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Original Article

Investigating the Antibacterial Effect of *Prunus scoparia* (Spach) C.K.Schneid. Extract on Anaerobic Bacteria of Gum Infection

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ABSTRACT

Oral anaerobic bacteria cause a wide range of oral diseases. Research results have proven that there is a strong connection between periodontal bacteria and oral anaerobic bacteria with some serious human cancers, oral cavity infections, rheumatoid arthritis, ankylosing spondylitis and even heart disease. *Prunus scoparia*is one of the wild medicinal plants of Iran, which is widely distributed in Lorestan province. Local people of Lorestan use it in traditional medicine to treat diabetes, inflammatory diseases and microbial infections. In this study, the methanolic extract of *P. scoparia* was tested on several anaerobic bacteria. The results showed that the methanol extract of the *P. scoparia* can inhibit the growth of *Porphyromonas gingivalis*, *Streptococcus mutans* and bacteria at a dilution of 40 mg/ml and the growth of *Lactobacillus acidophilus* bacteria at a dilution of 25 mg/ml. The minimum inhibition concentration of vancomycin for *P. gingivalis* and *Streptococcus mutans* is 0.002 mg/ml and for *L. acidophilus* is 0.008 mg/ml. MIC values of metronidazole for *Streptococcus mutans*, *L. acidophilus* and *P. gingivalis* are 0.008, 0.032 and 0.064 mg/ml, respectively. So, the results demonstrated that the methanolic extract of this plant can be used in the treatment of many diseases related to anaerobic bacteria.

INTRODUCTION

Cavities and periodontal diseases are amongst the most common human infectious disease caused by oral bacteria such as *Porphyromonas gingivalis*, *streptococcus mutans*, *Lactobacillus acidophilus*, *Pseudomonas*, *Staphylococcus aureus*, *Candida* species and etc. By this order, it is concluded that plaque forming disease causing tartar or calculus, might be the most common human infectious diseases. Some types of the bacteria such as *Enterococcus faecalis*, *S. sanguinis*, *Eikenella corrodens*, *Actinomyces viscosus*, *P. gingivalis*, *Streptococcus mutans* and *L. acidophilus* are proven to be effective on causing cavities and periodontal diseases [1]. Periodontal diseases do not follow the same pattern of spreading worldwide [2]. The epidemiological studies suggest that 5 to 20 percent of people around the world suffer from periodontitis [3-5]. It is said that periodontitis has at least affected one tooth in 80 percent of the human population [6]. Based on a WHO study, the rate of periodontitis outbreak is highly increasing in developing countries [7]. In 2002, 20 percent of the Iranian 18 years old, had slight periodontal pocket and up to 1 percent had the advanced stage [8, 9]. Also, 43 percent of the 34-35-year-old, had the slight and up to 10 percent of them, had the advanced stage. Periodontitis can affect the general health as well. Chronic periodontitis can be considered a risk factor for cardiovascular diseases, preterm labors, diabetes, arthritis rheumatoid, gastrointestinal and pancreatic cancers [10]. There are several methods to prevent cavities and periodontal diseases; one that has been used for a long time, is maintaining dental hygiene using chemical compounds such as mouthwash. Mouthwash can contain fluoride, alcohol, detergent and other antibacterial compounds [11]. One other

method is using toothpaste. Toothpastes contain fluoride and other antibacterial compounds such as triclosan and citrate royande or synthetic antibacterial compounds like povidone Iodine, fluorides, phenol compositions and chlorhexidine vestil pyridinium [12]. These chemical compounds can affect one's health, resulting in some side effects such as nausea, diarrhea and tooth discoloration [13]. Some antibiotics such as ampicillin, erythromycin, penicillin, tetracycline and vancomycin are also greatly used in dentistry, in order to inhibit the growth of bacterial pathogens. Nowadays, the rate of antibiotic resistance in oral bacteria is rising [14]. Due to deficiencies in chemical compounds and antibiotics today, using certain herbal medicine and natural antibiotics is proposed, as more practical replaced treatments in toothpastes and mouthwash [15]. *Prunus scoparia* (Spach) C.K.Schneid.Spach.or wild almond tree, belongs to the Angiosperm clade, Rosaceae family and *Amygdalus* genus. It is a xerophytic shrub which grows mostly in Alborz and Zagros mountains of Iran. At first, its young branches are smooth and light brown, which will turn into light or dark gray after a while. Lorestan is a hometown for wild almond shrubs and they have the capacity to fight against the Pathogenic factors and insects, but unfortunately, the ecological and environmental tensions such as climate change, droughts, leaf and wood eating insects and the hemi parasitic plant called Viscum, would do permanent harm to this plant annually. Although the domesticated species of *Amygdalus* genus are well known in terms of chemical composition and biological properties and are widely used in food, pharmaceutical and cosmetic industries, not many studies have been conducted on wild species. It is somehow similar to other species of almond in chemical ingredients. Sweet almond contains 3 to 4 percent water, about 54 percent oil, about 25 percent nitrogen binding substances and sugar, resin, mucilage and nutrients. It also has an enzyme called emulsin. In bitter almonds, the percentage of oil is less, but the emulsin is more. In addition, bitter almonds have a special heteroside called amygdalinoside in the amount of 1 to 3%. The main difference between bitter and sweet almond, is that the bitter almond contains a substance called amygdaline. Amygdaline is also found in other fruits pyrene such as apricot, cherry and plum. Amygdaline can be hydrolyzed to glucose, benzaldehyde and hydrocyanic acid. Amygdaline is a cyanogenic glycoside which has antineoplastic effects [16, 17]. Studies on anaerobic microorganisms are one of the most important and challenging investigation areas because anaerobic situation (with no oxygen) makes the conditions for conducting studies difficult. Up until today, there has been so little attention to the antibacterial effects of this plant. Because of the importance and outbreak of periodontal diseases in public health, the importance of on time and safe treatments, the connection between periodontal diseases and indications of other diseases and also the bacterial etiology of the disease as the main reasons of failed treatments, we are in need of a safe and protected treatment [18]. Based on the importance of anaerobic bacteria in forming periodontal diseases, antibacterial effects of *P. scoparia* and so far, no recent study on the effects of *P. scoparia* on periodontal pathogens, this study tries to discuss the effects of *P. scoparia* extract on the bacteria causing gingivitis, such as *Streptococcus mutans*, *P. gingivalis* and *L. acidophilus*. This study may help researchers as a new method in further treatment of this disease.

MATERIALS AND METHODS

Plant Material

Specimenes used in this study were collected from wild growing populations of *P. scoparia* from natural sites of Sefidkouh, Khorramabad area, Iran. Voucher specimens (LUMH-1109) were deposited in the Lorestan University of Medical Science Herbarium, Lorestan, Iran. After identification the samples were dried in the shadow at room temperature.

Extraction

The aerial parts of plants were air-dried at room temperature in the shadow. 10 grams of dried and pulverized plant material were macerated with 200 ml of methanol to obtain methanol extract that condensed using a rotary evaporator apparatus at 45 °C.

Determining the Minimum Inhibitory Concentration

The antimicrobial effects of *P. scoparia* extract and vancomycin, metronidazole, benzydamine mouthwash and carvone (the active ingredient of

peppermint oil) were tested on three oral anaerobic bacteria. The strains used in this research, including *P. gingivalis*, *Streptococcus mutans*, and *L. acidophilus*, were prepared from the Faculty of Health, Tehran University of Medical Sciences. The minimum inhibitory concentration for microorganisms sensitive to methanol extract, antibiotics and carvone was obtained using the dilution method. First, serial dilutions of 1, 2, 4, 8, 16, 32, 64, 80, 256 and 400 mg/ml of *P. scoparia* extract were prepared. Then, 100 microliters of enriched Brucella broth agar culture medium were poured into the wells of the 96 well plate, 5 microliters of bacterial suspension with 0.5 McFarland dilution and 100 microliters of extract and vancomycin, metronidazole, carvone and benzydamine mouthwash were added. DMSO solvent was used as a negative control. Then the microplates were incubated for 48 hours at 35 ºC in an anaerobic jar.

Determining the Minimum Bacterial Concentration

To determine the minimum concentration of bacterial lethality, 5 µl of the contents of the wells in which the bacteria did not grow were inoculated into the nutrient agar medium and incubated for 48 hours at 35 ºC in an anaerobic jar.

RESULTS AND DISCUSSION

The results of the evaluation of the antimicrobial properties of the *P. scoparia* plant showed that the methanol extract of the *P. scoparia* can inhibit the growth of *P. gingivalis* and *Streptococcus mutans* bacteria at a dilution of 40 mg/ml and the growth of *Lactobacillus acidophilus* bacteria at a dilution of 25 mg/ml (Table 1). *P. scoparia* methanol extract at a dilution of 80 mg/ml caused the death of *S. mutans* and at dilutions of 64 and 256 mg/ml it killed *L. acidophilus* and *P. gingivalis*, respectively. The minimum inhibitory concentration of vancomycin for *P. gingivalis* and *S. mutans* is 0.002 mg/ml and for Lactobacillus acidophilus is 0.008 mg/ml. MIC values of metronidazole for *Streptococcus mutans*, *Lactobacillus acidophilus* and *P. gingivalis* are 0.008, 0.032 and 0.064 mg/ml, respectively. The MIC values of benzydamine mouthwash for *S. mutans*, *L. acidophilus* and *P. gingivalis* are 1, 10 and 20 mg/ml, respectively. The MIC values of carvone for *L. acidophilus*, *S. mutans* and *P. gingivalis* are 12, 20 and 40 mg/ml, respectively (Table 1). Also, vancomycin MBC values for *P. gingivalis* and *S. mutans* bacteria are equal to 0.008 mg/ml and for *L. acidophilus* bacteria are equal to 0.016 mg/ml. MBC values of metronidazole for *Streptococcus mutans* bacteria are equal to 0.008 mg/ml and for *L. acidophilus* and *P. gingivalis* bacteria are equal to 0.032 mg/ml. The MBC values of benzydamine mouthwash for *Streptococcus mutans*, *L. acidophilus* and *P. gingivalis* are 1, 10 and 20 mg/ml, respectively. The MBC values of carvone for *Streptococcus mutans*, *L. acidophilus* and *P. gingivalis* were 10, 12 and 20 mg/ml, respectively. The results related to the minimum inhibitory concentration and the minimum Bactericidal concentration of *P. scoparia* methanol extract, vancomycin, metronidazole, benzoamidine mouthwash and the active ingredient carvone are shown in Figure 1 and 2, respectively.

Table 1 Antimicrobial activities of *Prunus scoparia* (Spach) C.K.Schneid. (mg/ml)

		P. gingivalis	*L. acidophilus*	*S. mutans*
A. scoparia	MIC	40	25	40
	MBC	256	64	80
Carvone	MIC	40	12	20
	MBC	20	20	12
Benzydamine	MIC	20	10	1
	MBC	20	10	1
Vancomycin	MIC	0.002	0.008	0.002
	MBC	0.008	0.016	0.008
Metronidazole	MIC	0.06	0.08	0.008
	MBC	0.032	0.032	0.008

The results showed that the methanol extract prepared from the native *P. scoparia* of Lorestan province had an inhibitory effect on all the studied bacteria. But in all cases, at higher concentrations than antibiotics and carvone, it inhibited the growth and death of bacteria. In the present study, vancomycin, metronidazole, benzydamine mouthwash, and carvone were used as control groups in order to compare the effectiveness of *P. scoparia* on preventing the growth of periodontal pathogens. The antimicrobial effect of essential oils and extracts of different medicinal plants on different microorganisms has been reported in different regions [19]. So far, there has been no study on the antimicrobial effect of the extract of the *P. scoparia* on different bacteria, so this is the first time that these results are reported and it is not possible to compare the results with previous studies. However, the therapeutic effects of different medicinal plants on periodontitis have been evaluated in many studies. After investigating the effects of eucalyptus extract, Nagata *et al.* reported that this plant has antibacterial activity against several pathogens effective in periodontitis, such as *P. gingivalis* and *P. intermedia*. Also, another study showed that chewing gum containing eucalyptus reduces gum bleeding, pocket depth and platelet aggregation [20].

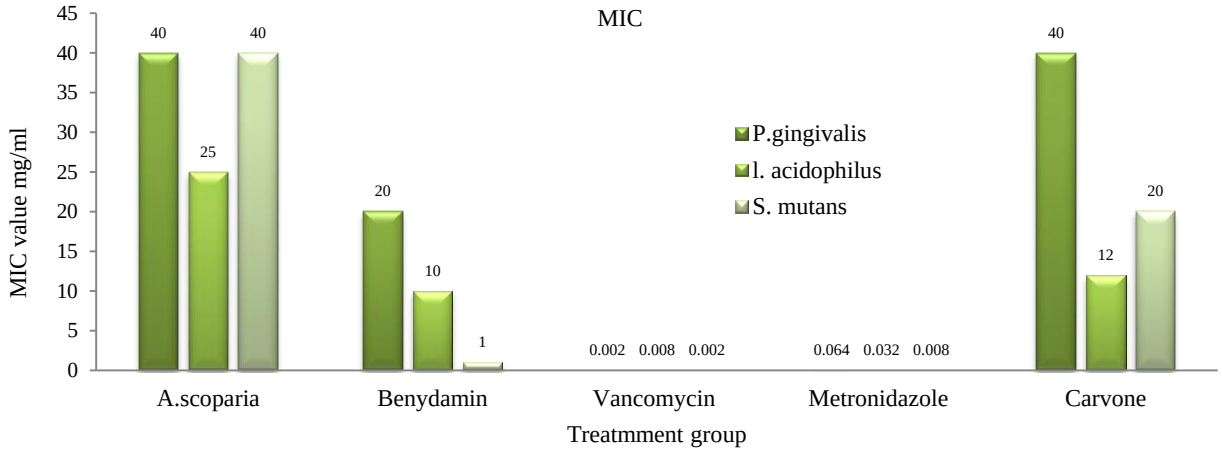

Fig. 1 The minimum inhibitory concentration of the methanol extract of *Prunus scoparia* (Spach) C.K.Schneid., vancomycin, metronidazole, benzoamidine mouthwash and carvone

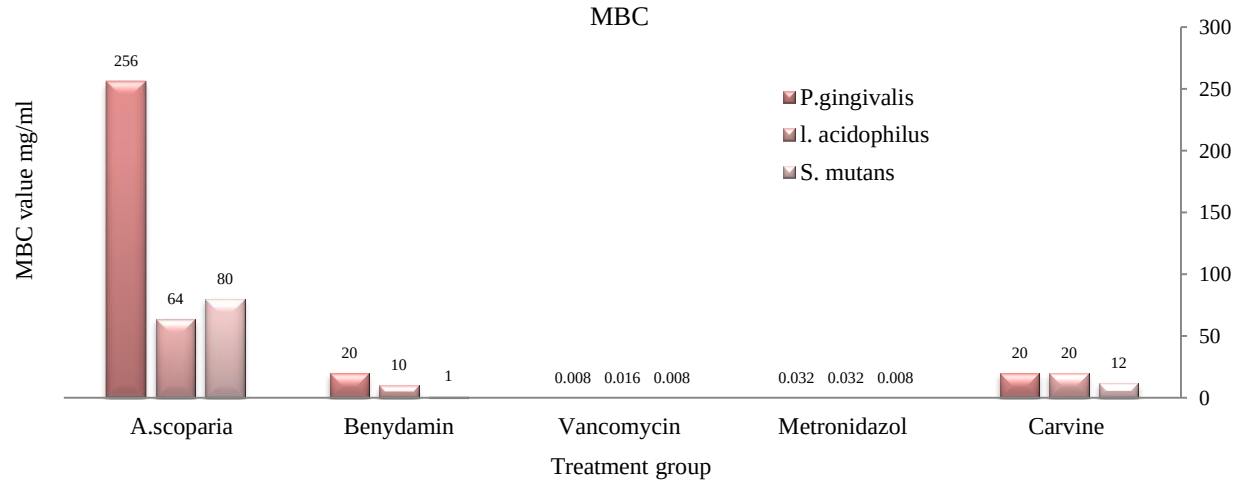

Fig. 2 The minimum bactericidal concentration of the methanol extract of *Prunus scoparia* (Spach) C.K.Schneid., vancomycin, metronidazole, benzoamidine mouthwash and carvone.

Chatterjee and Pai in two separate studies, investigating the therapeutic effects of neem plant, reported that the use of this plant in periodontitis patients reduces BOP and plaque, and in a 21-day follow-up, this plant had similar effects to chlorhexidine [21, 22]. In another study, Pai *et al.* mentions this plant as a factor in preventing the

progress of periodontitis due to the reduction of bacteria and plaque [23].

Other studies have investigated the antibacterial effect of medicinal plants. Chamomile flower has antibacterial effects against *P. gingivalis* and can be used as a mouthwash in the treatment of patients with periodontitis [24]. Garlic has antimicrobial activity against periodontal pathogens including *A. comitans* bacteria [25] and its local use prevents the growth of *A. comitans* and *Fusobacterium* [26]. Cranberry extract inhibits adherence and biofilm formation by *P. gingivalis* [27, 28]. Green tea is effective in preventing the growth of anaerobic bacteria that cause periodontitis, including *P. gingivalis* and *Provetella* [29]. In the present study, the antibacterial effect of *P. scoparia* methanol extract on *P. gingivalis*, *Lactobacillus acidophilus* and *Streptococcus mutans* bacteria and its comparison with common antibiotics used in the treatment of periodontal lesions, i.e. metronidazole and vancomycin, as well as benzydamine mouthwash and carvone, were conducted. The results showed that *P. scoparia* methanol extract can prevent the growth of *P. gingivalis* and *Streptococcus mutans* bacteria at least in a dilution of 40 mg/ml and *Lactobacillus acidophilus* in a dilution of 25 mg/ml. 80 mg/ml caused the death of *Streptococcus mutans* bacteria and 64 and 256 mg/ml dilutions caused the death of *Lactobacillus acidophilus* and *P. gingivalis* bacteria, respectively. The results of a study by Asiri and colleagues in 2016 support our findings. They concluded that herbal mouthwashes have shown significant improvement in protecting oral health. Therefore, they can replace chemicals such as chlorhexidine mouthwash, which has more side effects [30]. But the findings of the study by Haffajee in 2008 and Bhat in 2013 are contradictory. By comparing chlorhexidine mouthwash and herbal mouthwashes in measuring the growth of periodontal pathogens and reducing plaque growth, they concluded that chlorhexidine has better effects [31, 32]. Gupta and colleagues also found herbal toothpastes to have similar effects in controlling plaque and gingivitis compared to conventional fluoride toothpastes [33-38].

CONCLUSIONS

According to the obtained results, the antibacterial effect of the extract prepared from the *P. scoparia* on *Streptococcus mutans* is significant, and *P. gingivalis* is the most resistant bacterium to this extract compared to the other two bacteria studied. Compared to conventional antibiotics for the treatment of periodontal infections, such as metronidazole and vancomycin and benzydamine mouthwash, large concentrations of this extract are needed, which is necessary for use, its benefits compared to side effects and the limitations of using these antibiotics, especially in special situations such as pregnancy and breastfeeding. Therefore, the local and systemic use of this herbal medicine, either in the form of various medicinal forms or in the form of mouthwash and toothpaste, can play an effective role in improving periodontal diseases. The active substance of carvone also needs a significant concentration to start the effect, which indicates that compounds such as active substances are not superior for antibiotic effects compared to extracts, which are the impurity combination of all plant components extracted by different solvents**.**

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