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Isolation of Major Active Antibacterial Compounds of Sumac Fruit (*Rhus coriaria* L.)

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Abstract

Background: Infectious diseases are still one of the main causes of death according to the World Health Organization (WHO) reports. Plants due to their biochemical metabolites have been considered as one of the important sources for investigation in this field. Ethnobotanical and ethnopharmacological researches are considered effective in developing new anti-infectives. Sumac (*Rhus coriaria* L.) has been used as an anti-infective agent by ancient Iranian medical sages.

Objectives: The aim of this study was to isolate bioactive agents of sumac epicarp with probable antibacterial activity.

Materials and Methods: Grounded epicarp of sumac fruit was fractionated with different solvents. The fractions were dried and subjected to antibacterial investigation. Ethyl acetate fraction showed the strongest antibacterial activity. This fraction was further investigated through TLC-bioautography which led to the isolation of two crystallized compounds. The structure of these compounds (1 and 2) was identified using spectroscopic techniques. Isolated compounds were tested for antimicrobial activities.

Results: Compound 1 which was named 1,2-dioxo-6-hydroxycyclohexadiene-4-carboxilic acid was isolated from *R. coriaria* L. for the first time. It showed antibacterial activity against *Staphylococcus aureus* (minimum inhibitory concentration [MIC] = 0.02%). Compound 2 which was identified as gallic acid showed weak antibacterial effects on both gram-positive and gram-negative bacteria (MIC > 0.1%).

Conclusion: This is the first report about the chemical structures of antibacterial constituents of *R. coriaria* L. Previous studies have shown anti-methicillin-resistant *S. aureus* (MRSA) activity of sumac total extract. Compound 1 as the most effective anti-*S. aureus* component of sumac extract would be responsible for this activity and could be the subject matter for future investigations.

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Background

Plants are important sources of biochemical compounds not only in the development of drugs but in the production of many other agrochemicals, cosmetics, flavors, and food stocks.[1] Ethnopharmacological information is an effective asset in the evaluation and confirmation of traditional uses of medicinal plants.[2]

Despite progress in antibiotic therapy, infectious diseases are still one of the main causes of death worldwide. According to the World Health Organization (WHO) report, 26% of all deaths was due to the microbial infections.[3] On the other hand, plants have an unlimited capacity to be used in synthesizing aromatic substances, some of which have shown antibacterial effects.[4]

Iran with its various geographical climates presents vast varieties of medicinal plants.[5] Sumac (*Rhus coriaria* L.) is among the herbs used as a drug in Iranian traditional medicine. The fruit of this plant was utilized by famous Iranian physicians including Rhazes and Avicenna for the treatment of two infectious ailments, namely, diarrhea and purulent ear.[6,7] Therefore, this is rational to suppose sumac as a source of antibacterial substances. Previous studies have shown that total and some fractions of sumac extract show antibacterial effects against gram-positive and gram-negative bacteria.[8-12] These studies revealed that polar fractions have the most antibacterial activity but none of them determines which compound(s) is/are responsible for this activity.

Objectives

This study intended to identify and isolate antibacterial ingredients of sumac fruit by the aid of conventional purification and spectroscopic techniques.

Material and Methods

Plant Materials

Sumac fruit (*R. coriaria* L.) was obtained from Tehran botanical market and authenticated by Professor Gh. Amin in the herbarium of Faculty of Pharmacy, Tehran University of Medical Sciences, in comparison with original samples. According to the policy of the herbarium, no specific number is given for such a sampling but the sample is kept for occasional checking during the study.

Extraction

Epicarps of sumac fruits were separated from the seeds by sifting ground fruits. The content of fine powder of epicarps was fractionated by solvent partitioning with percolation method at room temperature. Light petroleum ether, dichloromethane, ethyl acetate, and methanol were used respectively to separate the content of epicarp by polarity. The fractions were dried by rotary evaporator, weighed, and kept in cool and dry place for further investigations.

Microorganisms and Growth Conditions

The standard strains of *Staphylococcus aureus* (6538-P) and *Escherichia coli* (ATCC 8739) were kept in 20% glycerol in phosphate buffered saline (PBS) at −70°C. Active cultures were generated by inoculating 100 µL of the thawed microbial stock suspension into 5 mL nutrient broth (Merck, Germany) followed by overnight incubation at 37°C. Freshly synchronized cultures of bacterial strains were prepared by transferring 100 µL of the vegetative cells successively into Muller Hinton broth and incubating for 24 hours at 37°C. The cells were harvested by centrifugation at 1600 g for 10 minutes, washed with PBS, spun at 1600 g again and diluted in sterile water to obtain 10^8 bacteria/mL as estimated by the surface plate counting method.[13]

Preliminary Antibacterial Assay and TLC-Bioautography

Fractions of epicarp were dissolved in their solvents and loaded on sterile blank discs 6 mm in diameter to create 20 mg extract disc. Ten microliter of the suspension of each bacterium (10^8 bacteria/mL) was poured on Muller Hinton agar plates. Then, the prepared discs were placed on the plates. After incubation at 37°C overnight, plates were examined for any zone of growth inhibition. Ethyl acetate as the most active fraction was spotted on thin layer chromatogram for more purification using thin layer of silica and the mixture of chloroform/ethyl acetate/methanol (4:4:1) as solid and mobile phases respectively. To find active spots, overlay bioautography was done by the method described by Wilkinson.[14] Briefly, the chromatogram was overlain with Muller Hinton agar and after agar gelation, the microorganisms were seeded on the surface of the culture. Following the incubation at 37°C overnight, zones of inhibition were observed. Purity of active spots checked by two dimensional TLC showed that one of the active spots was not pure. This spot was separated further by TLC using methanol/acetic acid/chloroform (1:1:2) as mobile phase. Antimicrobial activity of this new chromatogram was also checked via overlay bioautography.

Isolation and Identification

To prepare further active spots, preparative thick layer chromatography (PTLC) was run with the same mobile phase mentioned above and active spots were pared from chromatogram and suspended in methanol to separate silica from active compounds. Finally, methanol was evaporated by vacuum at 30°C and two compounds (1 and 2) were crystallized. The structures of crystallized compounds were identified using IR, ^{1}HNMR, ^{13}CNMR, and mass spectroscopy.

Minimum Inhibitory Concentration and Minimum Bactericidal Concentrations Determination

To determine the minimum inhibitory concentration (MIC) against *S. aureus* and *E. coli*, serial dilutions of active spots obtained via PTLC were prepared between 7.5 to 1000 µg/mL in Muller Hinton broth. Final concentration of bacteria in individual tubes was 10^6 CFU/mL and control tubes contained no test samples. After overnight incubation at 37°C, the test tubes were examined for possible growth and MICs of the samples were determined as the lowest concentration that ended with no growth.[8] Tubes containing concentrations above the MIC were streaked onto Muller Hinton agar plates to achieve minimum bactericidal concentrations (MBC) of the sample against the tested strains.

Results

Antibacterial Evaluations

In this study, preliminary antibacterial activity of the fractions of sumac extract was evaluated by disc diffusion method measuring the inhibition zones around discs. The results of this test showed strong activity of ethyl acetate fraction against both *S. aureus* and *E. coli* (Table 1). TLC-bioautography of ethyl acetate fraction showed that two out of three major spots of the fraction with the R_f of 0.12 and 0.65 had antibacterial activity. The spot with the R_f of 0.12 inhibited the growth of *S. aureus* by the concentration of 0.02% while it showed no effect on *E. coli*. Another active spot was effective on both bacteria in concen-

Table 1. Antibacterial Activity of Sumac Fractions

Fractions	Solvent Polarity Index	Yield of Extraction (%)[a]	Antibacterial Activity (Inhibition Zone)[b]	
			S. aureus	*E. coli*
Light petroleum ether	0.1	8.4	-	-
Dichloromethane	3.1	2.4	9	-
Ethyl acetate	4.4	28.3	18	12
Methanol	5.1	27	19	8

[a]Yield of extraction was calculated as weight of dried fraction by weight of the plant starting material.
[b]The results are given in millimeter.

trations more than 0.1%. None of the spots had detectable bactericidal effects in logical concentrations.

Chemical Analysis

The characteristics of compounds 1 and 2 are given bellow:

Compound 1: ^{1}H NMR (D_2O): 6.67 (bs), ^{13}C NMR (D_2O): 182.93, 176.75, 161.77, 159.77, 149.56, 128.87, 116.21. Mass: m^+ (%), 168 (22), 154 (19), 125 (100), 108 (90), 97 (95), 79 (80). IR (KBr): υ, 3431 (OH), 1720 (C=O), 1613 (C=C), 1198 (C-O), 1019 (C-O).

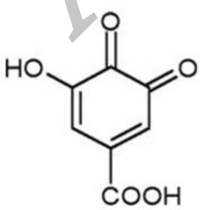
Compound 1 with R_f of 0.12 was identified as 1,2-dioxo-6-hydroxycyclohexadiene-4-carboxilic acid which is presented for the first time. Physicochemical characteristics and spectra of this compound are presented in Figures 1 and 2.

Compound 2: ^{1}H NMR (D_2O): 6.94 (s), Mass: m^+ (%), 170 (58), 153 (25), 126 (100), 108 (28), 79 (32), 53 (28). IR (KBr): υ, 3400 (OH), 1670 (C-O), 1620 (C=C), 1250 (C=C), 1100 (C-O).

Compound 2 with R_f of 0.65 was identified as gallic acid through comparison of its data with those given in literature.[15,16]

Discussion

Phenolic acids and quinones are well known antimicrobials among herbal second metabolites. A phenolic acid is one of the simplest bioactive phytochemicals which consists of a single substituted phenolic ring. Because of oxidizing ability, it inhibits enzymes possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins.[4]



Chemical name	1,2-dioxo-6-hydroxycyclohexadiene-4-carboxilic acid
Molecular formula	C7H4O5
Chemical group	Quinones
Molar mass	168 g/ml
Appearance	Brown crystals
Melting point	>300 °C
Solubility in water	1.5 g/100ml

Figure 1. Physicochemical Characteristics of Compound 1.

Quinones are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds are colorful and responsible for the browning reaction in cut or injured fruits and vegetables. They provide a source of stable free radicals and can form irreversible complexes with nucleophilic amino acids in proteins that often cause their function loss and subsequent cell death. Surface-exposed adhesions, cell wall polypeptides, and membrane-bound enzymes are probable targets of quinone oxidization.[4]

Sumac contains a representative for each of the mentioned phytochemicals. 1,2-dioxo-6-hydroxycyclohexadiene-4-carboxilic acid (compound 1) and gallic acid (compound 2) are phenolic acids. Compound 1 also belongs to quinones. The differences between the spectra of antibacterial activity of these compounds are related to their chemical structures. Compound 1 is more polar than compound 2 (gallic acid) and therefore, it cannot pass through gram negatives' cell walls; so its antibacterial effect is limited to gram-positive bacteria. Gallic acid affects both gram-positive and gram-negative bacteria, but because of its relatively weak oxidizing activity, its antibacterial activity is not so strong. Gallic acid has been found in another species of genus *Rhus,* namely, *R. glabra* L.[17]

Compound 1 is represented for the first time in *R. coriaria* L. according to our bibliographic survey. Previously, Italian scientists had shown that compound 1 can be produced by in vitro radical oxidation of gallic acid.[18] They used sulfate radical anions to promote the reaction in laboratory conditions (Figure 3). It is probable that the compound 1 has been produced via oxidation reaction in a biological environment.

Considering the novelty of compound 1, this molecule can be the subject matter for more investigations on gram-positive bacteria especially resistant strains of *S. aureus* (e.g. methicillin-resistant *S. aureus* – MRSA). Our previous studies revealed that sumac total extract has the same antibacterial effect on both Methicillin-susceptible *S. aureus* (MSSA) and MRSA (unpublished data). We also indicated that sumac total extract has synergistic effect with some antibiotics against MRSA and therefore, makes MRSA susceptible to commercial antibiotics.[19] Because most of anti-*S. aureus* effect of sumac total extract is related to compound 1 (based on the result of present study), this component would be responsible for anti-MRSA activity and is to be considered for more investigations.

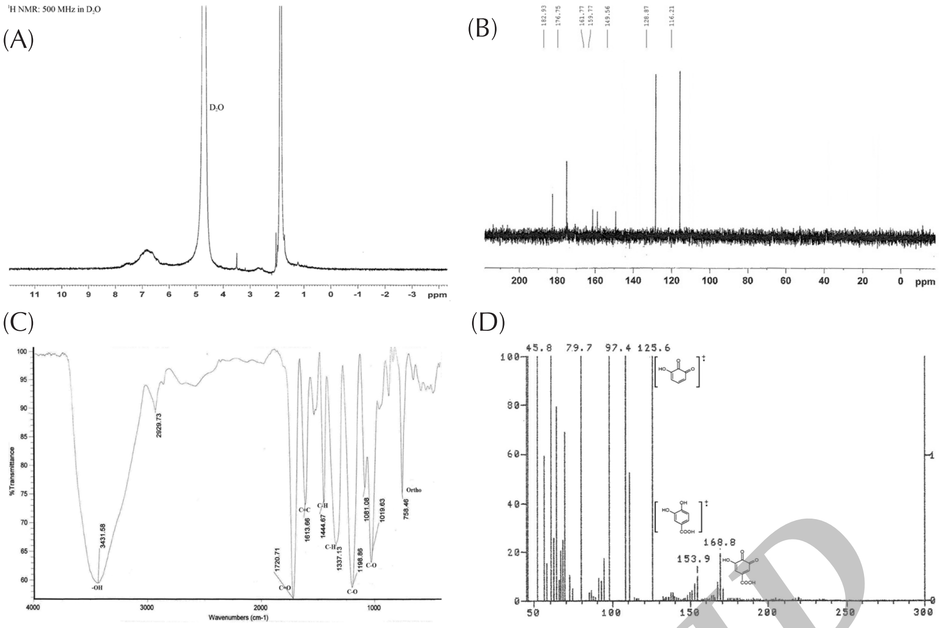


Figure 2. Spectra of Compound 1. (a) Proton NMR; (b) Carbon NMR; (c) IR; (d) Mass Spectrum.

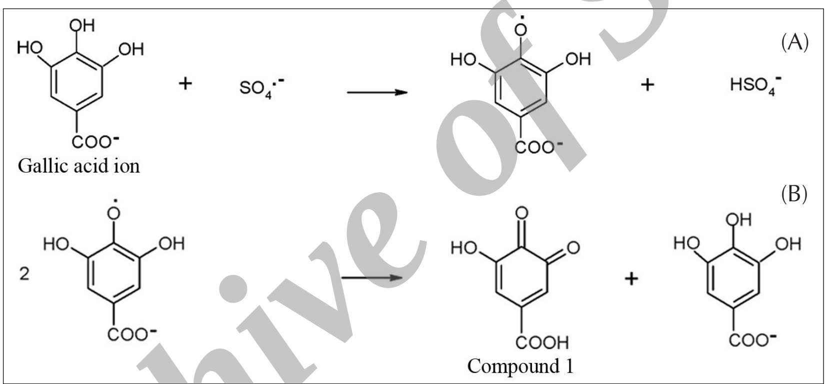


Figure 3. In Vitro Radical Oxidation of Gallic Acid Produces Compound 1.

Authors' Contribution

Study concept and design: GA, MRF, and MMAA; Acquisition of data: MMAA and HJ; Analysis and interpretation of data: MA and HRME; Drafting of the manuscript: HF, MMAA, and AB; Study supervision: GA, MRF, and HF.

Ethical Approval

The research followed the principles of Basel Declaration.

Conflict of Interest Disclosures

The authors have declared that no conflict of interests exists.

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