

Antifungal Efficacy of Rhamnus Prinoides Leaf Extract as an Intracanal Irrigant Against Candida Albicans: An in Vitro Study

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ABSTRACT

Background: *Candida albicans* is one of the most prevalent fungal pathogens associated with persistent endodontic infections and root canal failures. The search for biocompatible, plant-based intracanal irrigants with antifungal properties has gained increasing attention in dental microbiology and endodontic therapy. **Objective:** This study aimed to evaluate the antifungal efficacy of *Rhamnus prinoides* leaf extract as a natural intracanal irrigation solution against *Candida albicans*, comparing its effectiveness to conventional endodontic irrigants. **Methods:** The aqueous and ethanol extracts of *Rhamnus prinoides* leaves were prepared and tested against *Candida albicans* using agar well diffusion and minimum inhibitory concentration (MIC) assays. The antifungal activity was compared with sodium hypochlorite (NaOCl) and chlorhexidine (CHX), which are commonly used in root canal disinfection. The cytotoxicity of the extracts was assessed using MTT assay on human gingival fibroblast cells to ensure biocompatibility. **Results:** The ethanol extract of *Rhamnus prinoides* exhibited a significant zone of inhibition ($p < 0.05$) against *Candida albicans*, comparable to 2% chlorhexidine, while the aqueous extract showed moderate antifungal activity. The MIC values confirmed that higher concentrations of the extract effectively inhibited fungal growth. Cytotoxicity analysis demonstrated acceptable biocompatibility at clinically relevant concentrations. **Conclusion:** *Rhamnus prinoides* leaf extract demonstrates promising antifungal properties as an alternative intracanal irrigant against *Candida albicans*. Its natural bioactive compounds may serve as a potential adjunct in endodontic disinfection protocols, reducing reliance on synthetic irrigants while maintaining antimicrobial efficacy and biocompatibility. Further research is warranted to explore its mechanism of action, long-term stability, and clinical applications in endodontics.

Keywords: *Candida Albicans*, Plant Extracts, *Rhamnus Prinoides*, Sodium Hypochlorite

INTRODUCTION

In endodontic treatment, chemomechanical debridement primarily aims to eliminate or drastically decrease the amount of bacteria in an infected root canal. Complete success in this endeavor may be elusive due to the root canal area's complex shape and bacterial colonization of specific locations, including dentinal tubules, ramifications, and auxiliary canals [1]. Fungi are a type of microbe that can be found in infected root canals. Despite the use of suitable chemomechanical debridement, fungi may still be resistant to standard root canal therapy. It significantly contributes to the treatment failure of periapical diseased teeth [2]. *Candida albicans* is a eukaryotic commensalism fungus that settles as part of the microbiota of the human digestive tract, which includes the mouth cavity.³ The microbe transforms into a harmful state by modifying its virulence features, such as its ability to switch between blastospore and hyphal forms. This enables it to infiltrate human tissue and evade immersion by macrophages. Thigmotropism, which is the ability to adhere, enables *C. albicans* to enter the inner layers of dentinal tubules and create a biofilm within 48 hours. This biofilm is capable of surviving in various pH levels, highly alkaline environments, and extreme ecological situations, hence facilitating persistent infection [3, 4]. The efficacy of endodontic treatment is heavily reliant on the complete eradication of germs from the root canal system. Therefore, it is crucial to utilize chemical irrigating solutions during root canal preparation that include sufficient antibacterial and antifungal

qualities [4]. Sodium hypochlorite (NaOCl) is a commonly used solution in endodontics for irrigating root canals. It is renowned for its capacity to break down organic tissues during the process of chemomechanical root canal cleaning [5]. Conversely, its ability to harm critical tissue is an unintended consequence known as cytotoxicity [6]. In order to overcome the limits of the currently available endodontic irrigant, it is imperative to develop an irrigant that possesses a wide range of antimicrobial properties, a rapid disinfection time, and excellent biocompatibility [7]. Herbal products are gaining popularity in dental and medical practice because they are biocompatible, have enhanced antibacterial activity, and possess antioxidant and anti-inflammatory properties [8]. The World Health Organization (WHO) defines a herbal medication as a synthetic substance or prepared substance containing medicinally active components derived from plants [9]. Conventional endodontic irrigants have side effects, and intracanal pathogens are becoming more resistant to these materials. Antimicrobial and anti-inflammatory chemicals were sought after in the medical plant due to these reasons. One of these medicinal plants is *Rhamnus prinoides*, which belongs to the *Rhamnaceae* family and may include polyphenols, terpenoids, tannins, alkaloids, saponins, and flavonoids in its methanol fraction. The anti-inflammatory, antibacterial, and antioxidant effects of this plant depend on these metabolites [10]. *Rhamnus prinoides* has been the subject of investigations exploring its

diverse ethnomedicinal applications. The various components of this substance are utilized in the management of gonorrhea, brucellosis, malaria, and infections of the ear, nose, and throat. It is also employed in the treatment of tonsillitis [11-13]. Research into the effectiveness of Rhamnus prinoidea alcoholic extract as an intracanal disinfectant against *Candida albicans* in permanent tooth root canals was the driving force behind the current investigation.

MATERIAL AND METHODS

The research proposal was approved by the Research Ethics Committee/College of Dentistry/University of Baghdad according to a decision report (Ref. number: 727 in 28-12-2022). This approval contained application form and check list, study protocol and patient information sheet and consent form (Arabic and English version).

Teeth Selection and Preparation

A total of thirty permanent teeth with a single root were collected from adolescent individuals under the age of 16 for the purpose of orthodontic extraction. This was done after getting a signed informed consent (containing all the patient's rights and the purpose of the study) from the children's parents/ guardians. The study eliminated teeth that had fractures, internal or external resorption, or calcification. Periodontal curettes were utilized to delicately eliminate soft tissue, plaque, and bone from the root surfaces. Subsequently, the teeth were sterilized using a 5.25% sodium hypochlorite solution for a duration of sixty minutes, followed by storage in a 0.2% thymol crystal solution [14]. The cemento-enamel junction (CEJ) was used for crown dissection, and the root lengths of the teeth were reduced to 14 mm. After verifying the distance from the apical foramen—one millimeter—the K-file confirmed the working length of each tooth, which was then subtracted by one millimeter [15]. The canals were prepared using Pro-Taper rotary files up to the master apical file, F3. A 5 ml syringe and endodontic needle were used to irrigate the canals with 5.25% NaOCl in between each use of the rotary files. After the initial preparation, a 30-gauge double-sided vented endodontic needle was used to irrigate each canal for three minutes with 1 ml of 17% EDTA, 5 ml of 0.9% normal saline solution, and 1 ml of NaOCl. In the end, each canal was sprayed with five milliliters of saline solution. After the biomechanical instrumentation was finished, composite restorative fillings were used to close the apical foramen. The root surfaces were then given two coats of fingernail polish [16]. After that, the teeth were autoclaved in screw-cup glasses at 121°C with 15 IB for thirty minutes [17].

The Plant Material Extraction

The Biology Science Department of Iraq's University of Diyala conducted the plant identification and extraction. Using an electrical grinder, fifty grams of clean and dry *Rhamnus prinoidea* leaves were powdered and added to 90% methanol in a thimble at 60°C for 6 hours of extraction (Soxhlet extractor method). Then the extract was incubated with 70% methanol for two days. Following that, Whatman No. 1 filter paper was used to filter the extract. After filtering the methanol extract with filter paper once more, it was treated with 1% HCl. Then, diethyl ether was poured to the funnel and permitted to sit for 24 hours. After removing the

diethyl ether layer, the mixture was split into two layers: one containing the remaining diethyl ether, which was collected and brought to a pH of 8 [18]. Then, a lyophilizer (Operan, Korea Vacuum Limited, Korea) was used to dry the extract from alcohol and to get the extracted powder, which was applied in a container where it was mixed with deionized water and vibrated in a water bath for 15 minutes to get the extract irrigation solution.

Isolation of *Candida Albicans*

A signed informed consent was taken from the children's parents before taking multiple samples from inside the root canal of their children (aged 7-14 years) who needed root canal treatment. *C. albicans* was identified using colony morphology and a Germ Tube Test, which is specific for isolating *C. albicans* [19].

Antimicrobial Activity

An agar well diffusion method was used to assess the antimicrobial properties of Rhamnus prinoidea leaves extract against several pathogens [20]. Aseptically, 25 milliliters of Muller-Hinton (MH) agar was transferred to sterile petri plates. A sterile wire loop was used to remove the microbial species from their stock cultures [21]. Once the organisms had been cultured, a sterile point was used to bore five 6-mm-diameter wells into agar plates. Each of the five wells contained a different concentration of the extract: (0.062, 0.125, 0.25, and 0.5 mg/ml). The control well contained deionized water. We measured and recorded the average zone of inhibition diameter after incubating the plates containing the extract and the organisms under test overnight at 37°C [22]. (Fig.1).

Minimum Inhibition Concentration (MIC) of the Rhamnus Prinoidea

Mueller-Hinton broth was used for micro-broth dilution assays to determine the plant extract's MIC against *C. albicans*. The extract's concentrations were: (0.0156, 0.0312, 0.0625, 0.125, 0.250 and 0.5 mg/ml). After aerobic incubation for 24 hours at 37°C, the lowest concentration of plant extract that showed neither visible microbial growth nor turbidity in the micro-broth dilution assay was considered as MIC [23]. (Fig.2).

Minimum Bactericidal Concentration (MBC) of the Rhamnus Prinoidea

In order to acquire the MBC of the plant extracts, 100 µL of each well's culture was subcultured on MHA plates at 37°C for 24 hours following the micro-broth experiment, which was considered as the lowest concentration of the extracts that reduces the viability of the initial microbial inoculum by 99% [24].

Samples Inoculation

Candida albicans colonies were cultivated for 4 hours in 5 ml of Brain/Heart Infusion Broth (BHI) at 37°C. A turbidity of 0.5 was achieved by applying the McFarland standard [25]. The microbial suspension was then injected into the mechanically expanded root canal lumen using a sterile micropipette.

After that, the teeth were placed in brain heart infusion broth and cultured at 37°C for two weeks to allow a well-known and significant microbial colony to form [26]. The culture medium was replaced every alternate day to avoid nutrient depletion and accumulation of toxic end products [27].

Effectiveness of Irrigants as Antimicrobials

After the incubation period, each tooth sample was delicately taken out of the tubes. After a minute of entering and removing a sterile #25 paper point from the root canals, a pre-irrigating sample (S1) was collected [28]. The teeth were then randomly assigned to one of three groups, each consisting of ten teeth: the control group received 5 ml of 0.9% normal saline as irrigation, the second group received 5 ml of 2.5% NaOCl, and the third group received 5 ml of *Rhamnus prinoides* leaves extract at a concentration of 0.5 mg/ml. Every sample was irrigated for 5 minutes. Endodontic needles and sterile 5 ml plastic syringes were used to inject each solution into the canal lumen. All irrigation operations were performed by the same operator in a sterile environment at room temperature [7]. After that, samples taken after irrigation were collected using sterile paper points (S2). Each sample, S1 and S2, was vortexed for 30 seconds in an Eppendorf tube with 1 ml of normal saline [28]. A subsequent diluting step was carried out. Plates of Sabouraud agar were cultivated for 24 hours at 37°C after each dilution was pipetted. A value of CFU/ml

was determined by counting the number of Colony Forming Units (CFU).

Statistical Analysis

Data in this study were analyzed using the GraphPad Prism software (GraphPad Software, San Diego, CA, USA). To test normality distribution of collected numerical data, Shapiro-Wilk test was used. Paired t test was used to compare the mean in Pre and post irrigation for each group, and One-Way ANOVA, Dunnett's T3 post hoc test were used to compare the mean reduction between groups. The significance level was set at $p < 0.05$.

Results

The maximum growth inhibition zone of *Rhamnus prinoides* leaves extract against *C. albicans* in the agar diffusion test was noticed at 0.5 mg/ml (19 mm), while the minimum inhibition zone was at 0.062 mg/ml (15 mm), as shown in Figure 1. The MIC value of *Rhamnus prinoides* leaves extract against *C. albicans* was 0.25 mg/ml (Figure 2), while the MBC was 0.5 mg/ml.

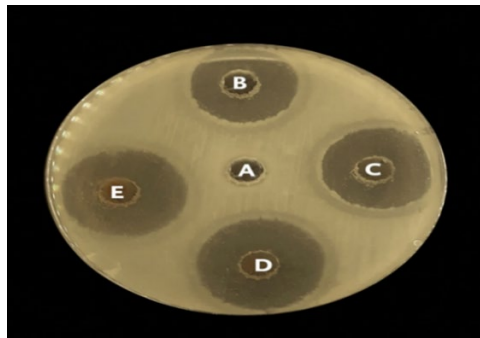


FIGURE 1: Antimicrobial Activity of *Rhamnus prinoides* against *Candida albicans*. A (deionized water): 6mm; B (0.062 mg/ml): 15mm; C (0.125 mg/ml): 16mm; D (0.25 mg/ml): 18mm; E (0.5 mg/ml): 19mm.

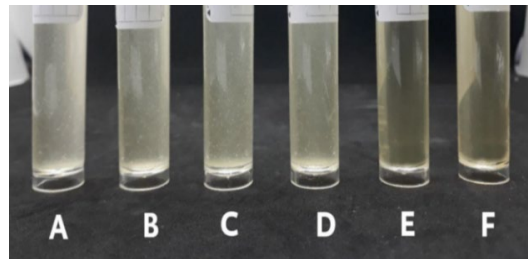


FIGURE 2: Minimum Inhibition Concentration (MIC) of *Rhamnus prinoides* leaves extract against *Candida albicans* by broth dilution: (A) 0.0156 mg/ml (+); (B) 0.0312 mg/ml (+); (C) 0.0625 mg/ml (+); (D) 0.125 mg/ml (+); (E) 0.25 mg/ml (-); (F) 0.5 mg/ml (-).

All three irrigation solutions used in this study significantly decreased the mean value of *C. albicans* (CFU), as shown in Table 1. The NaOCl group exhibited the largest effect size, followed closely by the *Rhamnus prinoides* leaf extract group, while the normal saline

group demonstrated the least impact size. The groups treated with normal saline, NaOCl, and *Rhamnus prinoides* leaf extract differed significantly from one another, whereas the ones treated with the latter two did not. (Table 2).

TABLE 1: Descriptive and Statistical Test of Candida Albicans (CFU $\times 10^6$) among groups.

		Pre	Post	P value	Efficiency (Mean $\pm$ SD)
Normal Saline	Mean	3372.50	2677.20	0.01*	16.51 $\pm$ 8.03
	$\pm$ SD	2825.03	2178.19		
2.5% NaOCl	Mean	3367.60	98.20	0.00*	93.82 $\pm$ 5.01
	$\pm$ SD	2821.16	52.60		
Rhamnus Prinoides	Mean	3373.10	211.60	0.00*	89.86 $\pm$ 5.63
	$\pm$ SD	2824.99	152.71		
F		0.00	13.36		
P value		1.00 NS	0.00**		

*Significantly different resulting from paired t-test

** Significantly differences resulting from one-way ANOVA test and post hoc Tukey's tests

Table 2: Multiple Comparisons of *Candida Albicans* Post Irrigation Using Dennett's T3

Groups	Mean Difference	P Value
Normal Saline	2579.00	0.013*
2.5% NaOCl	2465.60	0.017*
<i>Rhamnus prinoidea</i>	-113.40	0.130

* Significant difference $p < 0.05$

Discussion

The use of herbs is gaining popularity in dentistry and medical practices for the treatment of many conditions [29]. The phrase "phytotherapy, ethnopharmacology, or phytomedicine" refers to the use of herbs to cure a variety of illnesses [30]. Herbal medicine has seen remarkable expansion in recent years. The benefits of using herbal alternatives include simplicity of access, longer shelf life, affordability, minimum toxicities, and the lack of resistant microbes observed so far [31]. Due to their biocompatibility, greater anti-inflammatory, antimicrobial properties and antioxidant effects, herbal alternatives to standard root canal therapy are becoming more popular [30, 31]. Traditionally, NaOCl was the most often used irrigant for endodontic therapy because it possesses antimicrobial properties and the capacity for disintegrating tissues [32]. Various adverse effects of NaOCl include its cytotoxicity when extrusion outside the tooth apex, foul odor and taste, ability to bleach clothing, dis-acceptance by the child, powerful metallic corrosion, harm to oral mucosa [33]. Due to the adverse effects of most traditional synthetic intracanal medications and irrigant, there has been a rise in research into herbal irrigants [34]. There are a number of herbal remedies with antimicrobial properties; one of these is *Rhamnus prinoidea* leaf extract. This effect might be due to the synergistic or additive effects of certain plant compounds [35]. *Rhamnus prinoidea* also includes tannins, which are antimicrobial and antioxidant, and triterpenoids, which have anti-inflammatory and antibacterial effects, according to previous research [36]. Meanwhile, certain compounds found in *Rhamnus prinoidea* leaf extract may have a destructive effect on the microbial cell membrane or on the enzymes required for microbial development [37]. The current investigation on *Rhamnus prinoidea* leaf extract against *C. albicans* indicated that the MIC was 0.25 mg/ml and the

MBC was 0.5 mg/ml. The MBC of the leaf extract of *Rhamnus prinoidea* employed in this investigation was considerably different from the LC50 value used in a previous study, which established the cytotoxic concentration at 5000 µg/ml, in terms of cytocompatibility [18]. In this study, *Rhamnus prinoidea* leaf extract showed comparable antifungal effects against *C. albicans* to NaOCl as an intracanal irrigant solution. This finding is consistent with earlier studies indicating that demonstrated *Rhamnus prinoidea* leaf extract has high anti-inflammatory and antioxidant properties [35-37]. This conclusion must be considered in light of the fact that *Rhamnus prinoidea* leaf extract was tested against a single microbe, as opposed to actual clinical situations where polymicrobial endodontic infection exists in the root canal system. Additionally, irrigation methods that work in vitro may not be successful in vivo against the same microorganism. Furthermore, more laboratory and clinical experiments are required to assess biocompatibility, cytotoxicity, and safety before *Rhamnus prinoidea* leaf extract can be definitively suggested as an intracanal irrigating solution.

Conclusion

The efficacy of *Rhamnus prinoidea* leaf extract as an irrigant against *C. albicans* was comparable to that of 2.5% NaOCl. Additional in vitro and in vivo studies are required to validate the efficacy of *Rhamnus prinoidea* extract as an irrigant solution against various microbiological species.

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