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RESEARCH ARTICLE

Chemical Composition and Antibacterial Activities of Sumac Fruit (*Rhus coriaria*) Essential Oil on Dental Caries Pathogens

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Abstract:

Introduction:

Dental caries is an infectious disease, and various microorganisms are involved in its progression. The common antibacterial agents against oral pathogens have many side effects and their excessive use cause drug resistance. Therefore, the identification of natural compounds and medicinal plants with antibacterial activity has been considered by the researchers. The Sumac (*Rhus coriaria*) is one of the native plants of Iran, which used as a food flavoring.

Aims & Objectives:

The aim of this study was to evaluate the antimicrobial activity of Sumac essential oil on oral pathogens, including *Streptococcus mutans*, *Lactobacillus rhamnosus,* and *Actinomyces viscosus*.

Materials & Methods:

In this study, the Sumac essential oil was prepared and its antibacterial activity was evaluated by disk diffusion, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC) methods on established terminology strains of *S. mutans*, *L. rhamnosus,* and *A. viscosus*.

Results:

The obtained results showed that the Sumac essential oil has high inhibitory effects against *S. mutans*, followed by *L. rhamnosus* and *A. viscosus*. The predominant compound in the Sumac essential oil was related to Beta-caryophyllene.

Conclusion:

In conclusion, Sumac essential oil has an appropriate antibacterial activity and can be used in the pharmaceutical industry to produce antibacterial agents and mouthwash, against oral infectious diseases and dental caries.

Keywords: Antimicrobial activity, Essential oil, Sumac, Oral pathogens, Agar disk diffusion, Broth micro-dilution, Gas chromatography/mass spectrometry.

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1. INTRODUCTION

Dental caries is an infectious disease, which can dissolve and damage teeth calcareous tissue. Dental caries can cause loss of teeth, pain, and mouth odor. The main known etiologic factors for dental caries are *streptococci*, *lactobacilli*, and *Actinomyces* [1]. Prevention and treatment of dental caries using common antibacterial agents cause alteration of salivation oxidation potential, weak lysozyme activity, allergic reactions, and reduces the resistance of the body to pathogenic factors [2].

Medicinal plants play a special role in traditional medicine. Recently, the trend towards herbal medicine has increased worldwide due to the side effects of chemical antibacterial drugs [3]. Also, excessive use of antibiotics and other chemical antibacterial compounds cause antibiotic resistance. Therefore,

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natural resources, especially edible medicinal plants, are currently considered as antibacterial agents [4]. On the other hand, many hygienic, cosmetics, and pharmaceutical manufacturers are willing to use natural compounds in their products, and many studies have been performed on antimicrobial effects of medicinal plants essential oil or extracts [5]. Recently, many studies reported that essential oil and extracts of traditional herbs have inhibitory effects against various pathogenic microorganisms [6, 7].

Sumac (*Rhus coriaria*) is commonly found in the Mediterranean region, North Africa, Southern Europe, Afghanistan, and Iran. Also, this plant is found in different regions of Iran, such as Azerbaijan, Qazvin, and Hamadan [8]. In past years, the fruit of Sumac was used to improve digestive problems, and treatment of dermatitis, fever, diarrhea, and cancer. Sumac is also known as a diuretic, disinfectant, and an appetizer [9]. Sumac effectively inhibits alpha-amylase and increases glucose tolerance in diabetic patients. It also can inhibit xanthine oxidase and reduce the amount of uric acid in patients with gout. Sumac contains flavonoids, anthocyanins, gallic acid, flavones (such as quercetin, myricetin, and camphorol), nitrate, and nitrite. It also contains fatty acids such as malic acid, palmitic acid, stearic acid, oleic acid, and linoleic acid. Moreover, Tannins form an important part of polar compounds in the Sumac essential oil and extract [10]. Sumac has significant effects in preventing gram-positive and gram-negative pathogenic bacteria. Previous studies have shown that essential oil and extracts of Sumac leaf and fruit have appropriate antimicrobial effects against *bacilli*, *staphylococci*, *enterococci,* and *lactobacilli* [11, 12].

According to the significance of dental caries worldwide, and also the appropriate antimicrobial activity of Sumac as a medicinal plant, the aim of this study was to evaluate the chemical composition and antibacterial activities of Sumac fruit (*Rhus coriaria*) essential oil against dental caries pathogens.

2. MATERIALS AND METHODS

2.1. Preparation of Essential Oil

Sumac fruit was collected from Urmia city in July 2018 and approved by the Herbarium of the Islamic Azad University, Urmia Branch. In this study, 100 gr of Sumac fruit was dried in a dark place with airflow, and then powdered. The obtained powder was added to a balloon containing 600 ml distilled water. The essential oil was prepared using a Clevenger instrument (Zarrin Pirax, Iran) and filtered by a sterile 0.4 µm filter. The obtained essential oil was stored at 4°C until required [5].

2.2. Preparation of Bacterial Strains

The studied oral pathogens in this study include *S. mutans* (PTCC 1683), *L. rhamnosus* (PTCC 1637), and *A. viscosus* (PTCC 1202). These established terminology bacterial strains were purchased from the Iranian Biological Resource Center-Persian Type Culture Collection (IBRC-PTCC).

2.3. Evaluation of Antibacterial Activity

2.3.1. Agar Disk Diffusion

Evaluation of the antimicrobial activity of Sumac fruit essential oil was performed using the agar disc diffusion method. Initially, the standard 0.5 McFarland microbial suspensions (approximate 1.5×10^8 microorganisms per 1 ml) was prepared and cultured on blood agar medium (Merck, Germany). In this study, antimicrobial susceptibility disks (Padtan teb, Iran) containing different concentrations of Sumac fruit (50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%) were used. A disk containing the solvent of essential oil (distilled water) was considered as negative control. Also, penicillin (6 µg), enrofloxacin (5 µg), amoxicillin (25 µg), and florfenicol (30 µg) antibiotic disks (Padtan teb, Iran) were used as positive controls. Finally, the diameter of the inhibition zone was measured after 48 hours incubation at 37°C [13, 14].

2.3.2. Broth Micro-dilution

Evaluation of the antimicrobial activity of Sumac fruit essential oil was performed using the broth micro-dilution method. Initially, the studied concentrations of Sumac fruit essential oil were prepared using sterile Brain Heart Infusion (BHI) medium (Merck, Germany) in sterile tubes. The microbial suspensions with standard 0.5 McFarland concentration were added to each tube. The bacterial strains without studied essential oil were considered as positive controls, and mediums without the bacterial strains were considered as negative controls. The prepared tubes were incubated at 37°C for 24 hours, and the least concentration of studied essential oil without opacity was considered as the Minimum Inhibitory Concentration (MIC). Moreover, 25 µl of each tube contents were cultured on blood agar medium for 48 hours at 37˚C, and the minimum concentration without bacterial growth was considered as the Minimum Bactericidal Concentration (MBC) [7].

2.4. Evaluation of Essential Oil Compounds

The gas chromatograph (Shimadzu-QP2010, Japan) with the ZB-WAX column (length 20 m, inner diameter 0.18 mm, thickness 18.1 µm) was used to identify the compounds of the Sumac fruit essential oil. The essential oil of Sumac fruit was diluted with normal hexane and 1 µl was injected into Gas Chromatography/Mass Spectrometry (GC/MS). The initial temperature of the oven was 50°C, maintained at this temperature for 5 minutes (thermal gradient: 3°C per minute) and then the temperature was increased to 240°C. The final temperature of the oven was 300°C and maintained at this temperature for 3 minutes (thermal gradient: 3°C per minute). The temperature of the injector was 300°C and split/splitless (1 to 50). Helium (99.9999%) was used as the carrier gas at a flow rate of 1ml/min. Then, mass spectrometry (Agilent 5973, USA) (length 20 m, inner diameter 0.25 µm, thickness 0.25 mm) was used. The temperature of the ionization chamber was 150°C, the temperature of the detector was 230°C, the ionization energy was 70 eV, and the mass analyzer was Quadrupole. The scan mass range was 40 m/z to 550 m/z. The mass spectrometry was used to determine the compounds of the essential oil of Sumac fruit. The spectral values were compared with Kovatz index values in the standard tables and the compounds of the essential oil of Sumac fruit were identified according to data and information available in the GC-MS library. The total running time of the GC-MS analysis was performed in 24 minutes.

3. RESULTS

3.1. Antibacterial Activity of Sumac Essential Oil

3.1.1. Agar Disk Diffusion

According to the obtained results, the largest inhibition zone created by Sumac essential oil was related to *S. mutans* (19.0 mm), followed by *A. viscomosus* (16.3 mm) and *L. rhamnosus* (13.6 mm). The studied essential oil created a higher inhibition zone compared to amoxicillin. Also, the largest inhibition zone was related to florfenicol. The solvent of essential oil (negative control) did not show any inhibition zone. The obtained results from the agar disk diffusion assay are shown in Table **1**.

3.1.2. Broth Micro-dilution

According to the obtained results, *S. mutans* and *A. viscosus* showed the highest sensitivity (MIC = 1.56% and MBC = 3.12%) and *L. rhamnosus* showed lower sensitivity (MIC = 3.12% and MBC equal to 6.25%) to the Sumac fruit essential oils.

3.2. Compounds of Sumac Essential Oil

According to the obtained results, the percentage of essential oil in Sumac fruit was 0.7% weight/volume (W/V). In total, 54 compounds were identified in the Sumac fruit essential oil, in which beta-caryophyllene (20.2%) was the predominant compound. Also, the frequency of mono-terpenoid, sesquiterpenoid, diterpenoid, and aliphatic compounds was 10.2%, 34.6%, 12.1%, and 20.7%, respectively. The obtained results from GC-MS are shown in Table **2**.

Table 1. The inhibition zone diameter of Sumac fruit essential oil on dental caries pathogens.

Bacterial Strain	Inhibition Zone Diameter (mm)				
	Essential Oil	Penicillin	Enrofloxacin	Amoxicillin	Florfenicol
Streptococcus mutans	19.0 ± 1.6	21.6 ± 2.4	23.3 ± 4.3	18.3 ± 3.6	27.3 ± 2.5
Lactobacillus rhamnosus	13.6 ± 1.9	16.0 ± 3.1	15.6 ± 1.5	15.6 ± 3.1	17.6 ± 3.1
Actinomyces viscosus	16.3 ± 3.3	18.3 ± 1.5	17.0 ± 1.6	17.3 ± 2.6	22.6 ± 1.9

Table 2. The obtained compounds of Sumac fruit essential oil using GC/MS.

No.	Compounds	Frequency	No.	Compounds	Frequency
1	Hexanal	0.1%	28	2-trans-4-sis-decadienal	1.7%
2	Alfa-pineno	1.3%	29	Ondcanal	1.7%
3	Camphene	0.1%	30	Hepthenyl-acrolein	5.5%
4	2-heptanone	0.1%	31	α-terpinyl acetate	0.4%
5	Beta-pineno	0.1%	32	2-andesnal	1.0%
6	1-octene-3-ol	0.1%	33	Beta-caryophyllene	20.2%
7	Beta-myrcene	0.1%	34	Isoamyl benzoate	0.5%
8	Octanal	0.1%	35	Alfa-humulene	2.1%
9	p-cymene	0.1%	36	Trans-geranylacetone	1.0%
10	Limonene	0.4%	37	Germacrene	0.3%
11	sis-ocimene	0.1%	38	Beta-selinene	0.3%
12	trans-ocimene	0.1%	39	Alpha-murolene	0.3%
13	Octanol	0.1%	40	Alfa-selinene	0.3%
14	Terpinolene	0.1%	41	trans, trans-farnesol	1.0%
15	Linalool	1.8%	42	Ladene	0.8%
16	Fenchol	0.2%	43	trans-nerolidol	1.4%
17	Camphor	0.1%	44	Caryophyllene oxide	3.1%
18	trans-2-nonenal	0.3%	45	Globulol	0.7%
19	Borneol	0.3%	46	10-epi-gamma-eudesmol	2.3%
20	Isopinocampheol	0.1%	47	Alfa-eudesmol	0.8%
21	Alfa-terpineol	5.1%	48	Hexahydroxyfarnesylacetone	1.0%
22	Decanal	0.4%	49	Cembrene	11.1%
23	Linalyl acetate	0.1%	50	Icosane	0.4%
24	trans-2-decenal	2.2%	51	Heneicosane	0.5%
25	trans-3-caren-2-ol	0.1%	52	Docosane	2.8%

(Table 2) cont.....

No.	Compounds	Frequency	No.	Compounds	Frequency
26	Vitispirane	0.2%	53	Tricosane	2.2%
27	Bornyl acetate	0.5%	54	Heptacosane	0.1%
A	**Monoterpenoid**	**10.2%**	**C**	**Diterpenoid**	**12.1%**
B	**Sesquiterpenoid**	**34.6%**	**D**	**Aliphatic compound**	**20.7%**

4. DISCUSSION

Recently, many studies have evaluated the antimicrobial effects of essential oil and extracts of various medicinal plants and reported an appropriate anti-fungal, anti-parasitic, antibacterial, and anti-viral properties [13, 15]. Therefore, the herbal essential oil has been used in pharmacology, medical microbiology, phytopathology, and food, fruit, and vegetable storage [16]. The excessive use of antibacterial agents causes antibiotic resistance and increases the prevalence of resistance genes among pathogenic microorganisms [17]. The unavailability and high cost of new generation antibiotics and its limited effects lead to increased mortality caused by pathogenic microorganisms. Therefore, identification and preparation of novel compounds with antimicrobial activity from natural sources are important in the control of infectious diseases [18]. Therefore, in the present study, the antibacterial activity of Sumac fruit against the most important pathogens of dental caries, such as *S. mutans*, *L. rhamnosus,* and *A. viscosus* was evaluated for the first time in the world, by agar disk diffusion and broth micro-dilution methods.

The obtained results from the agar disk diffusion method showed that Sumac fruit essential oil inhibited the growth and proliferation of *S. mutans* more efficiently than the *L. rhamnosus* or *A. viscosus*. Also, Sumac fruit essential oil caused a higher inhibition zone in *S. mutance* as compared to amoxicillin. Many studies have investigated the antibacterial activity of essential oil and extracts of various medicinal plants against gram-negative and -positive bacteria and fungi. A study by Talei *et al*. (2004) reported that the largest inhibition zone caused by the extraction of Sumac fruit was related to *S. aureus* (12 mm) [19]. Another study by Gabr *et al*. (2014) reported that the largest inhibition zone caused by methanolic extraction of Sumac fruits was related to *P. aeruginosa* (46 mm) and *S. aureus* (45 mm) [11]. According to the mentioned studies, the essential oil and extracts of Sumac fruit have an antibacterial activity against gram-positive and gram-negative pathogenic bacterial strains.

The obtained results from broth micro-dilution showed that essential oil of Sumac fruit has a high inhibitory effect against *S. mutans* and *A. viscosus*. Many studies have investigated the inhibitory effects and bactericidal concentrations of essential oil and extracts of various medicinal plants against gram-negative and -positive pathogenic bacterial strains. Moreover, many studies showed a high resistance in gram-negative bacterial strains to Sumac fruit essential oil [12, 20, 21]. Pajohi-Alamoti *et al*. (2016) and Abu-Shanab *et al*. (2005) reported that the essential oil and extraction of Sumac fruit have higher antibacterial activity against gram-positive bacterial strains, as compared to gram-negative [20, 21].

The obtained results from the GC-MS method showed that the major Sumac fruit essential oil compounds were related to beta-caryophyllene (20.2%). Beta-caryophyllene is a plant alkaloid. Previous studies reported appropriate antimicrobial activity of Beta-caryophyllene against different pathogenic bacterial strains [22]. Also, Sumac fruit essential oil has an inhibitory effect against *Salmonella* strains. The beta-caryophyllene is the most frequent compound of Sumac fruit essential oil, which suggests that it can be responsible for the antibacterial activity of Sumac fruit essential oil [23]. Also, several biological activities are attributed to beta-caryophyllene, such as anti-inflammatory, antioxidant, anticarcinogenic, and local anaesthetic activities. Beta-caryophyllene has been found in the essential oil of many medicinal plants [24]. The essential oil of medicinal plants such as *Heracleum persicum*, *Piper nigrum,* and *Rosmarinus officinalis* show inhibitory effects against gram-positive and -negative bacterial strains and contain a large amount of beta-caryophyllene. Therefore, beta-caryophyllene may have a role in the inhibitory activity of Sumac fruit essential oil [16, 25]. However, 50% over the essential oil compounds in Sumac fruit are terpenoids other than beta-caryophyllene, and these compounds may play important role in its antimicrobial function, which may play a role in its antimicrobial function. Therefore, investigation of antimicrobial activity of identified terpenoid compounds seems necessary, separately.

CONCLUSION

In general, our results showed that Sumac fruit essential oil has an inhibitory effect against dental caries bacterial strains (*Streptococcus mutans*, *Lactobacillus rhamnosus,* and *Actinomyces viscosus*). Therefore, it may be used in the pharmaceutical industry to produce antibacterial, disinfectants, and mouthwashes to control oral infectious diseases and dental caries.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article are available within the article.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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