ceftazidime, Gentamicin, Imipenem Meropenem, Aztreonam, and according to Vitek 2 system all results were identical and the isolates were resistant to 11 antibiotics. Qualitative phytochemical screening of the Sumac (*Rhus coriaria* L.) extracts: The qualitative phytochemical screening of Sumac aqueous and alcoholic extracts that was investigated, revealed the presence of various phytochemical components in the extracts as shown in Table (1).

Table 1: Qualitative phytochemical analyses of Sumac aqueous and alcoholic extracts.

Phytochemical component	Aqueous extracts	Alcoholic extracts
Flavonoids	+ +	+++
Alkaloids	_	+
Glycosides	++	+++
Phenols	++	+++
Terpenoids	-	+ +
Steroids	-	_
Saponins	++	++
Resins	+	++
Tannins	++ +	++ +
Coumarin	_	_

Legend: +++ (Much abundant), ++ (less abundant), + (minute), - (absent).

Antibacterial activity of Sumac fruits extracts: The antibacterial activity results of the *Sumac* fruit aqueous extract showed that the aqueous extract of sumac fruit has antibacterial activity against chosen isolates of MDR bacteria compared with untreated control as shown in Table (2).

Table 2: Antibacterial activity of the *Rhus coriaria* L. fruits aqueous extract against bacterial isolates.

Concentration (%)µg /ml	Mean ± SE x10^5 CFU/ml			
	S. aureus	*E. faecalis*	*P. aeruginosa*	*A. baumannii*
Control	15000 ±0.00 a	15000 ±0.00 a	15000 ±0.00 a	15000 ±0.00 a
30	0.00 ±0 .00d	0.00 ±0.00 d	0.00 ±0.00 c	0.00 ±0.00 d
15	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 c	0.00 ±0.00 d
7.5	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 c	0.00 ±0.00 d
3.75	180.00 ±11.54 c	85.00 ±20.21 c	0.00 ±0.00 c	40.00 ±11.54 c
1.875	420.00 ±11.54 b	220.00 ±11.54 b	16.00 ±0.57 b	160.00 ±34.63 b
LSD value	18.442 **	24.705 **	19.660 **	32.623 **
P-value	0.0001	0.0001	0.0001	0.0001

Means having with the different letters in Same column differed significantly. ** (P≤0.01).

Table 3: Antibacterial activity of the *Rhus coriaria* L. fruits alcoholic extract against bacterial isolates.

Concentration (%) µg /ml	Mean ± SE x10^5 CFU/ml			
	S. aureus	*E. faecalis*	*P. aeruginosa*	*A. baumannii*
Control	15000.00 ±0.00 a	15000.00 ±0.00 a	15000.00 ±0.00 a	15000.00 ±0.00 a
30	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d
15	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d
7.5	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d
3.75	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d
1.875	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d	0.00 ±0.00 d
0.94	20.00 ±5.77 c	13 ±0.88 c	11.00 ±0.57 c	12.00 ±3.46 c
0.47	38.00 ±6.92 b	22.00 ±1.15 b	20.00 ±0.57 b	48.00 ±6.92 b

Means having with the different letters in Same column differed significantly. ** (P≤0.01).

The results of Table (2) showed that it was not possible to account for the bacterial colonies in the control dishes due to the heavy growth of bacteria in the dishes, and they were equal to TNTC (too numerous to count" more than 300 colonies). While dishes treated with concentrations (30, 15, 7.5) µg/ml showed complete inhibition of growth. While the minimum inhibitory concentration (MIC) for all isolates was (1.878%), The aqueous extract inhibited all chosen bacterial isolates with highly significant differences (P≤0.01) compared to the control dish as shown in the table, where the least significant difference was (18.442 **) for *S. aureus* isolate, and then for *P. aeruginosa* isolate (19.660 **), then (24.705 **) for the *E. faecalis*, and *A. baumannii* (32.623 **) with high significant differences (P≤0.01), and Gram-negative bacteria were more sensitive to the aqueous extract with a slight difference from the Gram-positive bacteria as shown in the table. The antibacterial activity of the Rhus *coriaria* L. fruits alcoholic extract. The results shown in Table (3) showed that alcohol extract of Rhus *coriaria* L.(Sumac) fruits has antibacterial activity against a chosen isolates, at concentrations (30, 15, 7.5, 3.75, 1.875, 0.94, and 0.47) µg/ml, and the concentration from (30 to1.875%) was the (MBC) to all clinical isolates. But at a concentration (0.94%) was sub MIC effect and the concentration (0.47 %) has MIC effect in all clinical isolates, where the least significant difference was)8.061**) for *P. aeruginosa* isolate, and for *E. faecalis* isolate was (8.172 **), then (9.637 **) for the *S. aureus* and finally for *A. baumannii* was (10.403 **), with highly significant differences (P≤0.01) in comparison with the control dish as shown in the table and for all concentrations used.

Anti-biofilm activity of Sumac fruits extracts:

The inhibitory effect of the aqueous and alcoholic sumac fruits extracts on the biofilm-formation ability of MDR biofilm-forming isolates was examined at the MIC concentrations used for each extract (in aqueous extract was1.878%, and in alcohol extract was 0.47%). And as shown in tables (4, and 5), and figures (1, and 2) there is a sharp decrease in biofilm productivity compared to control, where all the selected isolates were strongly biofilm producers. The results showed each all the aqueous and alcohol extracts had an inhibitory effect on the ability of bacteria to biofilm formation with highly significant differences (P≤0.01), and from the tables and graphs, it is clear that the bacterial isolates were somewhat more sensitive to the alcoholic extract compared to the aqueous extract.

Table 4: Anti-biofilm activity of the Sumac aqueous extracts against the bacterial isolates.

Bacterial isolates	Mean ± SE of biofilm formation inhibition		T-test
	Control	Aqueous extract	
S. aureus	1.64 ± 0.003	0.48 ± 0.01	0.377 **
E. faecalis	2.61 ± 0.05	1.30 ± 0.02	0.304 **
P. aeruginosa	2.08 ± 0.003	0.10± 0.004	0.472 **
A. baumannii	2.86 ± 0.02	1.49 ± 0.007	0.537 **
** (P≤0.01).			

Bacterial isolates	Mean ± SE of biofilm formation inhibition		T-test
	Control	Alcoholic extract	
S. aureus	1.64 ± 0.003	0.14 ± 0.004	0.522 **
E. faecalis	2.61 ± 0.05	0.59 ± 0.02	0.704 **
P. aeruginosa	2.08 ± 0.003	0.03 ± 0.001	0.492 **
A. baumannii	2.86 ± 0.02	1.70 ± 0.001	0.437 **
** (P≤0.01).			

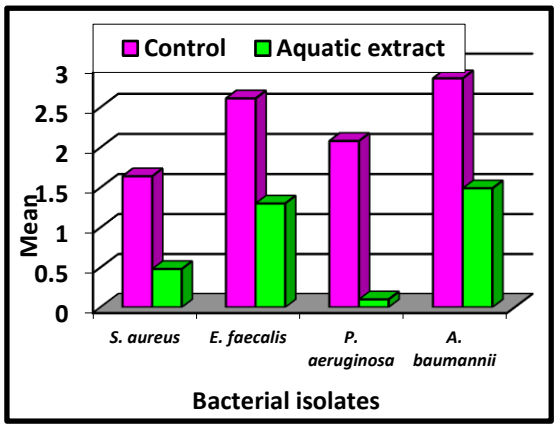

Fig 1: Anti-biofilm activity of the Sumac aqueous extracts against the bacterial isolates.

Table 5: Anti-biofilm activity of the Sumac alcoholic extracts against the bacterial isolates.

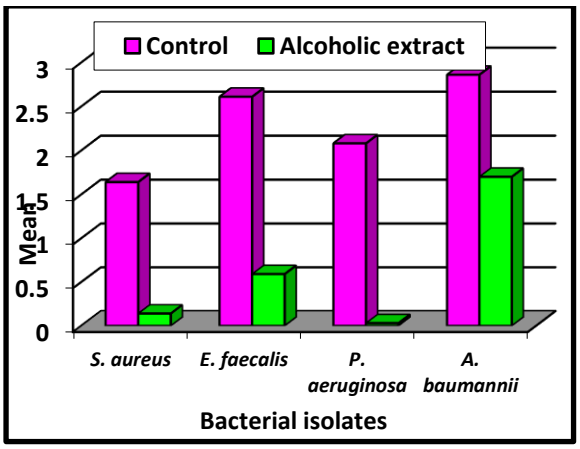

Fig 2: Anti-biofilm activity of the Sumac alcoholic extracts against the bacterial isolates.

Discussion:

A wide range of environmental stressors, including the existence of antibiotic compounds, may be met by bacteria's amazing genetic flexibility. As a result, bacteria that live near antimicrobial-producing species have developed mechanisms that allow them to persist despite the presence of the antibiotic molecule. Thus, antibiotic resistance spread and caused a threat to global health. This prompted the search for alternative therapeutic agents to antibiotics (16, 17). According to the results of the current study, the phytochemical components of Sumac fruit aqueous extract showed the presence of flavonoids, glycosides, phenols, steroids, saponins, resins, and tannins. While the phytochemical components of Sumac fruit alcoholic extract showed the presence of flavonoids, glycosides, phenols, terpenoids, steroids, saponins, resins, tannins, and a minute presence of alkaloids. With similar results, the phytochemical groups of Sumac extract *in vitro* were analyzed by (18), and the results indicated that the Sumac extract contains tannins, phenols, saponins, flavonoids, alkaloids, and phlorotannin. *Rhus coriaria* contains phenols and tannins, and as in many, the researchers explained the action of hydrophobic property of phenolic compounds in impairing the cellular function and membrane integrity as mentioned in (19), and also interpreted that the aqueous extracts of *R. coriaria* contain phenols, tannins, and others integrates and these may have an influence on the enzymatic system of bacteria especially those that prevent the plasmid replication or may effect on the cell membrane, especially on mesosome which is considered as the attachment point for plasmids. The effect of tannins may be related to their ability to inactivate microbial adhesions, enzymes, cell envelope transport proteins, etc. they are also complex with polysaccharides (20).The abundance of water-soluble tannins in sumac is well known to have antibacterial properties. These results were in agreement with the study of (21). The current study proved that the alcoholic extract has an inhibitory effect against the selected isolates compared to the aqueous extract, and with lower concentrations. This is due to the alcoholic extract's high content of active secondary metabolites especially phenolic compounds as in table (1). The study by (22) showed that the methanolic extract of Sumac fruit contains a higher total content of phenols than the aqueous extract (environmental drugs and antioxidants). In another study (23) the phenolic content of sumac was evaluated and methanol, ethanol, a mixture of methanol-ethanol, and distilled water were used for extraction. ethanol shows the best results and sumac had the highest phenolic content as compared to other extracts ,These results are in agreement with the results of the current study . And Since biofilm is the most dangerous virulence factor in pathogenic bacteria isolated from wounds and burns, it was worth examining the inhibitory activity of aqueous and alcoholic extracts of sumac fruits against biofilm productivity. The results showed each of the aqueous

and alcohol extracts had an inhibitory effect on the ability of bacteria to biofilm formation with highly significant differences (P≤0.01). These results agreed with the study results of (24), where an experiment was conducted on the inhibitory effect of Sumac fruits alcoholic and aqueous extracts on the biofilm formation by several Gram-positive and negative isolates (*E. coli*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*), the extracts appeared an inhibitory effect on the biofilms formation of all the isolates used.

A previous study (25) stated that natural phytochemical compounds have an anti-biofilm effect, such as phenols, terpenes, and alkaloids (26) as phenolic consist of a group of compounds. There are seven subclasses of it, and among them are phenolic acids, quinines, flavonoids, flavones, tannins, and coumarin. Condensed tannins are one kind of tannin that possesses anti-biofilm function. Six major methods, including substrate deprivation, membrane rupture, binding to adhesion complex and cell wall, binding to proteins, contact with eukaryotic DNA, and inhibiting viral fusion, are used by all of these compounds to suppress biofilm growth (27). It is clear to us from the foregoing that the active secondary metabolites present in both aqueous and alcoholic extracts target one or more stages of biofilm formation, thus losing the ability of bacteria to cause the virulence caused by biofilm production.

Conclusions:

The current study provides valuable results for the use of extracts of medicinal plants and herbs, including Sumac extracts, to treat pathogenic bacteria that have become more resistant to antibiotics. These plant extracts also contain natural compounds that can be used without causing any harmful effects on patients.

Authors' Contributions: Both authors Sally K. Abd. Alaameri and Huda S. A. Al-Hayanni have made a substantial, direct, and intellectual contribution to the work through the implementation of experiments, writing, and revision, and they approved it for publication.

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التأثير المضاد للبكتيريا والغشاء الحيوي لمستخلصات ثمار السماق ضد بعض البكتريا الممرضة المقاومة للأدوية المتعددة

سالي العامري
هدى الحياني

الخلاصة

خلفية البحث: أظهر انتشار البكتيريا المقاومة للأدوية المتعددة ومساهمتها في زيادة معدلات الاعتلال والوفيات بسبب الأمراض التي يصعب علاجها والتي تسببها هذه البكتيريا ، الحاجة إلى تطوير واستخدام عوامل بديلة مضادة للميكروبات للسيطرة على البكتيريا المقاومة للأدوية المتعددة. كان هناك اهتمام متزايد بالنباتات الطبية والأعشاب ومستخلصاتها لاكتشاف بدائل علاجية طبيعية جديدة. لذلك هدفت هذه الدراسة الحالية إلى معرفة الفعالية المضادة للبكتيريا للمستخلصات المائية والكحولية لثمار نبات السماق (*Rhus coriaria L*) ضد العزلات البكتيرية المقاومة للأدوية المتعددة وتأثير هذه المستخلصات على إنتاج الغشاء الحيوي كعامل ضراوة هام.

المواد والطرق: اختير نبات السماق (*Rhus coriaria* L) لهذه الدراسة وحضرت من ثماره مستخلصات مائية وكحولية, وقد اختبرت ضد أربع عزلات بكتيرية متعددة المقاومة للمضادات الحيوية ومنتجة للأغشية الحيوية موجبة وسالبة لصبغة غرام وهي (*Staphylococcus aureus*, *Enterococcus faecalis*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*) معزولة من مرضى عراقيين راقدين في مستشفيات مدينة الطب من الفترة تشرين الأول 2021 الى شباط 2022.

النتائج: اثبتت الدراسة الحالية ان المستخلص المائي والكحولي لنبات السماق *Rhus coriaria* L فعال كمضاد للبكتيريا والغشاء الحيوي ضد العزلات البكتيرية المقاومة للمضادات الحيوية المتعددة المدروسة وبجميع تراكيزه التي تم اختبارها مع وجود فروقات معنوية واضحة. كذلك أظهرت الدراسة ان المستخلص الكحولي اكثر فعالية كمضاد بكتيري ومضاد للأغشية الحيوية اكثر من المستخلص المائي لثمار نبات السماق.

الاستنتاجات : تقدم الدراسة الحالية نتائج قيمة لاستخدام مستخلصات النباتات والاعشاب الطبية ومنها مستخلصات نبات السماق لعلاج البكتيريا الممرضة والتي أصبحت أكثر مقاومة للمضادات الحيوية. كما تحتوي هذه المستخلصات النباتية على مركبات طبيعية يمكن استخدامها دون إحداث أي آثار ضارة على المرضى.

مفتاح الكلمات: نشاط مضاد للبكتيريا ، نشاط مضاد للغشاء الحيوي ، بكتيريا مقاومة للأدوية المتعددة ، *Rhus coriaria* L، السماق.