Ann Med Res 2021;28(3):516-9

Annals of Medical Research

DOI: 10.5455/annalsmedres.2020.05.487

Original Article

Antimicrobial activity of sapindus mukorossi and saponaria officinalis extracts on streptococcus mutans and enterococcus faecalis

Meltem Mert Eren[1], Benin Dikmen[2], Cansu Vatansever[3], Huseyin Servi[4], Hulki Caner Yegin[5], Gunce Ozan[6]

[1]Department of Restorative Dentistry, Faculty of Dentistry, Altinbas University, Istanbul, Turkey
[2]Department of Restorative Dentistry, Faculty of Dentistry, Medipol University, Istanbul, Turkey
[3]Department of Pharmaceutical Microbiology, Faculty of Pharmacology, Altinbas University, Istanbul, Turkey
[4]Department of Pharmaceutical Botanic, Faculty of Pharmacology, Altinbas University, Istanbul, Turkey
[5]Department of Department of Endodontics, Faculty of Dentistry, Altinbas University, Istanbul, Turkey
[6]Department of Restorative Dentistry, Faculty of Dentistry, Istanbul University, Istanbul, Turkey

Abstract

Aim: Studies on the antimicrobial effectiveness of saponins against oral pathogens are conflicting. Therefore, this study aimed to evaluate the antimicrobial activity of saponins from three different extracts of *Sapindus mukorossi* and *Saponaria officinalis* plants against *Streptococcus mutans* and *Enterococcus faecalis*.

Materials and Methods: The fruit of *Sapindus mukorossi* and the root of *Saponaria officinalis* materials were sliced into small pieces and macerated with hexane, ethyl acetate (EtOAc) and methanol (MeOH), in this order. The antimicrobial activity of the extracts against *Streptococcus mutans* and *Enterococcus faecalis* was determined by the broth microdilution method based on the minimum inhibitory concentrations (MIC) of each extract.

Results: Except for the hexane extract of *Saponaria Officinalis*, all extracts of both plants had an inhibitory activity against the tested microorganisms. Higher MIC values were obtained against *Enterococcus faecalis* than *Streptococcus Mutans*. The lowest MIC values for both *Sapindus mukorossi* and *Saponaria officinalis* were against *Streptococcus* mutans (4 mg/ml).

Conclusion: Saponins extracted from *Sapindus mukorossi* and *Saponaria officinalis* have a potential antibacterial activity against oral pathogens. They may be used as an ingredient in dental agents.

Keywords: Antimicrobial activity; *Enterococcus faecalis;* minimum inhibitory concentration; saponin; *Streptococcus mutans*

INTRODUCTION

Dental caries is described as one of the most common infectious diseases in humans. The biological nature of a carious lesion is a microbial infection caused primarily by *Streptococcus mutans (S. mutans)* (1,2). Additionally, *Enterococcus faecalis (E. faecalis)* has been isolated frequently from deep dentin caries (3). If dental caries is not treated, it may result in root canal infection and inflammation of the periapical tissues, and the strongest bacteria found in infected root canal is *E. faecalis* (4). Moreover, *E. faecalis* is one of the most commonly isolated bacteria in failed endodontic cases (3).

Herbal medicine has better compatibility and safety, for that reason, it is preferred, especially in developing countries (5). Since plants are known to cause inhibition of microbial growth, many studies have been focused on the antimicrobial activity of medicinal plants (6). Saponin is one of the most effective plant compounds in terms of antibacterial activities. Saponins are glycoside compounds and have many biological properties like haemolytic and antimicrobial effects. It is known that the antibacterial activities of saponins differ based on the type of the bacterium, and saponins from different sources differ in their biological activity because of their different chemical structure (7). *Sapindus mukorossi (S. mukorossi) and Saponaria officinalis (S. officinalis)* are some examples of plants that are saponin-rich sources (6,8).

S. mukorossi is a deciduous tree of tropical and subtropical regions of Asia (9). The major components of its pericarp are saponins. Sapindus saponins have great foaming ability, as well as antimicrobial, anti-inflammatory, antidermatophytic and molluscicidal

Received: 14.05.2020 **Accepted:** 02.07.2020 **Available online:** 19.03.2021
Corresponding Author: Meltem Mert Eren, Department of Restorative Dentistry, Faculty of Dentistry, Altinbas University, Istanbul, Turkey **E-mail:** meltemmert@hotmail.com

activities. The most important advantages of Sapindus saponins are easy availability in a wide range of sources and inexpensiveness (8). *S. officinalis* is a plant that grows along roadsides, in meadows and near old home sites. This plant also contains large amounts of saponins and has some biological effects such as anti-inflammatory, haemolytic, anticancer and antifungal activities (6,10).

Although there are studies (11,12) that investigated the antimicrobial activity of saponins from *S. mukorossi* against *S. mutans* and *E. faecalis*, to the best of our knowledge, there is no study that evaluated the effectiveness of saponins from *S. officinalis* on *S. mutans* and *E. faecalis*. Moreover, the results of studies about the antimicrobial effectiveness of saponins against *S. mutans* are conflicting. While Aneja et al. (11) reported no inhibitory activity of *S. mukorossi* against *S. mutans*, Jyothi and Seshagiri (13) found that saponins extracted from *Bauhinia purpurea* and *Madhuca longifolia* have a potential antibacterial activity against *S. mutans*.

To clarify the effect of saponins from different sources on oral pathogens, the aim of this study was to evaluate the antimicrobial activity of saponins from three different extracts of *S. mukorossi* and *S. officinalis* plants against *S. mutans* and *E. faecalis*. We hypothesized that all extracts of both plants have antibacterial activity against the tested oral pathogens.

MATERIALS and METHODS

Ethical Considerations

The present in vitro study does not involve any human or animal subjects thus, no ethical approval is needed.

Plant Materials

The plant materials were purchased from a local market in Istanbul and authenticated for their identity in Altinbas University Department of Pharmaceutical Microbiology.

Test Organisms

S. mutans ATCC 24175 and *E. faecalis* ATCC 29212 were procured from Microbial Type Culture Collection (MTCC) in Altinbas University Department of Pharmaceutical Microbiology.

The fruit of *S. mukorossi* and the root of *S. officinalis* materials were sliced into small pieces. The plant material was macerated (3 times with each solvent) with hexane, ethyl acetate (EtOAc) and methanol (MeOH), respectively. Each extract was filtered by using filter paper and concentrated under reduced pressure by using a rotary evaporator to provide solvent free residue, and crude hexane, ethyl acetate and methanol extracts were obtained from the fruit or root parts of the relevant plants. All extracts were kept at 4°C in a refrigerator until the day they were used.

Broth Microdilution Method

Two dental caries and infection-causing bacteria *S. mutans* and *E. faecalis*, were cultured on brain heart infusion (BHI) agar at 37°C for 24 h aerobically. The minimum inhibitory concentration (MIC) was determined by the broth microdilution method. The extracts were weighed and dissolved in 10% dimethyl sulfoxide (DMSO) to prepare an extract solution of 8 mg/mL. After preparing the extracts, all extracts were sterilized by filtration by 0.45 µm Millipore filters. Each microorganism was suspended in the BHI broth, and the suspensions were adjusted to the 0.5 McFarland standard turbidity. The bacterial solutions were then diluted in the BHI broth by 1:100 fold. 100 µl of the BHI broth was introduced into the wells of a 96 round bottom well plate, and 100 µl of each extract was added into the first well of the plate and mixed. Subsequently, the extracts were diluted two times in each well through serial dilution until the last well. 100 µl of the BHI-extract suspension was discharged from the last well of the microplate to obtain equal volume in every well. After the serial dilution step, 10 µl of the bacterial solution was added into all wells. All plates were incubated aerobically at 37°C for 24 hours, and according to the turbidity in the wells, the MIC values of the extracts were determined. Chloramphenicol was used as a positive control, and each assay in the experiment was repeated two times.

RESULTS

The MIC values of the extracts of *S. mukorossi* and *S. officinalis* against *S. mutans* and *E. faecalis* are listed in Table 1. The lowest MIC values for both *S. mukorossi* and *S. officinalis* were against *S. mutans* (4 mg/ml). While both the methanol and hexane extracts of *S. mukorossi* showed the aforementioned 4 mg/ml MIC value, only the methanol extract of *S. officinalis* exhibited this low value.

Table 1. MIC values (mg/ml) of the fruit extracts of S. mukorossi and S. officinalis against S. mutans and E. faecalis

	MIC (mg/ml)					
	S. mukorossi			S. officinalis		
	Hexane	Ethyl acetate	Methanol	Hexane	Ethyl acetate	Methanol
S. mutans	4	8	4	-	8	4
E. faecalis	8	8	8	-	8	8

DISCUSSION

Different parts of plants may exhibit different saponin contents. Budan et al. (14) reported that the saponin content ranged from 224.0 mg/g in the aerial part extract to 693.8 mg/g in the root extract of *S. officinalis*. Additionally, Singh et al. (12) stated that the fruit extracts of *S. mukorossi* have more antimicrobial activities than leaf extracts. Based on these studies, we used the fruit of *S. mukorossi* and the root of *S. officinalis* as sources of saponin.

MIC is defined as the lowest concentration of an antimicrobial agent that will inhibit the growth of a microorganism after overnight incubation. Since MIC values are accepted as the gold standard for determining

the susceptibility of organisms to an antimicrobial agent, we preferred to use MIC to determine the antimicrobial effectiveness of the tested plant extracts (15).

The hypothesis that all extracts of both plants have antibacterial activity against the tested oral pathogens may be partially accepted, because all extracts of the plants except the hexane extract of *S. officinalis* had an inhibitory activity against the tested microorganisms. Therefore, saponins extracted from *S. mukorossi* and *S. officinalis* may be used to inhibit plaque formation and dental caries. However, the important point here is that the root of *S. officinalis* should not be macerated with hexane to perform antibacterial activities against oral pathogens.

The methanol extracts of both *S. mukorossi* and *S. officinalis* and also the hexane extract of *S. mukorossi* showed good antibacterial activity against *S. mutans*. The inhibitory activity of saponins from different sources against *S. mutans* was confirmed by previous studies (13,16,17).

On the contrary to this study, Aneja et al. (11) found no inhibitory activity of *S. mukorossi* against *S. mutans* despite the fact that they also used methanol extracts of the plant. This discrepancy may be explained by substrate differences; the saponin type of *S. mukorossi* fruits obtained from varying regions may differ. Mahar et al. (9) reported that single primer amplification reaction (SPAR) methods are useful to unravel the diversity among different populations of soap nut plants. Further studies should investigate the variation of saponin types between different *S. mukorossi* fruits obtained from different regions.

The higher MIC values for the hexane and methanol extracts of *S. mukorossi* and methanol extract of *S. officinalis* against *E. faecalis* may be explained by the fact that *E. faecalis* is more resistant to antibacterial agents in comparison to *S. mutans*. This finding was supported by the study conducted by Soekanto et al. (18), who found that a higher concentration of propolis fluoride was needed to kill *E. faecalis* in comparison to *S. mutans*.

It is important to note that the antimicrobial activity of saponins from different sources such as *S. mukorossi* against oral pathogens has been previously reported (11,12). However, data on the antibacterial activity of *S. officinalis* against *S. mutans* and *E. faecalis*, to the best of our knowledge, are reported here for the first time. According to the results of this study, it may be concluded that the ethyl acetate and methanol extracts of *S. officinalis* show antimicrobial activity against the tested oral pathogens.

CONCLUSION

Based on these results, it is possible to conclude that *S. mukorossi* and *S. officinalis* exhibit antibacterial activity against *S. mutans* and *S. officinalis*. Toxicological studies of these plants should be carried out because these plants may be used as a source of new dentifrices, cavity disinfectants or root canal irrigants.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: The present in vitro study does not involve any human or animal subjects thus, no ethical approval is needed.

REFERENCES

1. Balakrishnan M, Simmonds RS, Tagg JR. Dental caries is a preventable infectious disease. Aust Dent J 2000;45:235-45.
2. Loesche WJ. Role of Streptococcus mutans in human dental decay. Microbiol Rev 1986;50:353-80.
3. Nakajo K, Komori R, Ishikawa S, et al. Resistance to acidic and alkaline environments in the endodontic pathogen Enterococcus faecalis. Oral Microbiol Immunol 2006;21:283-88.
4. Łukomska-Szymańska M, Zarzycka B, Grzegorczyk J, Półtorak K, Sokołowski J, Łapińska B. Streptococcus mutans and Enterococcus faecalis as crucial pathogens of the oral cavity. Dental Forum 2016;2:47-52.
5. Kamboj VP. Herbal medicine. Current Sci 2000;78:35-39.
6. Sengul M, Ercisli S, Yildiz H, et al. Antioxidant, Antimicrobial Activity and Total Phenolic Content within the Aerial Parts of Artemisia absinthum, Artemisia santonicum and Saponaria officinalis. Iran J Pharm Res 2011;10:49-56.
7. Nabinejad A. Antibacterial effects of Saponaria officinalis extracts against avian pathogenic Escherichia coli (APEC). Afr J Agric Res 2013;8:2068-71.
8. Wei M, Wu H, Xie Y, et al. In vitro anti-microorganism activity and detergency of Sapindus mukorossi extract based on surfactive nature. J Taiwan Inst Chem Eng 2017;80:1-9.
9. Mahar KS, Rana TS, Ranade SA. Molecular analyses of genetic variability in soap nut (Sapindus mukorossi Gaertn.). Ind Crops Prod 2011;34:1111-8.
10. Sadowska B, Budzyńska A, Więckowska-Szakiel M, et al. New pharmacological properties of Medicago sativa and Saponaria officinalis saponin-rich fractions addressed to Candida albicans. J Med Microbiol 2014;63:1076-86.
11. Aneja KR, Joshi R, Chetan S. In vitro antimicrobial activity of Sapindus mukorossi and Emblica officinalis against dental caries pathogens. Ethnobot Leaflets 2010;14:402-12.
12. Singh R, Kumari N, Nath G. Original Research Article Free radicals scavenging activity and antimicrobial potential of leaf and fruit extracts of Sapindus mukorossi Gaertn. against clinical pathogen. Int J Phytomed 2016;8:22-8.
13. Jyothi KS, Seshagiri M. In-Vitro Activity of Saponins of Bauhinia Purpurea, Madhuca Longifolia, Celastrus Paniculatus and Semecarpus Anacardium on Selected Oral Pathogens. J Dent (Tehran) 2012;9:216-23.

14. Budan A, Bellenot D, Freuze I, et al. Potential of extracts from Saponaria officinalis and Calendula officinalis to modulate in vitro rumen fermentation with respect to their content in saponins. Biosci Biotechnol Biochem 2014;78:288-95.
15. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001;48:5-16.
16. Deshpande S, Kadam D. Phytochemical analysis and antibacterial activity of Acacia nilotica against Streptococcus mutans. Phytochem Anal 2013;5:236-8.
17. Ajagannanavar SL, Battur H, Shamarao S, et al. Effect of aqueous and alcoholic licorice (glycyrrhiza glabra) root extract against streptococcus mutans and lactobacillus acidophilus in comparison to chlorhexidine: an in vitro study. J Int Oral Health 2014;6:29-34.
18. Soekanto SA, Marpaung LJ, Himmatushohwah AD, et al. Efficacy of propolis fluoride and nano silver fluoride for inhibition of streptococcus mutans and enterococcus faecalis biofilm formation. Int J Appl Pharm 2017;9:51-4.