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Investigation of the antimicrobial effects of *Sapindus mukorossi* on endodontic pathogens

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ABSTRACT

Endodontic infections have a polymicrobial nature. Thus, eliminating the microorganisms from infected root canals without damaging healthy surrounding tissue is a major concern. *Sapindus mukorossi* (*S. mukorossi*) is a natural product with potential antimicrobial effects. The aim of this study was to evaluate whether various extract solutions of *S. mukorossi* have an antimicrobial activity against specific endodontic pathogens. Extracts were obtained from *S. mukorossi* fruit pericarps using methanol, ethanol, butanol and distilled water solvents. The inhibition zone, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined with disc diffusion assay, broth microdilution assay and agar dilution assay. Antimicrobial inhibitory activity was observed with all four different solvent extracts of *S. mukorossi* against *Fusobacterium nucleatum* American Type Culture Collection (ATCC) 25586, *Porphyromonas gingivalis* ATCC 33277 and *Actinomyces odontolyticus* (clinical isolate). The MIC values were ranged 10.24-10.24, 0.01-0.64 and 1.28-2.56 mg/mL, respectively. The MBC value was not detected for *Fusobacterium nucleatum* ATCC 25586. The MBC values were 0.02-1.25 and 2.56-5.12 mg/mL for *Porphyromonas gingivalis* ATCC 33277 and Actinomyces odontolyticus (clinical isolate), respectively. Antifungal activities were also observed with the four different solvent extracts of *S. mukorossi* against *Candida albicans* ATCC 10231 and *C. albicans* clinical isolates 1, 2 and 3. The inhibition zone diameter values were in the range of 18-21 mm. The MIC values for *C. albicans* ATCC 10231 and *C. albicans* clinical isolates 1, 2 and 3 were 0.2-0.4 and MFC values were 0.4-0.8 mg/mL, respectively. The antimicrobial effects of the *S. mukorossi* fruit pericarp extract inhibited the growth of *P. gingivalis, A. odontolyticus, F. nucleatum, and especially the C. albicans strains. S. mukorossi* extract has interesting potential as an antimicrobial agent against endodontic pathogens.

1. Introduction

Microorganisms play an important role in the initiation and progression of tooth pulp diseases. In the endodontic applications, such as root canals treatment or regeneration procedures, chemomechanical preparation with an antimicrobial solution is an important stage for the treatment success. Bacterial flora in this region are normally mixed, with a predominance of anaerobic

and facultative species. The presence of bacterial residue in the root canal and periapical regions, even if the treatment is adequate, may result in treatment failure in the long term (Sundqvist, 1992). Studies have shown that bacteria inhibit differentiation of human periodontal stem cells and induce pro-inflammatory cytokines, which play an important role in delaying periodontal tissue regeneration (Graves et al., 2011; Kato et al., 2014). This is especially important in endodontic regeneration processes.

A wide variety of disinfection agents have been developed and used to eliminate necrotic tissues and microorganisms from the root canals. For example, sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are frequently used for the elimination of necrotic tissues and microorganisms during root canal treatment. Despite NaOCl has been the conventionally primary choice in endodontic treatment over the years for its antimicrobial and tissue dissolving properties, alternative approaches are being sought mostly due to concerns regarding cytotoxicity, especially in cases of regeneration (Gatot et al., 1991; Onçağ et al., 2003; Spencer et al., 2007; Simbula et al., 2010). In recent years, the trend of using natural resources has led to investigation of alternative plant extracts, and many researchers have investigated the activity of new substances in root canal disinfection (Herrera et al., 2010; Castilho et al., 2013; Dutta and Kundabala, 2013; Caetano da Silva et al., 2014; Sathyaprasad et al., 2015).

Sapindus mukorossi (S. mukorossi) is a deciduous tree that is widely grown in tropical and sub-tropical regions of Asia, and it is a valuable medicinal plant. It is commonly known by several names, such as soap walnut, ritha, soapberry or washnut. The fruit of *S. mukorossi* (soap walnut) is used in folk medicine for the traditional treatment of diseases like excessive salivation, pimples, epilepsy, chlorosis, migraines, eczema and psoriasis (Shah et al., 2017). Phytochemical studies have shown the presence of saponins (10-11.5%), sugars (10%) and mucilage, with different levels of biological activity in the fruit. These substances give the plant important potential effects, such as antimicrobial, anti-inflammatory, antiprotozoal, anti-cancer, spermicidal and hepatoprotective actions (Ibrahim et al., 2006; Sharma et al., 2011; Upadhyay and Singh, 2012; Shah et al., 2017; Hu et al., 2018). Although some properties of *S. mukorossi* in the medical field have been investigated, comprehensive studies on its antimicrobial activity are still limited (Ibrahim et al., 2006; Sharma et al., 2013; Srinivasarao et al., 2015; Porsche et al., 2018).

The quantity of the active substances in the content of *S. mukorossi*, expecially saponin that can be responsible for antimicrobial effect may change depending on the method of extraction and the type of solvent (Huang et al., 2006; Ghagi et al., 2011). The aim of this study was to evaluate whether various extract solutions of *S. mukorossi* have the antimicrobial activity against some important bacterial and fungal strains that are responsible from endodontic infections.

2. Materials and methods

Preparation of *S. mukorossi* extracts

Extracts of *S. mukorossi* fruit pericarps in different solvents (ethanol, methanol, butanol or distilled water; Sigma-Aldrich, US) were obtained using the Soxhlet extraction method in the Chemistry Department of Akdeniz University. The type of solvents was selected based on previous study results by the researchers (Güçlüer et al., 2020). For this purpose, the dried pericarp of *S. mukorossi* fruits (Botanik City Centre, Ankara, Turkey) was pulverized, and the obtained powder (50 g) was placed in an extraction cartridge. Extraction was carried out for each solvent (700 mL) and completed after 20 cycles of solvent in the system. The remaining solvent was removed in the evaporator under reduced pressure, and the mixture was completely dried under a vacuum at 600°C for 24h. As a result, four different extracts were successively obtained using methanol, ethanol, butanol and distilled water solvents. The ultraviolet (UV) spectrum percentages of extracts in dimethyl sulfoxide (DMSO) were determined for quality control. For the antimicrobial tests, 204.8 mg/mL of stock solution was prepared from the extracts with 10% DMSO and distilled water.

Microorganisms

This study was approved by the Akdeniz University Faculty of Medicine Ethics Committee (No. 05.13.2015/238). Fourteen species comprising ten bacteria and four yeasts were included. Seven of them were obtained from the American Type Culture Collection (ATCC) and seven clinical isolates were obtained from the culture collection of the Department of Microbiology of Akdeniz University cryopreserved at -80°C. More specifically, the following strains were employed: *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Fusobacterium nucleatum* ATCC 25586, *Porphyromonas gingivalis* ATCC 33277, *Bacteroides fragilis* ATCC 25285, *Candida albicans* ATCC 10231, clinical isolates of *Actinomyces odontolyticus* (n=1), *E. faecalis* (n=3) and *C. albicans* (n=3) were used in this study.

Aerobic and facultative anaerobic isolates were grown on Colombia Agar with 5% Sheep Blood plates (Becton Dickinson [BD], Germany). Anaerobic isolates were grown on Brucella Blood Agar with Hemin and Vitamin K1 plates (BD, Germany; 5 μg/mL of hemin, 1 μg/mL of vitamin K1, 5% lysed sheep blood) and incubated in anaerobic conditions

(GasPak EZ, Anaerobe Gas Generating Pouch System with Indicator, BD, Germany) for 3-4 days at 37°C. The yeasts were grown in Sabouraud dextrose agar (SDA; BD). In addition, quality control study (with fluconazole) recommended by the Clinical and Laboratory Standards Institute (CLSI) were included to evaluate the suitability of the tests.

Disc diffusion assay (DDA)

The extracts were initially evaluated with Kirby-Bauer disc diffusion assay (DDA) for determining their antimicrobial activity according to the CLSI standards (CLSI M02-A12, 2015; CLSI M44-A2 2009). Aerobic bacteria suspension of 0.5 McF (1-2 x 108 colony forming unit; CFU/mL) turbudity was applied to Mueller-Hinton agar medium (BD, Germany). Twenty microlitres of 204.8 mg/mL of each extract solution were added and then placed in each sterile paper disc (6-mm diameter) (BD Sensi-Disc, Germany). The discs (4.1 mg extract/disc) previously inoculated with the test microorganisms (*E. faecalis, S. aureus, P. aeruginosa* strains) were placed in the Mueller-Hinton agar plates (BD, Germany). The inhibition zones were measured for each extract after incubation under aerobic conditions for 16-24h.

DDA was also performed as a preliminary assay for anaerobic bacteria. For this, 0.5 McF density bacterial suspensions from colonies (*F. nucleatum, P. gingivalis and B. fragilis*) were prepared in Brucella broth and placed in Brucella Blood Agar with Hemin and Vitamin K1 plates (BD, Germany). Discs (BD Sensi-Disc, 4.1 mg extract/disc) prepared as described above were placed in Brucella Blood Agar plates that were previously inoculated with the anaerobic test bacteria. The inhibition zones were measured for each extract after incubation under anaerobic conditions for 36-48 h. DMSO and distilled water were used as negative controls for all DDAs.

DDA prepared a 0.5 McF density suspension of Candida strains grown for 24 h on SDA plates and distributed on the Mueller-Hinton agar supplemented with 2% glucose and 0.5μg/ml methylene blue dye. Discs (BD Sensi-Disc, Germany) prepared as described above (4.1 mg extract/disc) were placed on the surfaces of Mueller-Hinton agar medium plates previously inoculated with the test microorganisms [*C. albicans* ATCC 10231 and *C. albicans* 1, 2 and 3 (clinical isolates)]. The inhibition zones were measured for each extract after incubation at 37°C under aerobic conditions for 16-24h.

Broth microdilution assay (BMA)

The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of the *S. mukorossi* extracts were evaluated using the broth microdilution assay (BMA) under sterile conditions for aerobic/facultative anaerobic bacteria (CLSI M07-A9. 2015) and yeast according to CLSI (CLSI M27-A3. 2008). In the BMA for bacteria, stock extract solutions (204.8 mg/mL) were serially diluted twofold with cation-adjusted (Ca^{+2} and Mg^{+2}) Mueller-Hinton broth (CAMHB; BD, Germany), and each dilution was dispensed into the wells. Aerobic bacterial stock solutions (0.5 McF; 1-2 x 108 CFU/mL) were prepared from fresh colonies with 0.9% NaCl, then diluted with CAMHB and was inoculated in sterile, U-bottom 96-well microplates. The last bacterial inoculum density was 5 x 105 CFU/mL and final volume was 100 µL in each test well. The microplates were incubated at 35°C for 24 h. The amount of growth in each well was compared with the positive control and the MIC values were obtained the lowest concentration that completely inhibits growth, and 100μLbacterial suspensions from inhibited test wells were sub-cultured in 5% blood agar to evaluate the MBC.

A similar procedure to the BMA for bacteria was used for yeast. Briefly, stock extract solutions (204.8 mg/mL) were serially diluted twofold (between 25.6 - 0.05 mg/ml) with Roswell Park Memorial Institute (RPMI) 1640 broth (buffered with 3-(N-Morpholino) propane sulfonic acid, MOPS; Sigma-Aldrich, USA), and each dilution was dispensed into sterile 96-well microplates. Yeast suspensions from overnight cultures were prepared with an initial density of 0.5 McF (1-5 x 106 CFU/mL). The inoculum was adjusted to a density of 0.5-2.5 x 103 CFU/mL with RPMI 1640 broth and tested against each extract concentration.

The microplates were incubated at 35°C for 24 h. The MIC values were determined as the smallest extract concentration at which no increase is apparent in visual turbidity (approximately 80% growth reduction compared with the positive control). The MFC values were determined by plating 10 μL from each negative well and from the positive control on SDA. MFC was defined as the lowest concentration yielding negative subcultures or only one colony. Fluconazole powder (Fluka Analytical, Sigma) was included in the test for quality control.

Agar dilution assay (ADA)

The MIC and MBC of extracts of *S. mukorossi* were determined using the agar dilution test for anaerobic strains in the sterile conditions according to CLSI (M11-A8. 2012). The bacteria were grown under anaerobic conditions in Brucella Blood Agar supplemented with Hemin and Vitamin K1 plates (5 μg/mL of hemin, 1 μg/mL of vit K1, 5% lysed sheep blood; BD, Germany). For ADA, Brucella agar media (BD) plates were prepared that included different concentrations (20.48-0.01 mg/ml) of extracts.

Anaerobic bacteria solutions (1-2 x 108 CFU/mL) were diluted with Brucella broth medium (BD) over 36-48 h of culturing of bacterial colonies, and were inoculated in the prepared Brucella agar plates containing extracts, then were incubated under anaerobic conditions (GasPak EZ, Anaerobe Gas Generating Pouch System, BD, Germany). The MIC (80% reduction of bacterial growth) and MBC (99.9% reduction of bacterial growth) values were determined by comparing the colonies with the control plate after 48 h of incubation at 35°C. If necessary, the incubation time was prolonged to 72h (for *P. gingivalis*, ATCC 33277). The standard *B. fragilis* ATCC 25285 strain was tested for ampicillin-sulbactam susceptibility as a quality control strain.

3. Results

The antibacterial activities observed with all four solvent extracts of *S. mukorossi* fruit pericarp against *F. nucleatum* ATCC 25586 (Fig. 1), *P. gingivalis* ATCC 33277 and *A. odontolyticus* (clinical isolate). The zone diameters are presented in Table 1. The MIC and MBC values for *P. gingivalis* ATCC 33277 and A. odontolyticus (clinical isolate) with ADA are presented in Table 2. Although growth inhibition exhibited a relatively high concentration in *F. nucleatum* ATCC 25586 with ADA, there was no bactericidal activity (Table 2).

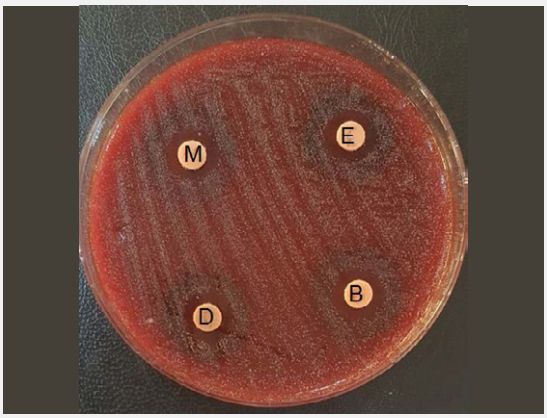

Fig. 1. DDA for *Fusobacterium nucleatum* (ATCC 25586); discs including methanol (M), ethanol (E), butanol (B) and distilled water (D) extracts.

When the different solvent extracts were compared, larger zone diameters with ethanol and methanol solvent extracts were observed against *F. nucleatum* ATCC 25586. Similarly, the extracts obtained with ethanol and methanol solvents were found to be more effective for *A. odontolyticus* (clinical isolate); the MIC and MBC values were determined to be twofold lower. For *P. gingivalis* ATCC 33277, the antibacterial activity of the extracts obtained with methanol, butanol, and ethanol solvents was found to be more effective (the zone diameters were higher and MIC and MBC values were lower compared to the distilled water extract).

Antifungal activity was observed against *C. albicans* ATCC 10231 (Fig. 2) and *C. albicans* clinical isolates 1, 2 and 3, as presented in Table 1, and higher inhibition zone diameter values were detected. At the same time, fungicidal activity was observed against *C. albicans* ATCC 10231 and all *C. albicans* clinical isolates and the MIC and MFC values are presented in Table 2.

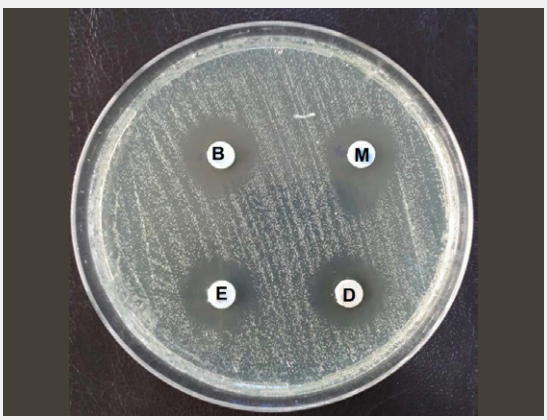

Fig. 2. DDA for *Candida albicans* (ATCC 10231); discs including methanol (M), ethanol (E), butanol (B) and distilled water (D) extracts.

When compared the extracts obtained with different solvents, the extracts obtained with ethanol and methanol solvents showed greater zone diameters for *C. albicans* strains with DDA. However, no difference was found between the MIC and MFC values.

According to the test results, no antimicrobial effect was detected against *E. faecalis* ATCC 29212 or E. faecalis clinical isolates 1, 2 and 3; *S. aureus* ATCC 29213; or *P. aeruginosa* ATCC 27853, even at the

Table 1. Inhibition zone diameter values (mm) of *S. mukorossi* extracts in Disc Diffusion Assay*.

S. mukorossi extracts	*F. nucleatum* (ATCC 25586)	*P. gingivalis* (ATCC 33277)	*C. albicans* (ATCC 10231)	*C. albicans* (clinical isolate 1)	*C. albicans* (clinical isolate 2)	*C. albicans* (clinical isolate 3)
Ethanol	14	15	19	20	21	19
Methanol	14	15	19	20	21	19
Butanol	12	15	18	19	19	18
Aqueous	12	14	18	19	19	18

*Disc Diffusion Assay not applied for A. odontolyticus. The results of bacteria for which inhibition zone was not created are not shown in this table.

Table 2. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration.

	Ethanol extract		Methanol extract		Butanol extract		Distilled water extract	
	MIC (mg/ml)	MBC or MFC*	MIC (mg/ml)	MBC or MFC*	MIC (mg/ml)	MBC or MFC*	MIC (mg/ml)	MBC or MFC*
F. nucleatum (ATCC 25586)	10.24	Ineffective¥	10.24	Ineffective¥	10.24	Ineffective¥	10.24	Ineffective¥
P. gingivalis (ATCC 33277)	0.32	0.64	0.01	0.02	0.01	0.02	0.64	1.28
A. odontolyticus (clinical isolate)	1.28	2.56	1.28	2.56	2.56	5.12	2.56	5.12
C. albicans (ATCC 10231)	0.4	0.8	0.4	0.8	0.2	0.4*	0.4	0.8
C. albicans (clinical isolate 1)	0.4	0.4*	0.4	0.4*	0.2	0.4*	0.4	0.4*
C. albicans (clinical isolate 2)	0.2	0.4*	0.4	0.4*	0.2	0.4*	0.2	0.4*
C. albicans (clinical isolate 3)	0.4	0.8*	0.4	0.4*	0.4	0.4*	0.4	0.8*

* Minimal fungicidal concentrations for C. albicans strains.
¥ Bactericidal effect was not detected with evaluated concentrations (51.2 mg/mL).

highest concentration (51.2 mg/mL) of *S. mukorossi* extract used in this study. Similarly, an antimicrobial effect was not observed against *B. fragilis* ATCC 25285 with DDA (containing 4.1 mg/disc) or ADA (51.2 mg/mL) with *S. mukorossi* extract.

4. Discussion

The presence of infection agents inside the root canal system or periapical tissue can alter the success rate of endodontic treatment. It has already been well established that the common endodontic irrigation solutions (e.g., NaOCl and CHX) are cytotoxic to pulp stem cells and oral tissues. It is evident that antimicrobial and biocompatible irrigants are needed for many endodontic applications, and many studies have been carried out in this field (Herrera et al., 2010; Trevino et al., 2011; Dutta and Kundabala, 2013; Sathyaprasad et al., 2015). In studies about *S. mukorossi,* its fruit pericarps have been reported to have anti-oxidant and anti- inflammatory activity, as well as a protective capacity on some tissue cells (Upadhyay and Singh, 2012; Shah et al., 2017; Ali et al., 2018). These effects can make the plant useful for endodontic applications. In this study, the antibacterial and antifungal effects of fruit pericarp extracts of *S. mukorossi* were investigated against significant endodontic infection agents in vitro and obtained a useful perspective on the antimicrobial efficacy.

Some solvents are used during the preparation of the plants to evaluate their in vitro properties. In the previous studies, ethanol (Ibrahim et al., 2006; Sharma et al., 2011; Srinivasarao et al., 2015; Hu et al., 2018), chloroform (Ibrahim et al., 2006; Porsche et al., 2018) and methanol (Shah et al., 2017; Porsche et al., 2018) were frequently used as a solvent for *S. mukorossi*. Although many researchers obtained the extracts of *S. mukorossi* using different solvents, these studies have reported limited information on differences in antimicrobial efficacy of this solvent extraction methods. Ibrahim et al. reported that chloroform and ethanolic extracts of *S. mukorossi* exerted antibacterial effects on *H. pylori*, while petroleum ether and benzene extracts did not show any antibacterial effect (Ibrahim et al., 2006). In this study, antimicrobial effect of *S. mukorossi* extracts obtained with ethanol, methanol, butanol or distilled water was evaluated against significant pathogen microorganisms of endodontic infections. First, DDA was applied for the preliminary study. The antibacterial (for *F. nucleatum)* and antifungal activities (for *C. albicans*) of the extracts with ethanol and methanol solvents were found to be slightly higher in DDA. Similarly, lower MIC and MBC values were determined for *A. odontolyticus* (clinical isolate) and P. gingivalis ATCC 33277 with extracts obtained from ethanol, methanol and butanol solvents. In this study, the extracts obtained with ethanol and methanol solvents were found to be more effective against related microorganisms.

The endodontic microbiota were predominantly anaerobes and facultative species. The bacterial species tested in this study, *F. nucleatum* and *P. gingivalis*, are Gram-negative, anaerobic bacterial species located in flora, and they may cause periapical infections from the pulpitis (Martinho et al., 2016). *A. odontolyticus* is another bacteria in this study, described as a Gram-positive anaerobic specie, highly detected in infected root canals and has pathogenic potential in periapical diseases (Abou-Rass and Bogen, 1998).

Previous studies about the activity of antimicrobial substances, such as plants, are usually expressed as inhibition zones by DDA in vitro. In previous studies the antimicrobial activity of plant extracts and phytochemicals were evaluated with antibiotic susceptible and resistant microorganisms. In this study, many plant extracts showed antibacterial activity against specific microorganism with zones of inhibition of 7 mm and above is acceptable (Nascimento et al., 2000; Dutta and Kundabala, 2013; Srinivasarao et al.,

2015). Srinivasarao et al. interpreted an 8 mm zone of inhibition for a plant extract as antimicrobial. The extracts of *S. mukorossi* showed inhibition zones of 12-14 and 14-15 mm for *F. nucleatum* ATCC 25586 and P. gingivalis ATCC 33277, respectively (Srinivasarao et al., 2015). Thus, it includes effective antibacterial compounds.

The determination of antimicrobial activity with MIC is accepted as a significant quantitative technique for in vitro studies with crude extracts (Alves et al., 2008). In this study, DDA was applied as a preliminary study and the MIC values were determined with ADA, which is the gold standard for anaerobic bacterial species. The sensitivities were observed for *F. nucleatum, P. gingivalis* and *A. odontolyticus*, which are three important periodontal bacterial pathogens, and the MIC values were determined as 10.24-10.24, 0.01-0.64 and 1.28-2.56 mg/mL, respectively (with different extracts). Aligiannis et al. accepted MIC values 0.28-1.27 mg/mL an extreme activity against a bacteria for a plant (Aligiannis et al., 2001). The bactericidal effect and MBC values for *P. gingivalis* ATCC 33277 and *A. odontolyticus* were also determined in our study. It appeared that *S. mukorossi* has a bacteriostatic effect against *F. nucleatum* ATCC 25586 compared with the other two anaerobic bacteria. Hence, sub-fractionationof this extract may be better for explaining the antibacterial effect.

It has been reported that *S. mukorossi* ethanol and chloroform extracts inhibit the growth of H. pylori in vitro (10-200 μg/mL by DDA) and in vivo (Ibrahim et al., 2006). In the literature, there has been no study evaluating the efficacy of *S. mukorossi* against Fusobacterium spp. and Actinomyces spp. and our study is the first to report the antibacterial effect of this plant's extracts on *F. nucleatum* and *A. odontolyticus*. Bacterial species evaluated in this study are representative of the different groups causing infections in root canals. *E. faecalis, S. aureus and P. aeruginosa* are usually associated with periapical lesions. *B. fragilis* is a Gram-negative anaerobic bacteria commonly found in endodontic infections (Pallotta et al.,, 2007). In this study, antibacterial effects of *S. mukorossi* extracts could not be detected against these bacteria. Srinivasarao et al. reported that *S. mukorossi* extract obtained with the thin layer chromatography method, showed antibacterial effect (8-mm inhibition zone) for *Bacillus subtilis, B. cereus, Pseudomonas aeruginosa and Escherichia coli*. It is thought that saponins, which are components of *S. mukorossi*, are responsible for its antimicrobial activity and other useful effects (Upadhyay and Singh, 2012; Srinivasarao et al., 2015; Ali et al., 2018; Hu et al., 2018). Another study reported that ethanolic extract of *S. mukorossi* has an antibacterial effect against the same bacterial species using the well diffusion method, drawing attention to the importance of saponin content in the plant (Sharma et al., 2013). There is also a study which the oleanane-type saponin fraction exhibits moderate antibacterial activity against Gram-positive bacteria (*S. aureus*) and no activity against Gram- negative bacteria (*P. aeruginosa and E. coli*). (Tamura et al., 2012). Overall, there has been a limited number of studies in this area. In our study, it could not be determinated the sensitivity to these bacteria. The reason for this may be that the extract did not divided into fractions (e.g., by the chromatographic method). Another possible reason may be that there was a relatively low amount of components responsible for antibacterial effects, such as saponins, in the extract.

C. albicans is one of the most common microorganisms in the flora of periapical lesions (Dutta and Kundabala, 2013; Sathyaprasad et al., 2015). In our study, the antifungal activity of *S. mukorossi* against *C. albicans* strains was determined with all extracts obtained with different solvents.

Aligiannis et al. and Tsuzuki et al. presented suggestions a classification for in vitro antimicrobial plant studies (Aligiannis et al., 2001; Tsuzuki et al., 2007). Briefly, with a MIC value up to 0.5 mg/mL, the antifungal activity is considered reliable. If the extracts show a MIC between 0.6 mg/mL and 1.5 mg/mL, the antifungal activity is moderate and MIC above 1.6 mg/mL, the antifungal activity is considered weak (Aligiannis et al., 2001; Duarte et al., 2005; Tsuzuki et al., 2007).

In this study according to the criteria outlined above, the MIC values determinated against *C. albicans* strains (0.4 and 0.2 mg/mL) suggested that *S. mukorossi* has promising antifungal activity. Indeed, this action was better than the observed antibacterial effects of all the extracts. These results also support the disc diffusion test results (18-21 mm). Studies have shown that triterpenoid saponins in the structure of *S. mukorossi* exhibited antifungal activity against *C. albicans* (Aneja et al., 2010; Upadhyay and Singh, 2012; Hu et al., 2018). In addition, the saponin fraction inhibited the dermatophyte fungi *T. rubrum, T. mentagrophytes, Sabouraudites canis, Epidermophyton floccosum* and *C. albicans* (Tamura et al., 2012). In another study, the efficacy of saponins isolated from *S. mukorossi* with the liquid chromatography technique was also shown against plant fungi (Porsche et al., 2018). The results of our study are consistent with other studies on the efficacy of *S. mukorossi* against *C. albicans* species.

Although many phytochemicals present in *S. mukorossi* have been isolated and identified by researchers, pharmacological studies on these components have been limited so far, and there is a need for extensive supplementary research on *S. mukorossi* to explain its mechanism of action. In addition, detailed analysis is required for isolating phytoactive components from *S. mukorossi* and

tracing their biological activities. If the phytoactive components are purified, the substances responsible for the antimicrobial effects can be detected more accurately. These results were obtained in vitro, and thus, they may not exactly reflect in vivo interactions. Safety assess and in vivo studies should be conducted to elucidate the effectiveness of *S. mukorossi*.

Conclusions

In this study, the antibacterial and remarkable antifungal activities of *S. mukorossi* fruit pericarps were determined. It was demonstrated that the fruit extracts of *S. mukorossi* inhibited the growth of *F. nucleatum, P. gingivalis, A. odontolyticus and C. albicans*. The extracts of *S. mukorossi* fruit pericarps were most effective against *C. albicans* strains. If advanced susceptibility tests are carried out separating the active substances, it may help to identify these effects better. These substances can be combined with different chemicals to increase their efficiency, especially when interacting with mixed bacterial flora, which are involved in most root canal infections.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication that could have influenced its outcome.

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