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Scientific Research

Comparison of the Antimicrobial Effect of Hydroalcoholic Extracts of Fruit and Root of *Prosopis farcta* on Cariogenic Streptococci and Bacteria Causing Infection and Food Poisoning

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ABSTRACT

Nowadays, due to the indiscriminate use of drugs and the consequent increase in antibiotic resistance, the tendency towards the use of medicinal plants is increasing because of their fewer side effects. Most plants produce compounds called secondary metabolites that possess numerous properties, including antimicrobial effects. On the other hand, dental caries is a common oral disease, and various factors, including nutrition, influence it. In this study, the antimicrobial effects of hydroalcoholic extracts of fruit (HEPFF) and root of *Prosopis farcta* (HEPFR) on cariogenic *Streptococci*, including *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus salivarius*, and *Streptococcus sobrinus*, as well as food poisoning bacteria (*Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus*) were investigated under laboratory conditions. The extracts were prepared using the ultrasound-assisted extraction method, and then their total phenolic content and total flavonoid content were determined separately. The antimicrobial properties of these extracts were evaluated by the well diffusion method and by determining (MIC) and (MBC) values. The results showed that (HEPFF) had higher total phenolic content and total flavonoid content compared to (HEPFR) ($P < 0.05$). Furthermore, (HEPFF) exhibited stronger antimicrobial activity against all examined strains compared to (HEPFR). Therefore, it can be concluded that (HEPFF) has desirable antimicrobial properties and can be utilized in the food and pharmaceutical industries.

1- Introduction

Dental caries is a multifactorial disease, infectious and microbial in nature, which causes the dissolution and destruction of dental calculus. The complications of this disease include tooth loss, pain and cosmetic defects. The main etiological agents known for dental caries are *Streptococcus mutans* and Lactobacilli [1]. In addition, the widespread use of industrially derived drugs and their improper use for the treatment of dental caries cause many side effects, sometimes resulting in toxic effects that are more serious than the diseases themselves [2]. Oral streptococci are an important component of dental plaque, and one of the most important members of this group is *Streptococcus mutans*, which has been associated with caries in several epidemiological studies and is thought to play a major role in the initiation of caries [3]. *Streptococcus mutans* is a gram-positive bacterium in the mouth that creates an acidic environment by metabolizing various carbohydrates. Extracellular glucan is the main pathogenic factor of this bacterium and is considered a cause of dental caries in humans and animals [4]. In recent years, extensive research has been conducted to evaluate the antimicrobial effect of various essential oils and extracts, which indicates the power and ability of these compounds to inhibit the growth of a wide range of pathogenic microorganisms [5].

The *Prosopis farcta* belongs to the family Leguminaceae and subfamily Mimosoideae. *Prosopis farcta* is one of the native plants of Iran that is used in traditional medicine to treat some heart diseases and high blood pressure. The reason for this is due to the presence of bioactive compounds in this fruit and root of this plant [6]. plant Secondary-metabolites disrupt the activity of microorganisms by reacting with the cell walls of microorganisms and disrupting the normal activity of their membrane system. Also, phenolic compounds disrupt the activity of the enzyme systems of microorganisms, preventing their natural activity and in this way, they can inactivate microorganisms and greatly reduce their activity [7].

So far, the antimicrobial effect of *Prosopis farcta* seed extract has been investigated on the microorganisms *Enterococcus faecalis* [8]; Also, the antimicrobial effect of ethanolic extract of the *Prosopis farcta* root has been determined on bacteria that cause food infection and poisoning (*Escherichia coli*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Bacillus subtilis*) [9]; the antifungal activity of methanolic extract of the *Prosopis farcta* root has also been studied against Trichophyton Mentagrophytes strains [10], but no study has been conducted on the comparative antimicrobial effect of HEPFF[1] and HEPFR[2] on dental caries-causing streptococci and bacteria that cause food infection food poisoning. Therefore, this study was conducted with the aim of comparing in-vitro antimicrobial effect of HEPFF and HEPFR on dental caries-causing streptococci and bacteria that cause food infection and poisoning.

2- Materials and Methods

The *Prosopis farcta* was collected from the Jahrom region of Iran. Then, it was identified and confirmed by the botanist of the Herbarium Center of the Faculty of Pharmacy, Islamic Azad University, Tehran Medical Sciences Branch with the herbarium code 458-PMPIA. Bacteria including *Streptococcus mutans* (ATCC 35668), *Streptococcus sanguinis* (ATCC 10556), *Streptococcus salivarius* (ATCC 9222), *Streptococcus sobrinus* (ATCC 27607), *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 14028), and S*taphylococcus aureus*

1 -Hydroalcoholic extract of *Prosopis farcta* fruitt

2- Hydroalcoholic extract of *Prosopis farcta* root

(ATCC 6538) were purchased from the Scientific and Industrial Research Organization of Iran in lyophilized form.

The laboratory materials used in this study included Folin-Ciocalteu reagent, aqueous sodium carbonate solution, gallic acid, sodium acetate solution, 2% w/v aluminum chloride solution, quercetin, and broth and plate count agar culture media were obtained from Merck, Germany.

2.1. Preparation of HEPFF and HEPFR

The root and fruit parts of the *Prosopis farcta* were separated, washed, and dried. To prepare HEPFF and HEPFR, the *Prosopis farcta* plant fruit and root were completely crushed and powdered with a laboratory grinder. After sieving with a 30 mesh according to the method of Frohlich et al. 2022, it was subjected to an ultrasound-facilitated extraction process (SONICS, Model VCX750, Sonics & Material Inc., Connecticut, USA) equipped with a titanium probe with a diameter of 13 mm. Each gram of powder was mixed with 35 ml of 70% ethanol; then, it was exposed to ultrasound waves with an amplitude of 85% (fixed frequency of 20 kHz and 750 W) at a temperature of 70 degrees Celsius for 25 minutes. The titanium probe tip was immersed to about 1 cm of the mixture and a thermostatic bath (Quimis, Q214M2) connected to the extraction cell was used to control the constant extraction temperature. After the extraction operation, the samples were filtered using a vacuum pump and the portion containing the solvent and extract mixture was transferred to a rotary evaporator under vacuum (IKA RV-10, IKA ® -Werke GmbH & Co. KG, Germany) and the solvent was evaporated. Subsequently, HEPFF and HEPFR were concentrated and stored at refrigerator temperature until further tests.

2.2. Evaluation of total phenolic content

The total phenol content of HEPFF and HEPFR was calculated using the Folin-Ciocalteu method. For this purpose, 20 µl of each sample was mixed with 1.16 ml of distilled water and then 100 µl of Folin-Ciocalteu reagent was added to it. After 5 minutes, 300 µl of 20% sodium carbonate solution was added. The resulting mixture was then allowed to react for 30 minutes and then the absorbance of each sample was measured by spectrophotometer (UNICO-2100, USA) at 760 nm. Finally, the total phenol content was expressed as milligrams of gallic acid equivalents per gram of sample (mg GAE/g) using a standard gallic acid chart [11].

2.3. Evaluation of total flavonoid content

The aluminum chloride colorimetric method was used to estimate the total flavonoid content of the samples. 80 µL of the extract was mixed with 80 µL of 2% (w/v) aluminum chloride solution and 120 µL of 50 g/L sodium acetate solution in a 96-well plate and incubated at room temperature for 2.5 hours. The absorbance was determined at a wavelength of 415 nm. Using a standard curve drawn with quercetin (0-50 µg/ml), the results were expressed as mass (mg) of quercetin equivalent (QE) per sample weight (mg QE/g) [12].

2.4. Evaluation of MIC and MBC

The antimicrobial activity of HEPFF and HEPFR was determined by the well diffusion method. Fresh streptococcal cultures with a turbidity of 0.5 McFarland were diluted 1:100 to obtain a turbidity of 106 [13]. Different concentrations of the extract sample were added to the prepared culture media to prepare different dilutions, and then 100 µl of each dilution, containing 100 µl of bacterial suspension, was poured into 96-well plates. Wells containing 200 µL of broth medium were considered as negative controls, wells containing culture medium and bacteria were

considered as positive controls, and wells containing 100 µL of medium and 100 µL of each dilution were considered as turbidity controls. Then, the samples were incubated in an anaerobic jar at 37°C for 24 hours. Finally, the turbidity was recorded at a wavelength of 630 nm by a microplate reader. MIC was considered as the lowest concentration of the substance that caused a 90% reduction in turbidity compared to the control group. Also, to determine MBC, before reading the number on the microplate reader, a loop was removed from the wells in which no growth was observed and cultured in agar medium, and the last dilution that was unable to grow in this medium was considered as MBC.

2.5. Statistical analysis

The experiments were conducted in a completely randomized design with three replications. The results of the different experiments were analyzed using SPSS.22 software to examine the significant differences between the data through one-way analysis of variance (One-way ANOVA) and Duncan's multiple range test was used to compare the means of the treatments at a probability level of 95% ($p<0.05$). The resulting graphs were also drawn using Excel software.

3-Results

3.1. Total phenol and flavonoid contents

The total phenol and flavonoid contents depended on the type of extracted extract (Table 1). Accordingly, HEPFF had a higher total phenol content (72.48%) and total flavonoid content (4.87%) compared to HEPFR ($p<0.05$).

3.2. MIC and MBC

Based on the results of Table 2, HEPFF showed strong antimicrobial activity against all the strains studied compared to HEPFR. The lowest MIC and MBC values in Gram-positive bacteria were for *Streptococcus mutans* and *Streptococcus sobrinus* in HEPFF (MIC, MBC = 15 mg/ml), indicating a stronger antimicrobial effect of this extract. In contrast, *Streptococcus sanguinis* and *Streptococcus salivarius* showed the highest resistance, especially against HEPFR; MBC = 240 mg/ml; MIC = 120 mg/ml). In Gram-negative bacteria, the lowest MIC and MBC values were for *Staphylococcus aureus* (MIC = 30 ; MBC = 60) and the highest for *Escherichia coli* (MIC = 120 ; MBC = 240).

4- Discussion

The *Prosopis farcta fruit* is more exposed to light because it is located in the aerial parts of the plant, which probably causes the formation of bioactive compounds in the fruit. Plants perform many of their vital activities through the reception of sunlight and lead to the production of secondary metabolites. Therefore, the possible better photosynthesis in the aerial part of the plant increases the antioxidant activity and also the total phenolic content and flavonoid compounds in *Prosopis farcta* fruit compared to its root [14]. It can be stated that HEPFF contains phenolic and flavonoid compounds in significant amounts. It has been reported that the most abundant bioactive compounds present in HEPFF are vitexin, isovitexin and luteolin [6] and [15]. The *Prosopis farcta* fruit has a high phenolic content and has antimicrobial and antifungal effects and antioxidant activity. [16]

In the present study, HEPFF showed the highest antimicrobial effect on *Streptococcus mutans* and *Streptococcus sobrinus*. Phenolic compounds (simple phenols, phenolic acids, coumarins, flavonoids, tannins, stilbenes, lignans, curcuminoids) disrupt the enzymatic

activities and normal activity of membrane lipids, as well as interact with membrane proteins, causing disruption of cellular transport and integrity in microorganisms, which inactivates or suppresses their activity [17]. Gram-negative microorganisms have thick peptidoglycan walls, which are more resistant to antimicrobial agents than the cell walls of Gram-positive microorganisms. Therefore, this resistance and thickness will increase the stability of Gram-negative bacteria to antimicrobial agents [18]. In fact, the greater susceptibility of Gram-positive bacterial species can be largely attributed to the presence of a thin, single layer of mucopeptide in their cell membrane, while the outer cell membrane of Gram-negative species is covered and protected by a complex lipopolysaccharide layer that can act as a barrier to the diffusion of hydrophobic antimicrobial agents throughout the cell [19].

Phenolic compounds are able to inactivate or suppress the activity of microbial cell structures, especially the cell walls of microorganisms, by reacting with them [18]. By reacting with the cell wall and interacting with enzymes, proteins, and membrane lipids, the normal activity of the cell wall is disrupted; by disrupting the cell wall and membrane, the integrity and balance of the microbial intracellular and extracellular space are disrupted, all of which lead to a decrease in the activity of microorganisms and even their death [20]. Antimicrobial effects of polyphenolic compounds present in the *Prosopis farcta* root, such as tannins, luteolin, and caffeic acid, have been reported [21].

Prevete et al. (2021) [9] also identified phenolic compounds in Olea europaea leaf extract as the cause of its antimicrobial activity and reported that the extract had greater antimicrobial activity against Gram-positive microorganisms than Gram-negative microorganisms. Alizadeh et al. (2024) also confirmed the antimicrobial effect of ethanolic extract of the *Prosopis farcta* root on bacteria causing food infection and poisoning (*Escherichia coli*, *Shigella dysentery*, *Staphylococcus aureus* and *Bacillus subtilis*) and the total phenol and total flavonoid contents were reported as (61.55 ± 0.07 mg GAE/g) and (17.00 ± 0.08 mg QE/g), respectively [19]. Also, Pajohi et al. (2016) attributed the antimicrobial effect of aqueous extract of sumac fruit on pathogenic bacteria (*Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Escherichia coli*) at two temperatures of 4 and 25 to the presence of significant amounts of antioxidants, such as tannins and procyanidins [22]. Salari et al. (2019) attributed the antimicrobial effect of silver nanoparticles produced from the extract of the *Prosopis farcta* fruit to the content of total phenols and total flavonoids [10]. Salimi-Sabour et al. (2022) confirmed the antifungal activity of the methanolic extract of the *Prosopis farcta* root against Trichophyton Mentagrophytes strains [23]. Haji Ghasemi et al. (2023) attributed the antimicrobial effects of aqueous, ethanolic, and methanolic extracts of the backstage doll plant against four strains of *Escherichia coli*, *Salmonella typhimurium*, *Bacillus subtilis*, and *Staphylococcus aureus* to the bioactive compounds, the extraction method, and the properties of the solvent used [24]. Alizadeh Behbahani et al. (2025) determined the total phenol content (33.5 mg gallic acid per gram of essential oil) and flavonoid (14.60 mg quercetin per gram of essential oil) contents of *Roman chamomile* essential oil, and reported the highest antimicrobial effect of this essential oil on the gram-positive bacterium *Staphylococcus aureus* and its lowest effect on the bacterium Shigella dysentery, and attributed the antimicrobial effect of the essential oil to the phenolic compounds of the essential oil [25].

5. Conclusion

Resistance of microorganisms to chemical drugs is a major threat to human health; As a result, the search for natural antibacterial agents seems to be essential. On the other

hand, plants produce unique compounds to protect themselves from microbes, which have high potential for use as nutraceutical components. In this study, the antimicrobial effect of HEPFF and HEPFR on bacteria that cause dental caries, and bacteria that cause food infection and poisoning was compared. The results showed that the total phenol and flavonoid contents of HEPFF is significantly higher than that of HEPFR, which is actually the factor that creates the stronger antimicrobial properties of HEPFF compared to HEPFR. Based on this unique feature of HEPFF, its use in microencapsulated form, as a nutraceutical component, in the food (functional foods) and pharmaceutical industries (toothpaste and mouthwash, chewing gum, etc.) is suggested.

In terms of conflict of interest, “There is nothing to declare”
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Table 1. Results of total phenol and flavonoid content of hydroalcoholic extract of *Prosopis farcta* fruit and root (Mean ± SD)

TEST	SAMPLE TYPE	RESULT	
Total phenol content (mg GAE/g)	Root	1.41 [b])±	(112.23
	Fruit	1/30 [a])±	(193/58
Total flavonoid content (mg GAE/g)	Root	1.42 [b]) ±	(98.41
	Fruit	1.37 [a])±	(103.21

Different lowercase letters indicate significant differences for each test (P < 0.05).

Table 2. Results of MIC and MBC indices of hydroalcoholic extract of *Prosopis farcta* fruit and root against cariogenic *Streptococci* and *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* bacteria

TEST	SAMPLE TYPE	MIC (mg/mL)	MBC (mg/mL)
Streptococcus mutans	Root	30.00 ± 3.50 [b]	60.00 ± 5.20[b]
Streptococcus mutans	Fruit	15.00 ± 1.20 [a]	15.00 ± 1.20[a]
Streptococcus sanguis	Root	± 10.50 [b] 120.00	240.00 ± 21.00 [b]
Streptococcus sanguis	Fruit	60.00 ± 5.50 [a]	120.00 ± 11.00 [a]
Streptococcus salivarius	Root	120.00 ± 12.00 [b]	240.00 ± 25.00 [b]
Streptococcus salivarius	Fruit	60.00 ± 6.10 [a]	120.00 ± 10.80 [a]
Streptococcus sobrinus	Root	30.00 ± 2.90 [b]	60.00 ± 4.80 [b]
Streptococcus sobrinus	Fruit	15.00 ± 1.20 [a]	15.00 ± 1.20 [a]
Escherichia coli	Root	240.00 ± 22.00 [b]	480.00 ± 45.00 [b]
Escherichia coli	Fruit	120.00 ± 11.50 [a]	240.00 ± 22.00 [a]
Salmonella typhimurium	Root	120.00 ± 11.00 [b]	240.00 ± 20.00 [b]
Salmonella typhimurium	Fruit	60.00 ± 5.80 [a]	120.00 ± 9.50 [a]
Staphylococcus aureus	Root	60.00 ± 5.50 [b]	120.00 ± 10.20 [b]
Staphylococcus aureus	Fruit	30.00 ± 2.80 [a]	60.00 ± 5.10 [a]

Different lowercase letters indicate significant differences for each test (P < 0.05).

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چکیده

امروزه به دلیل مصرف خودسرانه داروها و پیرو آن افزایش مقاومت به آنتی بیوتیک ها، تمایل به سمت استفاده از گیاهان داروئی به دلیل عوارض کمتر رو به افزایش است. اکثر گیاهان ترکیباتی تحت عنوان متابولیت های ثانویه تولید می کنند که خواص بی شماری از جمله ضد میکروبی دارند. از طرفی پوسیدگی دندان در بین بیماری های دهان و دندان شایع بوده و عوامل مختلفی از جمله تغذیه بر آن اثرگذار است. در این پژوهش، مقایسه اثر ضد میکروبی عصاره های هیدروالکلی میوه (HEPFF) و ریشه کهورک (HEPFR) بر استرپتوکوک های عامل پوسیدگی دندان شامل: *استرپتوکوکوس موتانس*، *استرپتوکوکوس سانگویس*، *استرپتوکوکوس سالیواریس*، *استرپتوکوکوس سوبرینوس* و همچنین، باکتری های عامل مسمومیت غذایی (*اشریشیا کلای*، *سالمونلا تیفی موریوم* و *استافیلوکوکوس اورئوس*) در شرایط آزمایشگاهی مورد بررسی قرار گرفت. عصاره ها به روش استخراج تسهیل شده با فراصوت تهیه شدند و سپس، محتوای فنل کل و فلاونوئید کل در آن ها به طور جداگانه تعیین گردید. خواص ضد میکروبی این عصاره ها از طریق روش انتشار چاهک و محاسبه میزان MIC و MBC ارزیابی شد. نتایج نشان داد که HEPFF دارای محتوای فنل کل و فلاونوئید کل بالاتری در قیاس با HEPFR بود ($p<۰/۰۵$)؛ همچنین، HEPFF در مقایسه با HEPFR در برابر تمامی سویه های مورد بررسی، فعالیت ضد میکروبی قوی تری از خود نشان داد. بنابراین، می توان نتیجه گرفت که HEPFF خواص ضد میکروبی مطلوبی دارد و می توان از آن در صنایع غذایی و دارویی بهره مند شد.