

Effectiveness of a 20% Miswak extract against a mixture of *Candida albicans* and *Enterococcus faecalis*

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ABSTRACT

الأهداف: اختبار فعالية 20% من خلاصة السواك ضد خليط من فطريات الكانديدا، وبكتيريا الانتروكوكس باستخدام اختبار قابلية الأنابيب المخففة والذي يسمح بالاتصال المباشر بين المواد المختبرة والكائنات الحية الدقيقة.

الطريقة: جمعت عينات الدراسة وأجريت التجربة خلال الفترة من أغسطس 2009م إلى يناير 2010م في كلية الأسنان - جامعة الملك سعود - الرياض - المملكة العربية السعودية. وضعت كل من هذه الكائنات الحية الدقيقة في محلول وتعرض 20% من خلاصة السواك في حاوية صغيرة بلاستيكية، وقد تم حضن السواك مع هذه الكائنات الحية الدقيقة ل ساعة، 6 ساعات و 24 ساعة.

النتائج: أظهرت أن خلاصة السواك فعالة ضد الفطريات، وضد البكتيريا في جميع الأوقات المختبرة باستثناء تعرض بكتيريا الانتروكوكس وخليط من بكتيريا الانتروكوكس وفطريات الكانديدا ل 20% من خلاصة السواك في الساعة الأولى فقط.

خاتمة: لقد استنتجنا من هذه الدراسة أن 20% من خلاصة السواك هي مادة فعالة جداً كمضاد لفطريات الكانديدا، وبكتيريا الانتروكوكس.

Objectives: To examine the *in vitro* antimicrobial effect of a 20% Miswak extract against a mixture of *Candida albicans* (*C. albicans*) and *Enterococcus faecalis* (*E. faecalis*) using the dilution tube susceptibility test, which allows direct contact between the tested material and the microorganisms.

Methods: The study samples were collected and processed between August 2009 and January 2010 in the College of Dentistry, King Saud University, Riyadh, Saudi Arabia. Each microorganism was obtained in a suspension and exposed to a 20% Miswak extract in plastic tissue culture clusters containing 24 wells. Six wells were used per group. The Miswak extract was incubated with the microorganisms for one, 6, and 24 hours.

Results: This *in vitro* study showed that Miswak extract was an effective antifungal and antibacterial agent at all tested experimental time periods, except one hour exposure of a 20% Miswak extract to *E. faecalis* and a mixture of *E. faecalis* and *C. albicans*, which was ineffective in inhibiting their growth.

Conclusion: Twenty percent Miswak extract is an effective antifungal and antibacterial agent against *C. albicans* and *E. faecalis*.

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The role of bacteria in the initiation of pulpal and periapical inflammation has been well demonstrated.¹ The elimination of microorganisms in infected root canals indicates the success of endodontic treatment, as microorganisms colonizing in an oral environment can be conducive to pulpal and periapical pathosis.² *Enterococcus faecalis* (*E. faecalis*) is a microorganism commonly detected in asymptomatic, persistent endodontic infections. *Enterococcus faecalis* possess various survival and virulence factors, such as its ability to compete with other microorganisms, resist nutritional deprivation, and invade dentinal tubules. The prevalence of infection ranges from 24-77%.³ It is associated with different forms of periradicular disease including primary endodontic infections and persistent infections,⁴ and has also been associated with therapy-resistant root canal infections.⁵ The presence of *Candida albicans* (*C. albicans*) in the infected pulp and

periradicular areas has been demonstrated through the use of light and electron microscopy, as well as using culture techniques.^{5,6} The incidence of *C. albicans* in infected root canals has shown to vary between 7-55%,^{7,8} and occurs in approximately 11.4% of teeth with pulp lesions.⁹ It has also been reported that *C. albicans* can use dentin as a nutrient source.¹⁰ Treatment has failed in root canals of obturated teeth where yeast-like microorganisms are found.¹¹ Irrigant solutions are very important during root canal preparation, as they aid in the cleaning of the root canal, lubricate the files, flush out debris, have an antimicrobial effect and tissue dissolution, without damage to the periapical tissues as well as some solutions having a bleaching effect on the teeth.¹² The selection of an ideal irrigant depends mainly on its action on microorganisms and periapical tissue with the least side effects. Sodium hypochlorite and chlorhexidine are antimicrobial agents frequently used in the treatment of endodontic infection.^{13,14} However, there is limited information available on the effect of Miswak extract against *E. faecalis*. Thus, the objective of this study was to examine the antimicrobial activity of Miswak extract against *E. faecalis*, *C. albicans*, and a mixture of *C. albicans* and *E. faecalis* using the dilution tube susceptibility test.

Methods. This *in vitro* study collected and processed samples between August 2009 and January 2010 in the College of Dentistry, King Saud University, Riyadh, Saudi Arabia. Ethical approval was sought from the College of Dentistry Research Center, but was deemed unnecessary. Fresh roots of Arrak (*Salvadora Persica L.*, *Salvadoraceae*) were procured from the farms located in Tuhama Asir, Saudi Arabia. The roots were cut into small pieces and powdered. The percolation method (2.5 liter of 96% ethanol was used 6 times) was used on the extract (percolate) prepared from 1 kg of the Arrak sample. The meticulous extraction was continuously carried out until the material appeared colorless. The resulting extract (percolate) was then concentrated and the solvent (ethanol) was completely removed at low temperature and reduced pressure. The extract was stored at 4°C in a tightly closed container to preserve it from any contamination, deterioration, and/or decomposition. The stock solution of Miswak extract was prepared by dissolving 0.2 mg of the Miswak extract in 1 ml of distilled water to prepare a 20% Miswak concentration. The experiment was carried out for 3 separate groups (A, B and C). Group A was used to observe the growth of *C. albicans*, B for *E. faecalis*, and C for a mixture of *C. albicans* and *E. faecalis*. Each group test was performed in plastic tissue culture clusters containing 24 wells, each with an inner diameter of 16 mm. One mL of Miswak extract was placed at

the bottom of each culture well, to which one mL of *Candida* suspension was added in group A. At the same time, one mL of Sabouraud infusion broth media was mixed with one mL of *Candida* suspension in a culture well to serve as a positive control. For the negative control, 2 mL of Sabouraud infusion broth was placed in a culture well. The same procedure was carried out for groups B and C. Six wells were thus used per group. The culture clusters plates were then incubated at 37°C and evaluated after one, 6, and 24 hours. At the end of the incubation period, aliquots of 0.1 mL were withdrawn from each well and transferred to tubes containing 5 mL of fresh Sabouraud infusion broth. The tubes were then vortexed, incubated at 37°C, and observed for 7 days. Growth of the microorganisms was observed daily, as indicated by the presence of turbidity in the tubes. The presence of turbidity was determined, and the purity of the cultures was checked by the morphology of colonies onto blood agar plates for *E. faecalis*, and Sabouraud agar plates for the *C. albicans*. The results were recorded and statistically analyzed using the Kruskal-Wallis test. The data was entered in MS Excel and analyzed using Statistical Package for Social Sciences (SPSS windows standard version 11.0, SPSS Inc., USA) using the Kruskal-Wallis test.

Results. The positive control, in which there was no putative antimicrobial agent, exhibited growth of the microorganisms at all times tested. As expected, no growth occurred in the negative control group. The 20% Miswak extract was ineffective at inhibiting the growth of *E. faecalis* and a mixture of *E. faecalis* and *C. albicans* after one hour of exposure. Further, there was no significant difference in growth between Miswak extract treatment for one hour and growth in the positive control. The 20% Miswak extract was effective in complete inhibition of the growth of *C. albicans* at all times tested, and effective against *C. albicans* and mixture of *C. albicans* and *E. faecalis* after 6 and 24 hours of exposure as shown in Table 1.

Discussion. Several studies have tested the effect of intracanal medicaments and antiseptic agents on *C. albicans* including sodium hypochlorite, chlorhexidine solution, and calcium hydroxide paste,^{15,16} and have shown to be effective. Waltimo et al⁶ and Siqueira and Uzeda¹⁷ demonstrated that calcium hydroxide associated with a saline solution was ineffective in eliminating *E. faecalis* and *Fusobacterium nucleatum* cells inside dentinal tubules even after one week of contact. Miswak is a chewing stick commonly used as a brush to clean the teeth.¹⁸ It is made from the aromatic root of a small bush known as the Arrak (*Salvadora Persica L.*). Many studies demonstrated that these sticks

Table 1 - The effect of 20% Miswak extract against the microorganisms at different exposure times.

Microorganisms	Exposure time		
	One hour	6 hours	24 hours
<i>Candida albicans</i>	-ve	-ve	-ve
<i>Enterococcus faecalis</i>	+ve	-ve	-ve
<i>Candida albicans</i> and <i>Enterococcus faecalis</i>	+ve	-ve	-ve

-ve - no growth, +ve - growth

have antiplaque, antiperiopathic, and anticaries effects,¹⁹ as well as antibacterial properties against several types of bacteria that are frequently found in the oral cavity.²⁰ The Miswak extract (100 mg/ml) at 50% concentration has a fungistatic and bacteriocidal effect for up to 48 hours, however 50 mg/ml extract at 25% concentration showed no bacteriocidal or antimycotic properties.²¹ The antimicrobial activity of Miswak extract has been reported by some researchers. Al-Sabawi et al²² reported significant antimicrobial effect at 15% alcoholic Miswak extract, however, this was not significantly different from sodium hypochlorite and chlorhexidine. Darout et al²³ reported that Miswak extract had an antibacterial activity against *Streptococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*, which may be due to its nitrate content.

The dilution tube susceptibility test was the method used in this experiment to evaluate the inhibition of microbial growth, which is an effective method to evaluate antifungal as well as antibacterial properties of any filling material or solution.^{24,25} This method allows direct contact of the solution between *C. albicans* and *E. faecalis* and the agents to be tested. Efficiency of this methodology was confirmed by the growth of the microorganisms (*C. albicans* and *E. faecalis*) in the positive control. This study was limited to using 2 microorganisms, *C. albicans* and *E. faecalis*, due to their resistance to many intracanal medicaments, and 20% Miswak extract was used against these microorganisms to evaluate its antibacterial and antifungal properties. The effect of the Miswak extract against these microorganisms was evaluated in different contact times, namely, one, 6, and 24 hours to allow collection of the data, statistical analysis, and comparison. The results of the study showed that 20% Miswak extract was effective against the *C. albicans* at all tested times, which were confirmed in the findings of Al-Bayati and Sulaiman²⁶ who reported that Miswak extract revealed antifungal activity against *C. albicans*. However, 20% Miswak extract was ineffective in inhibiting the growth of *E. faecalis*, and mixture of *E. faecalis* and *C. albicans* after one hour of exposure, but was completely effective

in inhibiting their growth after 6 and 24 hours of exposure, which implies that short contact time of the tested solutions is not enough to inhibit growth of the bacteria and would require longer contact time of at least 6 hours. These findings are not documented in other studies.

In conclusion, the results of this study show that 20% Miswak extract can be an effective intracanal medicament due to its antifungal and antibacterial properties. Further study should be carried out using different concentrations, and different forms of Miswak extract such as gel or paste.

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Related topics

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