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Original Article

Human pulp tissue dissolution ability of different extracts of *Sapindus mukorossi:* An *in vitro* study

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ABSTRACT

Objective: Due to the many negative properties of sodium hypochlorite used in current root canal treatment, interest in biocompatible natural agents is increasing day by day. The aim of this study was to evaluate whether various extract solutions of *Sapindus mukorossi* have dissolution effects on human pulp tissues.

Methods: Primarily powder extracts were obtained by extracting fruit shells of *S. mukorossi* in different solvents (ethanol, methanol, buthanol and distilled water). The test solutions were prepared and randomly separated into six groups with 10 samples in each group: ethanol extract, methanol extract, butanol extract, distilled water extract of *S. mukorossi*, sodium hypochlorite (NaOCl) and the control group. Among these, *S. mukorossi* extracts were separated into two subgroups, depending on their concentration level (50 μg/mL and 100 μg/mL). The pulp tissues of freshly extracted human molars were used for dissolution test. The weights of the pulpal tissues were measured and recorded for two times after the samples were placed in the solutions. Statistical analysis for all descriptive statistics was performed using SPSS 22 ($P < 0.05$).

Results: Our results showed that maximum percent yield of preparation was obtained in methanol extract of *S. mukorossi*. Among all of the groups, the best dissolution capacity was seen in the NaOCl group (positive control group). Among *S. mukorossi* groups, the best tissue solvent solution was found in SMM group at 50 μg/mL and SMB group at 100 μg/mL.

Conclusion: The different extracts of *S. mukorossi* had a capacity to dissolve pulp tissue but this capacity was less than NaOCl. Therefore, further studies will enable the creation of a commercial solution for clinical use by increasing the effectiveness of *S. mukorossi* while combining it with other endodontic irrigation solutions.

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1. Introduction

Complexity of root canals makes the elimination of the microbial environment difficult (Sundqvist, 1992). Complete disinfection of root canal system with a combination of chemical agents and root canal instruments plays an essential role in the success of root canal treatment. Sodium hypochlorite (NaOCl), ethylenediaminetetraacetic acid (EDTA), and chlorhexidine are the most routinely used chemical irrigants in endodontics.

NaOCl is a gold standard due to its desirable tissue dissolving property, its rapid penetration into canal walls due to its low surface tension, antisepticity, easy availability, and low cost (Alacam, 2012). It is generally used at the concentrations ranging from 0.5% to 6%. Despite being a great antibacterial and a tissue solvent, this chemical solution is harmful to perapical tissues only if it is extruded from the apical foramen (Oncag, Hoşgör, Hilmioglu, Zekioglu & Eronat, 2003; Simbula, Dettori, Camboni & Cotti, 2010); and its toxicity increases with the concentration. Numerous studies (Becking, 1991; Gatot, Arbelle, Leiberman & Yanai-Inbar, 1991) have shown clinically that this solution damages the periodontal and periapical tissues. Furthermore, this solution has a taste and smell that can disturb the patient in treatment. On account of

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these adverse effects, studies recently focus more on the efficiency of bioactive materials in disinfecting procedures instead of NaOCl.

Sapindus mukorossi Gaertn. has become a bioactive material that is researched extensively as a treatment option in a range of medical areas. It belongs to the *Sapindus* species from the Sapindaceae family; also called ritha, doadni, or soap nuts. This tree grows in the upper Indo-Gangetic Plains, Shivaliks and lower Himalayan foothills at the altitudes of 200−1500 m (Aneja, Joshi & Sharma, 2010). The main components of *S. mukorossi* are saponin (10%−11.5%), sugar (10%), mucilage (10%) and flavonoids (Ibrahim et al., 2006). Lately, many pharmaceutical effects of this fruit's extract has been discovered, including antimicrobial (Ibrahim et al., 2006; Talwar et al., 2008), fungicidal (Tanaka, Tamura, Masuda & Mizutani, 1996; Tsuzuki et al., 2007), anti-inflammatory (Shah et, al.; Takagi, Park & Kato, 1980), hepatoprotective (Ibrahim et al., 2008), and anticancer effects of its component saponin (Liu et al., 2018; Man, Gao, Zhang, Huang & Liu, 2010; Rashed et al., 2013) are some of the known. The name *S. mukorossi* is derived from the Latin word *"sapo"*, which means soap. Saponins generally have a triterpenic or steroidal aglycone that produces a permanent foam when shaken in aqueous solutions and glycosides are capable of hemolysis of red blood cells (Kucukkurt & Fidan, 2008). In a recent study, the combination of *S. mukorossi* with another bioactive material with 1:1 ratio was proved more effective in the removal of smear layer from the root canal walls than the traditionally used 17% EDTA; And it is argued that this may be due to the saponin it contains (Chhabra, Gyanani & Kamatagi, 2015).

The aim of this study was to examine whether various extract solutions of *S. mukorossi* obtained with different solvents have tissue solvent effects on human pulp tissues. The null hypothesis of this study was that 'the different extract solutions obtained from S. *mukorossi* had not a dissolution effect on human pulp tissues'.

2. Materials and methods

2.1. Preparation of extracts of S. mukorossi in different solvents

Organic certified *S. mukorossi* fruit pericarps were obtained from Asian Organic Products Company (Asian Organic Products Co., Hatay, Turkey) as a packaged product. Ethanol, methanol, butanol, and distilled water were purchased from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). All chemicals were of analytical grade and used without further purification. The Soxhlet extraction method was used to prepare the extracts of *S. mukorossi* with different solvents (ethanol, methanol, butanol and distilled water). First, *S. mukorossi* were homogenized by grinding with a blender and then 50 g of homogenized powder *S. mukorossi* was placed in an extraction thimble. A total of 700 g of solvent was added to the extraction flask and the extraction was carried out at the boiling point (°C) of the solvent. The extraction process was completed after 20 cycles of the circulation of the solvent. The solvent in the obtained solvent-extract mixture was removed under vacuum first at room temperature and at 60 °C for 24 h. Finally, the obtained dry extracts were milled with a blender to obtain homogeneous powders.

2.2. Pulp tissue preparation

The study was approved by the Akdeniz University Faculty of Medicine Ethics Committee (no. 2012-KAFK-20/238). Freshly extracted human third molars were used in this study, which were scheduled to be extracted for orthodontic or surgical purposes. The teeth were free from decay, calcified canals and cracks. Obliterated teeth or teeth lacking dental pulp were excluded. Sample teeth were bevelled longitudinally from the mesial and distal sections, scalpel number 11 was placed on the bevels, and the pulpal tissue was exposed by dividing it into two parts using a dental tweezer. The exposed pulp tissue was rinsed with distilled water to clean the blood clot. Pulpal tissues were placed in 2 mL Eppendorf tubes, containing a buffered saline solution and were preserved below 4 °C until required.

2.3. Dissolution test

The test solutions were randomly separated into six groups, each containing 10 samples: ethanol extract of *S. mukorossi* (SME), methanol extract of *S. mukorossi* (SMM), butanol extract of *S. mukorossi* (SMB), distilled water extract of *S. mukorossi* (SMD), 2.5% NaOCl (positive control) group and distilled water (negative control). Among these, *S. mukorossi* extracts were separated into two subgroups, depending on their concentration level (50 µg/mL and 100 µg/mL) by the findings of the pilot study. In the experiment stage, refrigerated samples were kept at room temperature for 1 h. In order to ensure standardization, each pulpal tissue was shaped with the assistance of a scalpel, in a way that each weighed approximately (6.5 ± 0.2) mg and a total of 100 samples of pulpal tissue were obtained. The samples were rinsed with distilled water, and dehumidified using gauze patches. Their initial weights were measured and placed back in the tubes. Samples were kept at 37 °C in the experimental solution for 15 min (t_1), removed from the incubator, rinsed with distilled water and dehumidified using gauze patches. The weights (mg) of the samples were measured on the precision scale and the values were recorded. The samples were placed back in the eppendorf tubes and undergone the same first-step processes for another 30 min (t_2) except for the NaOCl group. The NaOCl was not subjected to the 30 min waiting period, as there was no tissue substance left after 15 min. After waiting for 30 min, the final weights of the samples were measured and the values were recorded.

2.4. Statistical analysis

Statistical analysis for all descriptive statistics was performed using SPSS 22 (SPSS Software, IBM Corp). Shapiro-Wilk test was used to test for normality. One-way analysis of variance followed by post hoc analysis using Bonferroni test was used to detect statistically significant differences among groups. The level of significance is $P < 0.05$.

3. Results and discussion

In this study, pulp tissue of human third molars which were extracted for various reasons was used. In previous similar studies (Clarkson, Kidd, Evans & Moule, 2012; Niewierowski, Scalzilli, Morgental, Figueiredo & Vier-Pelisser, 2015; Peña López, Conde, Estevez, Valencia de Pablo & Rossi-Fedele, 2018), different types of tissue (dental pulp from oxen, palatal mucosa and dental pulp from pigs) were used for the same purpose. Although the limitations concerning the supply and preparation process of extracted human tooth pulp, the conditions of a clinical environment was tried to mimic as much as possible with the method used in this study (Slutzky-Goldberg, Hanut, Matalon, Baev & Slutzky, 2013). The pulp samples were especially collected from the crown pulp to ensure the volumetric and dimensional standardization.

3.1. Preparation of extracts of S. mukorossi in different solvents

The principal content of the *S. mukorossi* is comprised of saponin (10%−11.5%), sugar (10%), and mucilage (10%). The content and quantity of the active substances, expecially saponin that can be responsible for dissolving organic tissues (Ghagi, Satpute, Chopade & Banpurkar, 2011; Huang, Tsai, Morris-Natschke, Tokuda

& Lee, 2006), may change depending on the method of extraction and the type of solvent (Aneja et al., 2010).

Therefore four diffrerent solvents such as ethanol, methanol, butanol and distilled water were chosen in the extraction. The applied extraction temperatures and yield rates were shown in Table 1. Our results showed that maximum percent yield was obtained in methanol extract (85%), followed by distilled water (71%), ethanol (65%) and butanol (62%). The photos of *S. mukorossi* fruit pericarps and dry extracts were shown in Fig. 1. This can highlight that methanol is efficient in extracting phytochemicals but the solvents used in the process can have far more critical roles on the extract qualities (Yan et al., 2008).

Table 1
Yield of *S. mukorossi* extracts by different solvents at applied extraction temperatures.

Solvents	Extraction temperature/ °C	Yield /%
Methanol	64	85
Ethanol	78	65
Butanol	118	62
Distilled water	100	71

3.2. Dissolution test results

The principal properties expected from an ideal irrigation solution are antimicrobial activity, water solubility, low toxicity to periradicular tissues, and tissue solvent ability (Niewierowski et al., 2015). Over the years, several chemicals and combinations have been investigated for potential use as endodontic irrigant. When the literature is examined, it can be seen that NaOCl, though toxic, named “gold standard” as it is an excellent antimicrobial agent and solvent of organic tissue. Therefore, NaOCl was used as a positive control group (Prada et al., 2019).

In this study, the best dissolution capacity was seen in the NaOCl group used as the positive control, which dissolved the whole of the pulp tissue. The measurements made after contact of pulp tissue samples with NaOCl for 15 min revealed that the tissue samples completely dissolved. The results of the study were demonstrated in Tables 2 and 3. These results are consistent with the previous studies (de Almeida, Leonardo, Gomes, Souza & Pappen, 2015; Slutzky-Goldberg et al., 2013).

Hypochlorous acid, an organic substance found in NaOCl functions as a solvent when it comes into contact with the pulp tissue and creates chloramine by way of combining hydrogen from the protein amino groups with chlorine. Hypochlorous acid and hypochlorite ions can cause the degradation of amino acids (Estrela, Estrela, Barbin, Spanó & Marchesan, 2002).

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. The aim in the most of the studies was to reduce the cytotoxic feature by adjusting the NaOCl concentration and temperature, activating the irrigation, and combining it with EDTA and other solutions. Moreover, in order to enhance its penetration into unattainable zones of the root canals and improve its overall effect, the addition of surfaceactive agents has been suggested (Peña López et al., 2018; Rossi-Fedele, Prichard, Steier & de Figueiredo, 2013). However, according to some studies (Clarkson et al., 2012; De-Deus et al., 2013; Jungbluth, Peters, Peters, Sener & Zehnder, 2012), the increasing penetration depth and additional pulp tissue dissolution activity of these agents in NaOCl is limited. No previous study was found about the tissue solving property of a natural agent on human tooth pulp in literature. This study assessed the tissue solvent activity of *S. mukorossi* which is a natural bioactive agent and an alternative to NaOCl.

Extract solutions prepared from different solvents of *S. mukorossi* displayed a statistically significant effect as a tissue solvent compared to the negative control group. Among *S. mukorossi* groups, the best tissue dissolving capasity was found in SMM (50 µg/mL) group with 57%. It was observed that the weight of pulp tissue samples decreased statistically significantly ($P < 0.05$) at first-step measurement (t_1) in all *S. mukorossi* groups. There was no significant weight loss between the time periods t_2 and t_3 (Table 3).

Saponin, a significant component of *S. mukorossi*, has a glycoside structure that shows considerable biological effects on cell membranes. Saponins are used frequently in cell-based studies for their pore-forming ability (Hu, Chen, Jiao, Khan & Li, 2018; Huang et al., 2006; Kucukkurt & Fidan, 2008). The surfactant and emulsion characters of the saponin have aroused curiosity in the literature (Almutairi et al., 2015; Ghagi et al., 2011). The surfactant structure of saponin reduces the surface tension and provides it with a foaming characteristic. According to the study, the effective cause of the solvent ability might be linked to the structure of saponin in *S. mukorossi*, which reduces surface tension, thus increasing membrane permeability. Many studies revealed that the surfactant addition to NaOCl performed a synergistic action and affected the tissue dissolving specification positively (Clarkson et al., 2012; Estevez et al.; Peña López et al., 2018).

In a study that was the only study in the field of dentistry and endodontics using the *S. mukorossi* extract, the removal of the smear layer was investigated, but the evaluation was made using another bio-surfactant combination in different ratios (Chhabra et al., 2015). Even though that study reveals significant data on the ability of the dissolving characteristics of *S. mukorossi* on organic and expecially inorganic tissues (smear alyer), it does not provide an opportunity to assess its use alone. Besides this in our study, it may have caused different amounts of bioactive ingredients (saponin, flavonoid, musilage, sugar) to form, which could provide a comparable assessment of tissue dissolving properties of the solutions. In particular, the investigation of the active substances that demonstrate the ability of tissue dissolving by making advanced fractions of *S. mukorossi*'s butanol extraction by chromatography can be promising in the preperation of natural irrigation solutions in endodontics. Therefore, further studies can

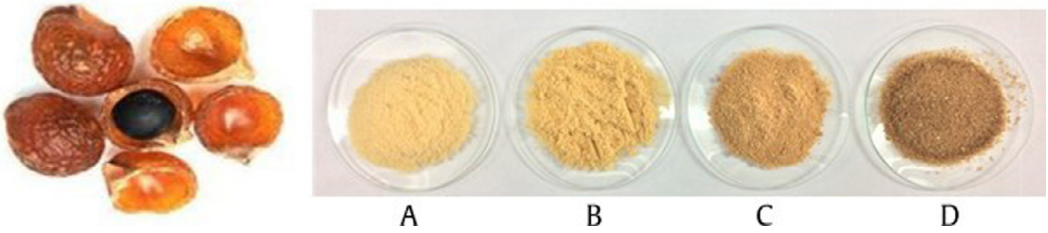


Fig. 1. *Sapindus mukorossi* fruits and dried extracts from ethanol (A), methanol (B), butanol (C) and distilled water (D).

Table 2
Amount of residual pulp tissue exposed to experimental solutions at different time periods (mean ± SD, $n = 10$).

Groups	Amount of residual pulp tissue/mg		
	0 min (t_1)	15 min (t_2)	45 min (t_3)
SME (50 μg/mL)	6.23 ± (0.60)[a]	3.39 ± (0.51)[b]	3.10 ± (0.49)[b]
SME (100 μg/mL)	6.19 ± (0.75)[a]	3.80 ± (0.72)[b]	3.34 ± (0.55)[b]
SMM (50 μg/mL)	6.30 ± (0.61)[a]	3.11 ± (0.47)[b]	2.82 ± (0.37)[b]
SMM (100 μg/mL)	6.01 ± (0.49)[a]	3.86 ± (0.76)[b]	3.25 ± (0.79)[b]
SMB (50 μg/mL)	6.22 ± (0.96)[a]	4.23 ± (0,90)[b]	3.39 ± (0.79)[b]
SMB (100 μg/mL)	6.56 ± (0.44)[a]	3.85 ± (0.82)[b]	2.82 ± (0.54)[b]
SMD (50 μg/mL)	6.37 ± (0.34)[a]	4.29± (0.38)[b]	3.85 ± (0.41)[b]
SMD (100 μg/mL)	6.63 ± (0.43)[a]	4.18 ± (0.79)[b]	3.50 ± (0.86)[b]
NaOCl 2.5% (positive control group)	6.53 ± (0.22)[a]	0[b]	0[b]
Distilled water (negative control group)	6.50 ± (0.86)[a]	5.67± (0.83)[a]	5.21 ± (0.90)[b]

Note: The groups of SME, *S.mukorossi* ethanol extraction solution; SMM, *S. mukorossi* methanol extraction solution; SMB, *S. mukorossi* butanol extraction solution; SMD, *S. mukorossi* distilled water extraction solution; NaOCl, sodium hypochlorite; [t_1, t_2, t_3 (min.)], the time of pulp tissue samples are weighed. Different letters in the each group indicate significant differences according to time at level of significance $P < 0.05$.

Table 3
Weight and rate of pulp tissue dissolved in experiment solutions at final time period.

Groups	Weight/mg	Rate/%
SME (50 μg/mL)	3.13[a]	50
SME (100 μg/mL)	2.85[a]	46
SMM (50 μg/mL)	3.48[a]	55
SMM (100 μg/mL)	2.76[a]	45
SMB (50 μg/mL)	2.83[a]	45
SMB (100 μg/mL)	3.74[a]	57
SMD (50 μg/mL)	2.57[a]	39
SMD (100 μg/mL)	3.13[a]	47
NaOCl 2.5% (positive control)	0[b]	100
Distilled water (negative control)	1.3[c]	19

Note: The groups of SME, *S. mukorossi* ethanol extraction solution; SMM, *S. mukorossi* methanol extraction solution; SMB, *S. mukorossi* butanol extraction solution; SMD, *S. mukorossi* distilled water extraction solution; NaOCl, sodium hypochlorite. Different letters indicate significant differences at level of significance $P < 0.05$.

enable the production of a commercial solution for clinical use by increasing the effectiveness of *S. mukorossi* while combining it with other enddodontic irrigation solutions as NaOCl or EDTA.

4. Conclusion

In this *in vitro* study, NaOCl exhibited the best tissue-dissolving effect among all solutions tested. The butanol and methanol extractions of *S. mukorossi* has a capacity to dissolve pulp tissue. Therefore, the next step of the study will be to explore the decomposition of the active ingredients related the dissolving capability of the butanol and methanol extraction of *S. mukorossi* by performing additional fractioning with chromatography.

Declaration of Competing Interest

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

Authors' contributions

The data analyzed during the current study are available from the corresponding author on reasonable request. Conceived and designed the study: GÖ. Performed the study: GÖ, BE, YE. Analyzed the data: KA, GÖ. Wrote the paper: GÖ, EK. All authors read and approved the final manuscript.

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