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Antimicrobial Properties of *Rhus coriaria* Seeds (Sumach)

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Abstract. Different solvent extracts of sumach seeds were first screened against few test organisms; Staphylococcus aureus, Bacillus subtilis, Mycobacterium phlei and Escherichia coli using the disc-agar diffusion technique. Five aqueous extracts (distilled water, saline 0.9% NaCl, pH 4 and pH 9 buffers and 1.5% H SO) were tested. Likewise, five crude organic solvent extracts (petroleum ether, ether, acetone, ethanol and methanol) were also tested using cold and hot extraction (Soxhlet). Acetone and alcohol extracts proved to be the most effective in antibacterial activity and gave higher yield of extractives. Acetone and alcohol extracts possessed broad-spectrum antimicrobial activity against all test organisms used (29 species), pathogenic, non-pathogenic and even Gram-negative bacteria and antibiotic-resistant strains (3 strains). The crude extracts were heat-stable; both boiling and autoclaving did not cause a significant loss in activity. They were alo stable in both alkaline and acidic conditions.

Introduction

Rhus coriaria seeds known as sumach (Family Anacardiaceae) is commonly used in Egypt in folk medicine. Ancient Arab scientists, Al-Antaky [1; pp.182] and Ibn Sina [2; pp.118-119] described the seeds to cure a variety of ailments. They recommended their use in treating eye diseases, wounds, sores, ulcers, as an astringent in dysentry and bowel disorders as well as for ring worm and skin diseases. At present, this plant material is still being used as a traditional remedy by many Egyptians, specially villagers and desert Bedouins, to cure different diseases such as the inflammatory conditions of the eyes (mucopurulent opthalmia, conjunctivitis and eye tumours and ulcers).

Most investigations dealt with the chemical constitution of sumach seeds such as lipids, oils, proteins, carbohydrates, polyphenols, etc. [3-7]. However, nothing could be traced in the literature about the antimicrobial activity of sumach seeds. In a preliminary screening of some plants commonly used in Egypt, the author [8] found

Antimicrobial Assay Methods

(i) Serial dilution method

The aqueous solution which was prepared from alcohol and acetone extracts as described above were used. A series of tubes containing equal amounts of Penassay broth (Difco) with graded concentrations (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}) of the solution were inoculated with 0.05 ml of 24 hr broth culture of the test organisms. The tubes were incubated at 37°C for 24 hrs and each tube was observed for the presence or absence of visible growth as compared with control broth tubes without inoculation. The minimal inhibitory concentration (M.I.C.) was determined by the turbidimetric method as described in the Difco Manual [10; pp. 203-204] average of duplicates was measured and calculated.

(ii) Agar diffusion method

The antibiotic diffusion assay method was used as described in the Difco Manual [10; pp. 203-204] and according to Schmidt and Moyer [11]. Four sterile filter paper discs (9 mm) were thoroughly moistened in the crude extracts (1 ml = 1 g powdered seeds) and then placed on agar plates seeded with different test organisms. The test plates were prepared by pouring 10 ml of Penassay base agar (Difco) and after solidifying, 5 ml of appropriate seed agar medium was distributed on the surface of solidified base agar. The seed agar was prepared by adding one ml of a 24-hour broth cultures of test organisms to 100 ml melted seed agar. The plates were incubated at 37°C for 18 to 20 hours. The zones of inhibition of growth were measured in millimeters. Average of 16 replicates (4 plates × 4 discs) were measured and calculated as shown in Tables 1, 2).

Media

The media were prepared as described in the Difco Manual [10; pp. 203-204] and according to the formula recommended by Schmidt and Moyer [11] for the assay of penicillin. Selected seed agar media were used for the growth of specific test organisms.

Test organisms

For determining the antibiotic spectrum of the crude sumach seed extracts, pathogenic and non-pathogenic bacterial cultures of Gram-positive, Gram-negative, sporeforming and acid-fact bacteria were used as listed in Table 2. These cultures were obtained from Animal Health Research Institute, Ministry of Agriculture, Egypt (AHRI); Naval Medical Research Unit No. 3, United States of America at

Table 1. Antibacterial activity of different crude sumach seed extracts*

Extract	Staph. aureus	Bacillus subtilis	Mycob. phlei	Esch. coli
Aqueous extracts:				
Distilled water	+	+	+	—
Saline (0.9% NaCl)	+	+	++	+
pH 4 water	+	+	++	—
pH 9 water	+	+	++	—
1.5% H_2SO_4	+	+	++	—
Organic solvent extracts: Hot Soxhlet (Total extracts):				
Petroleum ether	+	+	++	—
Ether	++	++	+++	+
Acetone	+++	+++	+++	++
Ethanol	+++	+++	+++	++
Methanol	+++	+++	+++	++
Hot Soxhlet (successive extracts):				
Petrol, ether	+	+	++	—
Ether	++	++	+++	+
Acetone	+++	+++	+++	++
Ethanol	++	++	+++	++

*Antibacterial activity against test organisms expressed as diameter of inhibition zone in millimeters is indicated by:

+ Diameter is less than 20 mm;) ++ diameter is between 20-30 mm, +++ Diameter is over 30 mm, — means negative result. (Average of 16 determinations).

Table 2. Antimicrobial spectrum of the acetone and alcohol extracts of sumach seeds (1 ml = 1 gm) using the agar diffusion method, expressed as diameter of inhibition zone in millimeters*

Test organism	Source and culture	Collection No.	Acetone** extract	Alcohol** extract
Gram-positive				
Staphylococcus aureus	PCI	209 P	34	30
Staphylococcus aureus	NAMRU	antibiotic-resistant	32	30
Staphylococcus albus	NAMRU	local isolate	36	32
Staphylococcus epidermidis	ATCC	14990	34	31
Staphylococcus citreus	NAMRU	local isolate	36	32
Sarcina lutea	PCI	1001	39	36
Streptococcus faecalis	NAMRU	local isolate	37	33
Streptococcus pyogenes	ATCC	10389	34	31
Corynebacterium bovis	AHRI,	local isolate	32	30
Corynebacterium ovis	AHRI,	" "	34	31
Corynebacterium pyogenes	AHRI,	" "	32	29
Spore-formers				
Bacillus subtilis	ATCC	6633	37	32
Bacillus mycoides	AHRI,	local isolate	38	32
Bacillus pumilis	AHRI,	" "	37	31
Bacillus polymyxa	AHRI,	" "	36	31
Bacillus megatherium	AHRI,	" "	37	32
Gram-negative				
Alkaligenes faecalis	NAMRU,	local isolate	28	25
Brucella suis	AHRI,	" "	30	26
Brucella abortus	AHRI,	" "	27	23
Brucella melitensis	AHRI,	" "	29	24
Enterobacter clocae	ATCC,	13047	27	23
Klebsiella aerogenes	NAMRU	local isolate	26	22
Proteus morganii	NAMRU	" "	27	23
Proteus vulgaris	ATCC	6380	26	21
Pseudomonas aeruginosa	ATCC	9721	24	19
Pseudomonas aeruginosa	NAMRU	antibiotic-resistant	21	17
Pseudomonas fluorescens	NAMRU	local isolate	27	22
Salmonella typhi	NAMRU	" "	28	23
Salmonella typhimurium	AHRI,	" "	29	25
Shigella dysentriae	NAMRU	" "	27	21
Acid-fast				
Mycobacterium phlei	AHRI,	local isolate	37	34

* Average of 16 determinations.

** Diameter of inhibition ≥ 30 mm is considered good activity.

that sumach seeds extracts revealed a pronounced antibacterial activity against Gram positive, Gram negative, spore-forming and acid-fast bacteria, namely, Staphylococcus aureus, Escherichia coli, Bacillus subtilis and Mycobactrium phlei. This investigation deals with the antimicrobial properties of the sumach seeds.

Materials and Methods

Extraction procedure

Different extracts of the powdered seeds were prepared and tested for their antimicrobial potency to determine the most efficient extracting solvent according to Carlson and Douglas [9]. Aqueous extracts: Saline solution (0.9% NaCl), 1.5% H_2SO_4, pH_4 and pH_9 acetate buffers were prepared. The pH_4 and pH_9 buffers were prepared by adding acetic acid and sodium carbonate respectively to distilled water. In addition, other organic solvents were also used as ethanol, methanol, ether, petroleum ether, chloroform and acetone. The finely grounds seeds (1 kg) were soaked in corked bottles in a ratio of plant to solvent (1:4 w/v). The mixtures were allowed to stand at room temperatures for 24 hours with frequent shaking. The 1.5% H_2SO_4 extract was neutralized with 4% NaOH just before testing. The extracts were filtered and tested for antibacterial activity. Since alcoholic and acetone extracts showed the highest antimicrobial activity, they were prepared for further work as follows:

Cold extraction

The finely ground seeds (1 kg) were soaked several times in an excess amount of ethanol or acetone.

Hot extraction

The finely powdered seeds (1 kg) were extracted in a Soxhlet extractor apparatus for 24 hours, using organic solvents separately or successively according to polarity : petroleum ether, ether, chloroform, acetone and finally ethanol.

Both the cold and hot extracts were filtered and concentrated under vaccum at 50°C, the residues were dried and weighed to calculate the yield.

The dry residue which resulted from alcohol and acetone extracts were dissolved in distilled water so that one ml. of the extract is equivalent to one g of the powdered seeds.

Abbassia Hospital for Fevers, Cairo (NAMRU); American Type Culture Collection (ATCC). The antibiotic-resistant strains and local isolate strains were obtained from both NAMRU and AHRI Research units.

Determination of heat stability

Aqueous solutions of crude acetone extract (1 ml = 1g of powdered seeds) were autoclaved at 15 lbs (121°C) and 21 lbs (128°C) for 15 and 30 minutes for both treatments. Aqueous solutions were also boiled for different periods (for 1/2 hr, 1 hr, $1^1/_2$ hr and 2 hrs) as shown in Table 3. The heat treated samples were compared to a fresh untreated solution of the crude extract as a control. All of the stability determinations were conducted by the agar diffusion method, using *Staph. aureus* and *Sarcina lutea,* which were the most sensitive test organisms; zones of inhibition were measured in millimeters. Average of 16 replicates (4 plates × discs) were measured and calculated as shown in Table 3.

Table 3. Effect of heat on the antibacterial activity of the aqueous solutions (1 ml = 1 g) prepared from the dry residue of the acetone extract of sumach seeds, expressed as diameter of inhibition zone in mm*

Heat treatment	Staph aureus	Sarcina lutea	Activity, % of control
Control	34	39	100
Boiling			
boiling for $^1/_2$ hour	**34**	**39**	**100**
" for one hr	34	39	100
" for $1^1/_2$	34	39	100
" for 2 hr	32	27	94
Autoclaving at			
15 lbs (121 °C)/15 min.	32	37	94
" " " /30 min.	30	34	88
21 lbs (128 °C)/15 min.	30	34	88
" " " /30 min.	28	31	80

* Average of 16 determinations.

Effect of pH on stability

Aqueous solutions of the crude acetone extract (1 ml = 1g of powdered seeds) were adjusted to different pH levels (pH 1-13) using solutions of H_2SO_4 or sodium carbonate. A pH-meter (Knick-Digital) was used to adjust pH. The solution were

kept at room temperature for 4 weeks. All solutions were neutralized before testing with Na_2CO_3 or H_2SO_4 solutions and were adjusted to pH 7. The antibacterial activity was then determined after neutralization against *Staphylococcus aureus* and *Sarcina lutea,* in comparison to a fresh untreated solution of the crude extract as a control. Mean average of 16 replicates (4 plates × 4 discs) were measured and calculated as shown in Table 4.

Table 4. Effect of pH on the antibacterial activity of the aqueous solutions (1 ml = 1 g) prepared from the dry residue of the acetone extract of sumach seeds, expressed as diameter of inhibition zone in mm*

pH range	Staph aureus	Sarcina lutea	Activity, % of control
Control	34	39	100
1–2	30	34	88
3–4	32	37	94
5–6	34	39	100
7	34	39	100
8–9	34	38	100
10–11	32	36	94
12–13	28	31	80

* Average of 16 determinations.

Results and Discussion

Antibacterial activity of solvent extracts

Different solvent extracts were screened for their antibacterial activity to determine the most effective solvent extract(s). The results are presented in Table 1. The aqueous extracts with distilled water, 1.5% H_2SO_4, pH_4 and pH_9 showed moderate activities against most test organisms except *E. coli,* while the saline extract showed activity against *E. coli* as well. Both cold (maceration) and hot (Soxhlet) organic solvent extracts (petroleum ether, ether, acetone, ethanol and methanol) exhibited stronger antibacterial activities than the aqueous ones. Acetone and alcoholic extracts showed the highest antibacterial activity. The yields of hot (Soxhlet) extracts were quantitatively higher, the yield of acetone extractive was 19%, while the ethanol extractive yield was 12%. The yield is expressed as the weight percentage (w/w) of the residue to the finely ground seeds.

(i) Antimicrobial spectrum

The antimicrobial spectrum of the more active crude sumach acetone and ethanol extracts against 29 species of bacteria is shown in Table 2. The extracts 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 bacteria (staphylococci, streptococci and corynebacteria), sporeformers (*Bacillus* species) and acid-fast bacteria (*Mycobacterium phlei*). The least sensitive organisms were the gram-negative bacteria (*Salmonella, Shigell,* Brucella ... etc). Generally, acetone extract has a stronger antibacterial activity than the alcohol extract, as evidenced from the diameter of the inhibition zone (Table 2).

(ii) The minimal inhibitory concentration

Quantitative estimation of the degree of antibacterial activity of dry residue obtained from either the crude acetone or ethanol extracts was made by the serial dilution method against 17 test organisms as presented in Table 5. The highest activity was obtained against Gram-positive and spore-forming bacteria; *Staphylococcus* (*aureus, albus* and *epidermidis*), *Streptococcus* (*faecalis* and *pyogenes*), *Sarcina lutea* and *Bacillus* (*subtilis, myocides* and *pumilis*). The minimal inhibitory concentration (M.I.C.) was 10^{-4} to 10^{-5}.

The Gram-negative bacteria were the least sensitive test organisms; *Escherichia coli, Klebsiella aerogenes, Salmonella* (*typhi* and *Typhimurium*) were inhibited at M.I.C. 10^{-3} to 10^{-4}, while *Pseudomonas* (*fluorescens* and *aeruginosa*) were inhibited at M.I.C. 10^{-2} to 10^{-3}. The acetone extract was more active than ethanol as evidenced from M.I.C. (Table 5).

Heat stability

The effect of heat on the antibacterial activity of crude extracts was tested to determine heat stability. The results are shown in Table 3. The crude sumach extracts were shown to be thermostable; boiling for different periods or even autoclaving up to 15 lbs (121°C) or 21 lbs pressure (128°C) caused a slight insignificant loss in activity.

pH stability

The effect of pH levels (pH 1-13) on the antibacterial activity of the curde sumach extracts was determined. The results are presented in Table 4. The extracts were shown to be stable (activity 100% of control) in neutral (pH 7), slightly acidic (pH 5-6) and slightly alkaline (pH 8-9) media. However, both strong acidic (pH 1-2)

and strong alkaline (pH 12-13) media caused a moderate loss in the antibacterial activity (12-20% loss).

Thus sumach seed extracts were shown to exhibit a strong antibacterial activity against all test organisms used; staphylococci, streptococci, corynebacterial and bacilli and moderate activity against *Salmonella, Shigella, Brucella,* etc. This would explain its different uses in folk medicine in Egypt especially in the treatment of dysentry, bowel disorders and eye diseases (purulent and mucopurulent ophthalmia).

Table 5. The minimal inhibitory concentration (MIC) of the dry residue obtained from acetone and alcohol extracts of sumach seeds (highest dilution of the dry residue inhibiting growth of test organisms), using the serial dilution method

Test organism	Source and culture	Collection No.	Acetone* extract	Ethanol* extract
Gram-positive				
Staphylococcus aureus	PCI	209 P	5	4
Staphylococcus epidermidis	ATCC	14990	5	4
Staphylococcus albus	NAMRU	local isolate	6	5
Sarcina. lutea	PCI	1001	6	5
Streptococcus faecalis	NAMRU	local isolate	6	5
Streptococcus pyogenes	ATCC	10389	5	4
Spore-formers				
Bacillus subtilis	ATCC	6633	5	4
Bacillus mycoides	AHRI	local isolate	5	4
Bacillus pumilis	AHRI	" "	5	4
Gram-negative				
Escherichia coll	ATCC	25922	4	3
Klebsiella aerogenes	*NAMRU*	*local isolate*	*4*	*3*
Pseudomonas fluorescens	*NAMRU*	" "	3	3
Pseudomomas aeruginosa	ATCC	9721	3	2
Pseudomonas aeruginosa	NAMRU	antibiotic-resistant	2	1
Salmonella typhi	NAMRU	local isolate	3	2
Salmonella typhimurium	AHRI	" "	3	2
Shigella dysentriae	NAMRU	" "	3	2
Acid-fast				
Mycobacterium phlei	AHRI	local isolate	6	5

(Average of two determinations "duplicates").

* The highest inhibiting dilutions are expressed as the logarithms:
$1 = 10^{-1}$, $2 = 10^{-2}$, $3 = 10^{-3}$, $4 = 10^{-4}$, $5 = 10^{-5}$, $6 = 10^{-6}$

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الخواص المضادة للميكروبات لمستخلصات بذور نبات السماق

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(ورد البحث في ١٤١٤/١٠/١٥هـ؛ وقُبل للنشر في ١٤١٦/٦/٢٢هـ)

ملخص البحث. تم إجراء الاختبار المبدئي لمستخلصات مختلفة من بذور نبات السماق على بعض السلالات من البكتيريا هي: ستافيلوكوكس أورياس وباسيلس سابتلس وميكو بكتريوم فلياي وإشيرشيا كولاي باستخدام طريقة الانتشار بوضع القرص على الآجار. وقد تم اختبار خمسة محاليل مائية للاستخلاص هي: الماء المقطر ومحلول ملح الطعام (ص كل) بتركيز ٩,٠٪ ومحلول مائي في الوسط الحمضي (رقم الأس الهيدروجيني ٤) ومحلول مائي في الوسط القلوي (رقم الأس الهيدروجيني ٩) وكذلك محلول حمض الكبريتيك المخفف بتركيز ١,٥٪. وكذلك تم اختيار خمسة مذيبات عضوية هي: الأثير والأثير البترولي والأسيتون وكحول الإيثيل وكحول الميثيل باستخدام الاستخلاص على البارد وكذلك الاستخلاص على الساخن بجهاز سوكسلت. وقد وجد أن كلاً من مستخلصي الكحول والأسيتون كان لهما أعلى إنتاجية كميًّا وأكبر تأثيرًا حيث إن لهما تأثيرًا واسع المدى على أنواع مختلفة من البكتيريا (٢٩ نوعًا) المرضية وغير المرضية وحتى السالبة لجرام والمقاومة للمضادات الحيوية (٣ سلالات). وقد تم اختبار خواص الثبات للمستخلصات الخام فوجد أن كلاً من الغليان والتعقيم ليس لهما تأثير يذكر على نشاط المستخلصات فضلاً عن ثبات هذه المستخلصات في كل من الظروف الحمضية أو القلوية.