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Nahla Ayoub (✉ naayoub@uqu.edu.sa)

Umm al-Qura University

Nadia Badr

Umm al-Qura University

Saeed Alghamdi

Umm al-Qura University

Arwa Alzahrani

Umm al-Qura University

Rahaf Alsulimani

Umm al-Qura University

Afnan Nassar

Umm al-Qura University

Rawabi Qadi

Umm al-Qura University

Noha Swilam

British University in Egypt

Ibtesam Afifi

Tanta University

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The Effectiveness of *Salavadora persica* (Siwak) Petroleum Ether Extract as an Intracanal Medicament used in Endodontic Therapy: An *in vitro* Study

Nahla Ayoub^{1*†}, Nadia Badr², Saeed Alghamdi¹, Arwa Alzahrani^{3*†}, Rahaf Alsulimani^{3*†}, Afnan Nassar⁴, Rawabi Qadi¹, Noha Swilam^{5‡}, Ibtesam Afifi^{6,7‡}

¹ Department of Pharmacology and Toxicology, Faculty of Medicine, Umm Al-Qura University (UQU), KSA.

² Department of Dental Biomaterials, Faculty of Dentistry, Umm Al-Qura University (UQU), KSA.

³ Dental students, Faculty of Dentistry, Umm Al-Qura University (UQU), KSA.

⁴ Department of Public Health, Faculty of Dentistry, Umm Al-Qura University (UQU), KSA.

⁵ Department of Pharmacognosy, Faculty of Pharmacy, British University in Egypt.

⁶ Department of Medical Microbiology and Immunology, Faculty of Dentistry, Umm Al-Qura University (UQU), KSA.

⁷ Department of Medical Microbiology and Immunology, Faculty of Dentistry, Tanta University, Egypt.

[†] These authors contributed equally to this work: Arwa Alzahrani^{3*†}, Rahaf Alsulimani^{3*†}

[‡] Noha Swilam^{5‡}, Ibtesam Afifi^{6,7‡}, also contributed equally to this work

* naayoub@uqu.edu.sa

27 * Corresponding author

28 Prof. Nahla Ayoub

29 Department of Pharmacology and Toxicology, Faculty of Medicine, Umm Al-Qura

30 University (UQU), KSA.

31 Abidiyyah, Makkah, PO Box 715

32 Phone: +966 (2) 550 1000

33 Email: naayoub@uqu.edu.sa

Abstract

Background: *Salvadora persica* L. (Siwak) of the family Salvadoraceae has been used for many centuries as oral hygiene tools, particularly in Saudi Arabia. The current study aimed at developing a new root canal medicament based on the extract of *Salvadora persica* (SP). The antibacterial activity against *Enterococcus faecalis* was determined compared with the gold standard intracanal medicament, Ca(OH)₂. **Materials and Methods:** Freeze-dried Siwak sticks were extracted with Petroleum ether to yield an oily extract (SPE). GC-MS analysis was carried out to identify SPE components, whereas 32 compounds were identified (89.09%), with main components of benzyl isothiocyanate (BITC) (33.32%) and steroids (34%). Firstly, the consistency of the experimental medicament was accomplished according to ANSI/ADA Specification no 57. SPE assessment as intracanal medicament was conducted through an *in vitro* assay on 45 single-rooted mandibular premolars inoculated of 5 µL of *E. faecalis*. Colony-forming units (CFU) were counted before the application of experimental intracanal medicament (CFU-1) and then after seven days of its application (CFU-2). Group I: Siwak extract-based intracanal medicament, Group II positive control group received Ca(OH)₂ paste, and Group III: negative control group received no medication. **Results:** CFU before and after using Siwak based medicament recorded statistically significant reduction ($P=0.006$) as well as those of Group II when Ca(OH)₂ was applied ($P=0.01$). On comparing both medicaments with Group III, there were significant differences; ($P=0.00$) and ($P=0.007$), respectively. Siwak oil extract-based medication proved complete eradication of *E. faecalis*. **Conclusion:** SPE has a good potential as an intracanal medicament, besides the cleaning and shaping during endodontic therapy. This finding could be referred to as the high content of BITC bactericidal against pulpal

59 and periodontal pathogens, in synergism with other antimicrobial components,
60 representing 70.71% of SP oil extract.

61

62 **Keywords:** *Salavadora persica* L., GC/MS analysis, antimicrobial activity,
63 *Enterococcus faecalis*, root canal treatment, intracanal medicament.

Introduction

The ultimate goal of an infected root canal preparation, including cleaning, shaping, and using the disinfectant solution, is to entirely remove bacteria, their byproduct, and pulpal remnants. The microorganisms in the root canal of the teeth are principally responsible for pulpal/periapical diseases. These microorganisms can survive and cause reinfection in the root canal with complex anatomy [1]. The pathological bacteria of root canals include (*Streptococcus sanguinis*, *Streptococcus mutans*, *Enterococcus faecalis*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, and *Prevotella intermedia*) [2]; however, the most causative species is *Enterococcus faecalis* [3, 4]. During multiple endodontic treatment visits, the intracanal medication is used to eradicate bacteria in adults and children's root canal systems. Accordingly, the pain and inflammation of pulpal and periapical tissues are reduced. This represents an optimal root canal disinfection protocol that guarantees a high success rate of root canal treatment. [5, 6]. The most commonly used intracanal medicament is calcium hydroxide Ca(OH)_2 because of its significant bactericidal effect and inhibition of inflammatory exudates [7].

There has been prosperity in phytotherapy and naturopathic medicine to treat the diseases due to their established properties of biocompatibility and availability [8]. *Salvadora persica* L. (Siwak, SP) is a plant whose roots, twigs, or stems have been used for many centuries as oral hygiene tools, particularly in Saudi Arabia. Using Siwak (tooth stick) for cleaning of mouth is advocated Islamic well-established beliefs. Recently, SPE proved an evidenced biocompatibility and antibacterial effect after histopathological and immunohistochemical investigation [9-12].

Many studies have been demonstrated that extracts of SP possess various antiplaque, antiperiopathic, anticaries, anti-inflammatory, and antimycotic effects [13, 14].

Extracts of SP also significantly affect intracanal irrigant both *in vitro* and *in vivo* [11, 15] because of its inhibitory effect on oral pathogen growth [16, 17]. According to the available literature, the target of root canal treatment (RCT) is to eradicate bacteria in the root canal system; the present study was designed as randomized control *in vitro* study to assess the effect of an experimental intracanal medicament based on SPE on the most causative bacteria for endodontic pulpitis/periodontitis; *Enterococcus faecalis*. The gold standard intracanal medicament Ca(OH)₂ was employed as control.

Materials and method

Materials:

The materials used in this study were *Salvadora persica* (Siwak, SP) sticks brought from Riyadh region in Saudi Arabia, Zinc oxide powder (Prevest DentPro Limited, unit II, Bari Brahmana, Jammus-181133, India), and Ca(OH)₂ endodontic medicament (MetaPaste) (META BIOMED CO.LTD., Korea)

The sticks of cultivated *Salvadora persica* SP (namely Miswak, Siwak or Arak) plant were collected from Riyadh region in Saudi Arabia. The plant was identified by Dr. Talal Dahan [18] and a voucher specimen (# 19750) was deposited in properly labeled polythene bags for future reference at the Herbarium Centre, College of Pharmacy, UQU, Saudi Arabia. The authors followed IUCN Policy Statement on Research Involving Species at Risk of Extinction.

Methods:

Preparation of SP petroleum ether extract (SPE)

The fresh plant sticks were freeze-dried and then ground to a fine powder using a commercially available food blender. The ground sample (500 g) was used to prepare

the extract. Consequently, petroleum ether extract was prepared by cold percolating 500 g of dried powder of the plant sticks in one L of petroleum ether for 72 h, and every 24 h fresh solvent was used. The solvent was removed and recovered in a rotary evaporator (BÜCHI Rotavapor RII, Büchi Labortechnik; Flawil, Switzerland) at 40 °C using a BÜCHI vacuum pump. At the last stage, the oily extract was freeze-dried to ensure that solvents were removed to yield 20 mL of an oily material (SP oil extract) with a powerful aromatic odor. SP oil extract was kept in a brown screw-capped tube in a -20 °C freezer until further analysis.

GC/MS Analysis

Mass spectra were recorded using Shimadzu GCMS-QP2010 (Kyoto, Japan) equipped with Rtx-5MS fused bonded column (30 m x 0.25 mm i.d. x 0.25 µm film thickness) (Restek, USA) and a split–splitless injector. The initial column temperature was kept at 50 °C for 3 min (isothermal) and programmed to 300 °C at a rate of 5 °C/min, and kept constant at 300 °C for 10 min (isothermal). Injector temperature was 280 °C. Helium carrier gas flow rate was 1.37 mL/min. All the mass spectra were recorded applying the following condition: (equipment current) filament emission current, 60 mA; ionization voltage, 70 eV; ion source, 220 °C. Diluted samples (1% v/v) were injected with split mode (split ratio) 1: 15.

Compounds identification

The identification of compounds was performed based on their retention indices relative to a homologous series of n-alkanes (C8-C28) injected under the same conditions and matching their mass spectra with National Institute of Standards and Technology (NIST) and Wiley library database as well as literature [19-23]

Collection of extracted teeth

The sample size for antimicrobial activity test was calculated based on data from an internal pilot study using G*Power version 3.1.9.2 for sample size analysis at $\alpha=0.05$ and 80% power which yielded a sample size of 15 samples/group. Forty-five single root premolars extracted teeth for periodontal or orthodontic reasons were obtained from the department of oral surgery at a different governmental and private hospital in Makkah city, Saudi Arabia. Teeth with previous endodontic therapy, fracture, crack, resorption, and root caries were excluded from the study. The present study followed the ethical guidelines for clinical investigation: ethical policy of the American Dental Association regarding the use of human subjects in clinical research and approved by the Institutional Review Board (IRB), Faculty of Dentistry, Umm Al-Qura University, KSA, (IRB # 122-19).

Preparation of the experimental Siwak-based extract endodontic medicament

Determination of consistency:

The first intended procedure was to determine the appropriate Powder/Liquid mixing ratio (Zinc oxide powder/ Siwak extract) for the experimental endodontic medicament. The purpose was to obtain a smooth and homogeneous material whose consistency is acceptable for clinical application. The consistency was comparable to the endodontic medicament used as a control, commercial calcium hydroxide paste.

Siwak oil extract was mixed with the zinc-oxide powder at different ratios till reaching the appropriate consistency compared to the control endodontic medicament (MetaPaste) using the one-inch string method. [24]. Some drops of the extract were placed on a clean glass slab, and the powder was added gradually using a clean stainless spatula. Various amounts of the extract and zinc oxide powder were mixed till obtaining the proper consistency. The paste was creamy in consistency but quite

heavy and stringed out at least an inch (2.5 mm) when the spatula is lifted from the mix. The adequate consistency was chosen, and its powder and liquid quantities were determined. It was found that one scoop of zinc oxide powder mixed with one drop of the Siwak oil extract resulted in the required consistency.

Preparation of extracted tooth

All 45 single-rooted premolars were decoronated to standardize their root length. The working length was established to be 14-16 mm and the roots were undergone rotary standardized instrumentation up to X3-ProTaper Next (Dentsply-Maillefer, Switzerland). An irrigant 2.5% NaOCl was used after each instrument and finally dried with size 25 absorbent paper points. Each root was placed in a closed test tube containing 3 mL of brain heart infusion (BHI) broth (SPML - Saudi Prepared Media Laboratory Co. Ltd.) and sterilized by autoclaving at 121°C for 20 min then incubated for 24 h at 37 °C to confirm sterility by absence of turbidity.

Preparation of *Enterococcus faecalis* suspension

Enterococcus faecalis ATCC 29212 strain was grown on Bile Esculin agar media (SPML - Saudi Prepared Media Laboratory Co. Ltd.) at 37 °C for 24 h. After growth, bacterial suspension was prepared from grown bacterial colonies by inoculation in Brain Heart Infusion (BHI) broth and adjusted to the optical density of approximately 1.5×10^8 CFU/mL by comparing its turbidity to a 0.5 McFarland standard spectrophotometrically.

Contamination of root canals:

Two mL of sterile BHI broth was removed from each root containing tube and replaced by 2 mL of the prepared bacterial suspension. The tubes were then closed and incubated at 37 °C for 48 h.

Colony forming units one (CFU-1)

After 48 h predetermined contamination period of root canals was elapsed, each root was removed from the tube under complete aseptic precautions and irrigated with sterile saline (100 µL). Later, a dry sterile absorbent paper point size 25 was inserted into the root canal and left for 5 min. Afterwards, these paper points were transferred individually to sterile test tubes, each containing 1 mL of sterile saline solution, vortexed for 30 sec, and then four serial dilutions were made for each tube. Aliquots of 10 µL of each dilution were plated onto Bile Esculin (BE) agar plates and incubated at 37 °C for 24 h. The grown bacterial colonies were counted and multiplied by their dilution factor to represent CFU-1 / mL.

Ex vivo testing of antimicrobial activity of the experimental medicaments

The 45 decoronated teeth were randomly allocated into 3 groups by simple random sampling using random digit table depending on medicament used:
Group I (n=16): Experimental Siwak extract based- medicament.
Group II (n=16): Ca(OH)₂ paste (positive control).

Group III (n=13): sterile physiological saline as negative control group.

Medicaments were placed into root canals with lentulo spiral inserted to the full working length. The root canal foramina were sealed by sterile cotton pellets and the orifices closed by temporary restoration; then, the samples were kept in an incubator at 37 °C for seven days.

Colony forming units-2 (CFU-2)

Seven days later, each root was irrigated with sterile normal saline then the previous technique was repeated to obtain CFU-2.

Statistical analysis:

Data were collected, tabulated, and statistically analyzed using Statistical Package for Social Science (SPSS v.20). $P < 0.05$ was considered as a level of significance. The Mean and standard deviation were tabulated and statistically analyzed using one-way ANOVA test regarding the CFU 1 of the three tested groups before using intracanal medication. Regarding the CFU- 2 of three groups, the mean and standard deviation were analyzed using One-Way non-parametric ANOVA (Kruskal–Wallis). Finally, the antimicrobial effect of different tested medications on *Enterococcus faecalis* was tabulated and statistically analyzed using Paired t-test.

Results:

Chemical composition of SP oil extract

Analysis of the SP oil extract by GC and GC/MS revealed that SP oil extract contained 32 compounds, of which benzyl isothiocyanate (33.32%), γ -sitosterol (25.76%), stigmasterol (5.92%), β -sitosterol acetate (2.28%), *n*-hexadecanoic acid (4.27%) and (Z)-11-octadecenoic acid (3.16%) were found to be the major constituents. Compounds are listed in order of their elution times on Rtx-5MS column; Table 1.

***Salvadora persica* oil extract endodontic therapy**

The statistical analysis of the CFU-1 of three tested groups before using intracanal medication was tabulated in Table 2. It was evident that there were no significant differences in bacterial counts among them ($P=0.359$). Table 3 and Fig.1 show the statistical analysis using Paired t-test of the number of the colony forming units of *Enterococcus faecalis* before and after applying the investigated medicaments at the root canal lumens; CFU-1 and CFU-2, respectively. The results showed a significant difference between CFU-1 and CFU-2 either in using experimental medicaments

Siwak oil-based medicament ($P=0.006$) or Ca(OH)_2 medicament ($P=0.011$). On the other hand, the bacterial count of negative control group showed a nonsignificant difference between CFU-1 and CFU-2 ($P=0.438$). Finally, the bacterial count's significant difference among the three tested groups was compared using one-way non-parametric ANOVA; Table 4. The values were insignificant difference between bacterial count after using the experimental SPE and Ca(OH)_2 medicaments ($P=0.210$). On the contrary, comparing both medicaments with negative control saline group, resulted in significant differences; ($P=0.00$) and ($P=0.007$), respectively.

Discussion

The chemomechanical cleaning and shaping of the root canals of the teeth are essential for the success of endodontic therapy [25, 26]. This combination of mechanical preparation with disinfection is effective in reducing bacterial counts. Yet, intracanal medication is a crucial procedure during root canal treatment [27].

In fact, the intention of root canal treatment is to eradicate bacteria and their by-products from the root canal system [28]. It is well documented that the use of biocompatible intracanal medications possessing antimicrobial properties between appointments destroy and eradicate bacteria in the root canal system. Consequently, the probability of success of root canal treatment would be significantly intensified [29]. It was proven that *Enterococcus faecalis* is the most predominant bacteria found in the infected root canal. Unfortunately, this species has the ability to survive in hard environment and implies a challenge to be completely exterminated from root canal system [30].

Calcium hydroxide is the gold standard intracanal medication used in the treatment of infected root canals, referring to its well-known antibacterial features and ease of

application [31]. Therefore, the present study compared the antimicrobial effect of the experimental intracanal medicament based on SPE to that of Ca(OH)_2 . The effect of both medicaments against *Enterococcus faecalis* was considered in the presence of a negative control group, saline, to specify the antimicrobial effect efficiently. In reality, Ca(OH)_2 is the most commonly used intracanal medicament and represents the first choice by dentists; Athanassiadis *et al* , Asnaashari, M *et al* [31, 32]. However, the antimicrobial effect of the experimental medicament SPE insignificantly surpasses that of Ca(OH)_2 ($P = 0.210$); Table 3. In the present investigation, SPE experimental intracanal medicament; (Group I) proved an effective eradication of *Enterococcus faecalis*. This finding was in agreement with the results of Al Qarni *et al.*, in 2019, who reported the usefulness of Siwak extract as endodontic irrigant against *E. faecalis* [33]. As well, it was evidenced that *Salvadora persica* has an inhibitory effect on the growth of the oral pathogen [34].

In this study, the chemical profile representing 32 compounds of *Salvadora persica* petroleum ether extract were analyzed, identified by GC/MS (89.09%) are listed in Table 1. The main component was found to be benzyl isothiocyanate (BITC) (33.32%). This component was reported as the most robust antibacterial component with high bactericidal activity against Gram-negative periodontal pathogens [35]. The potent antibacterial effect of the experimental medicament; Table 3, suggested that SPE targeted the bacterial membrane, whereas BITC has both lipophilic and electrophilic properties. It is reported that BITC penetrates through the outer bacterial membrane and possibly interfered with the bacterial redox systems, thus hampering the bacterium's ability to maintain its membrane potential. Such an effect of BITC has been demonstrated for mitochondrial membranes [36].

In addition, there are γ -sitosterol (25.76%), *n*-hexadecanoic acid (4.27%) and (Z)-11-octadecenoic acid (3.16%) are reported to as potent broad spectrum antimicrobial [37-41]. Moreover, it has been demonstrated that the SP extracts have antimicrobial effect and inhibited both Gram-positive and Gram-negative bacteria because the presence of carvacrol (0.57%) which is confirmed by Memar, M.Y., *et al*, 2017 [42]. Siwak extract components Table 1 showed the presence of *n*-tetradecane (0.21%), *n*-hexadecane (0.13%), 11-octadecenoic acid (3.16%), squalene (0.17%), stigmasterol (0.12%), sitosterol (2.28%) and sitostenone (0.54%). These components are potent antibacterial agents [43-49]. Therefore, Siwak petroleum ether extract's strong bactericidal effect is attributed to the antimicrobial compounds representing a percentage of 70.71% of the extract. It is worthy to mention that some components with anti-inflammatory and antioxidant effect are detected such as oleic acid (1.95%), 11-octadecenoic acid (3.16%), 2,2'-methylenebis (6-*tert*-butyl-4-methylphenol) (0.13%), squalene (0.17%), *n*-benzyloleamide (0.53%), *n*-benzylpalmitamide (0.36%) stigmasterol-5, 22-dien-3-ol (0.65%) lupeol (1.24%) and hexadecanoic acid, ethyl ester (0.13%) [45- 47] [50-55]. Besides, the use of zinc oxide powder might also contribute to the inhibitory effect of several oral pathogens[56]. Zinc as a metal has been used as bactericidal and bacteriostatic agents [57].

Conclusion

Referring to the present investigation findings, the authors report that a novel intracanal medicament based on *Salvadora persica* petroleum ether extract can be used efficaciously in endodontic therapy. Besides the regular cleaning and shaping procedures, this experimental endodontic medicament can eradicate the bacteria and their byproducts exclusively from the root canal system owing to its proficient antibacterial action.

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Author contributions

A.A., R.A., R.Q. A.N, N.S. and I.A. methodology, validation, wrote the main manuscript, N.A., N.B., and S.A. designed the study, prepared the tables and figures, revised the manuscript.

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Competing interests: The authors declare no competing interests

Data availability: The datasets analysed during the current study are available from the corresponding author on reasonable request.

Figure 1: A histogram representing CFU/mL*10⁴ before and after application of intracanal medicaments

326 Table (1): The chemical profile of *Salvadora persica* petroleum ether extract

Peak #	R _t	Component	Molecular formula	RI _{exp} ^a	RI _{lit} ^b	Content (%)	Identification ^c
1.	18.35	Carvacrol	C ₁₀ H ₁₄ O	1288	1288	0.57	MS, KI
2.	20.47	Benzyl Isothiocyanate	C ₈ H ₇ NS	1361	1359	33.32	MS, KI
3.	21.14	<i>n</i> -Tetradecane	C ₁₄ H ₃₀	1384	1400	0.21	MS, KI
4.	23.76	<i>n</i> -Pentadecane	C ₁₅ H ₃₂	1485	1500	0.38	MS, KI
5.	26.24	<i>n</i> -Hexadecane	C ₁₆ H ₃₄	1582	1600	0.13	MS, KI
6.	34.40	<i>n</i> -Hexadecanoic acid (Palmitic acid)	C ₁₆ H ₃₂ O ₂	1948	1946	4.27	MS, KI
7.	34.91	Hexadecanoic acid ethyl ester	C ₁₈ H ₃₆ O ₂	1972	1975	0.13	MS, KI
8.	36.12	Cyclic octaatomic sulphur	S ₈	2034	2055	0.99	MS, KI
9.	37.86	Oleic Acid	C ₁₈ H ₃₄ O ₂	2130	2130	1.95	MS, KI
10.	37.92	(Z)-11-Octadecenoic acid	C ₁₈ H ₃₄ O ₂	2133	2117	3.16	MS, KI
11.	38.15	(Z,Z)-9,12- Octadecadienoyl chloride (Linoleic acid chloride)	C ₁₈ H ₃₁ ClO	2146	2139	0.37	MS, KI
12.	38.24	(Z)-9-Octadecenoic acid ethyl ester (Oleic acid ethyl ester)	C ₂₀ H ₃₈ O ₂	2151	2168	0.45	MS, KI
13.	38.36	9-Octadecenoic acid ethyl ester	C ₂₀ H ₃₈ O ₂	2157	2171	0.14	MS, KI
14.	42.84	<i>p</i> -Cresol, 2,2'-methylene bis [6-tert-butyl-	C ₂₃ H ₃₂ O ₂	2403	2398	0.13	MS, KI
15.	48.95	Squalene	C ₃₀ H ₅₀	2796	2814	0.17	MS, KI
16.	49.51	N-Benzylpalmitamide	C ₂₃ H ₃₉ NO	2832	2880	0.36	MS

17.	51.09	Stigmasta-3,5-diene	C ₂₉ H ₄₈	2934	-	0.77	MS
18.	51.69	Stigmasterol acetate	C ₃₁ H ₅₀ O ₂	2972	2879	0.12	MS, KI
19.	51.87	3 β -Acetoxystigmasta-4,6,22-triene	C ₃₁ H ₄₈ O ₂	2984	-	0.24	MS
20.	52.06	<i>n</i> -Benzyloctadecenamide (<i>n</i> -Benzyloleamide)	C ₂₅ H ₄₁ NO	2996	2991	0.53	MS, KI
21.	52.17	Stigmasta-5,22-dien-3-ol, acetate	C ₃₁ H ₅₀ O ₂	3003	-	0.65	MS
22.	52.47	Clionasterol acetate	C ₃₁ H ₅₂ O ₂	3022	-	0.50	MS
23.	52.66	Stigmastan-3,5,22-triene	C ₂₉ H ₄₆	3034	2981	1.89	MS, KI
24.	52.94	β -Sitosterol acetate	C ₃₁ H ₅₂ O ₂	3052	-	2.28	MS
25.	53.28	Cholesterol	C ₂₇ H ₄₆ O	3074	3075	0.66	MS, KI
26.	54.89	Campesterol	C ₂₈ H ₄₈ O	3177	3131	0.70	MS
27.	55.40	Stigmasterol	C ₂₉ H ₄₈ O	3210	3213	5.92	MS, KI
28.	56.44	γ -Sitosterol	C ₂₉ H ₅₀ O	3277	3290	25.76	MS, KI
29.	57.47	Unidentified	-	3344	-	3.57	-
30.	58.21	3,5-Stigmastadien-7-one	C ₂₉ H ₄₆ O	3391	-	0.56	MS
31.	59.04	Sitostenone	C ₂₉ H ₄₈ O	3444	3435	0.54	MS, KI
32.	60.19	Lupeol	C ₃₀ H ₅₀ O	3518	3451	1.24	MS
% Total identified						89.09	

327

328 Compounds are listed in order of their elution times on Rtx-5MS column. ^a*RI*_{exp},
329 retention index determined experimentally relative to C8 – C28 *n*-alkanes on Rtx-
330 5MS column; ^b*RI*_{lit}, published retention indices; ^cIdentification, was based on
331 comparison of the compounds' mass spectral data (MS) and retention indices (RI)
332 with those of NIST Mass Spectral Library (2017), Wiley Registry of Mass Spectral
333 Data 8th edition and literature.

334 Table (2): The mean and standard deviation of bacterial count of the tested groups
 335 before application of the different intracanal medicament

Groups	Group I Siwak Mean± SD	Group II Ca(OH) ₂ Mean± SD	Group III Saline Mean± SD	ANOVA	
				F	P- value
CFU-1	48.9±53.7*10 ⁴	51.6±68.4*10 ⁴	56.3±59*10 ⁴	1.050	0.359

336

337 Table (3): The descriptive statistical analysis of the *Enterococcus faecalis* count
 338 before (CFU-1) and after (CFU-2) the application of the medicaments

339

Bacterial Count Groups	CFU-1 Mean $\pm$ SD	CFU-2 Mean $\pm$ SD	Paired t test
			p-value
Group I Siwak	48.9 $\pm$ 53.7*10 ⁴	0.0250 $\pm$ 0.1*10 ⁴	0.006
Group II CaOH (+ve control)	51.6 $\pm$ 68.4*10 ⁴	0.07 $\pm$ 0.25*10 ⁴	0.011
Group III Saline (-ve control)	56.3 $\pm$ 59*10 ⁴	50 $\pm$ 115*10 ⁴	0.438

340 Table (4): Comparison between three different tested groups after the application of
 341 the medicaments

Groups	Siwak VS Ca(OH)₂	Siwak VS Saline	Ca(OH)₂ VS Saline
(P-value)	0.210	0.00*	0.007*

References

1. Siqueira Jr, J.F., K.M. Magalhães, and I.N. Rôças. Bacterial reduction in infected root canals treated with 2.5% NaOCl as an irrigant and calcium hydroxide/camphorated paramonochlorophenol paste as an intracanal dressing. *Journal of endodontics*, **33**(6): p. 667-672 (2007).
<https://pubmed.ncbi.nlm.nih.gov/17509403/>
2. Tan, K.S., et al., Rapid method for the detection of root canal bacteria in endodontic therapy. *Journal of endodontics*, **41**(4): p. 447-450 (2015).
<https://europepmc.org/article/med/25576208>
3. Kakehashi Stanley HR, F.R., The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. *Oral Surg Oral Med Oral Pathol.*, ;20:340-9 (1965). <https://pubmed.ncbi.nlm.nih.gov/14342926/>
4. Rôças, I.N., J.F. Siqueira Jr, and K.R. Santos, Association of *Enterococcus faecalis* with different forms of periradicular diseases. *Journal of endodontics*, **30**(5): p. 315-320 (2004). <https://pubmed.ncbi.nlm.nih.gov/15107642/>
5. Bystrom, A., R. Claesson, and G. Sundqvist, The antibacterial effect of camphorated paramonochlorophenol, camphorated phenol and calcium hydroxide in the treatment of infected root canals. *Endodontics & dental traumatology*, **1**(5): p. 170. (1985)
<https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1600-9657.1985.tb00652.x>
6. Ramos, I.F.d.A.S., et al., Histopathological analysis of corticosteroid-antibiotic preparation and propolis paste formulation as intracanal medication

- 365 after pulpectomy: an in vivo study. *Journal of applied oral science*, **20**(1): p.
 366 50-56. (2012). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3928772/>
- 367 7. Portenier, I., *et al.*, The susceptibility of starved, stationary phase, and growing
 368 cells of *Enterococcus faecalis* to endodontic medicaments. *Journal of*
 369 *Endodontics*, **31**(5): p. 380-386. (2005).
 370 <https://pubmed.ncbi.nlm.nih.gov/15851934/>
- 371 8. Sivakumar, A., *et al.*, Herbendodontics–Phytotherapy In Endodontics: A
 372 Review. *Biomedical and Pharmacology Journal*, **11**(2): p. 1073-1082. (2018).
 373 [https://biomedpharmajournal.org/vol11no2/herbendodontics-phytotherapy-in-](https://biomedpharmajournal.org/vol11no2/herbendodontics-phytotherapy-in-endodontics-a-review/)
 374 [endodontics-a-review/](https://biomedpharmajournal.org/vol11no2/herbendodontics-phytotherapy-in-endodontics-a-review/)
- 375 9. Babay, N. and K. Almas, Effect of miswak extract on healthy human dentin:
 376 an in vitro study. *Saudi Dent J*, **11**: p. 46-52. (1999).
 377 <https://www.thejcdp.com/doi/pdf/10.5005/jcdp-3-3-27>
- 378 10. Barkhordar, R.A., *et al.*, Removal of intracanal smear by doxycycline in vitro.
 379 *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and*
 380 *Endodontology*, **84**(4): p.420-423 (1997).
 381 <https://www.sciencedirect.com/science/article/abs/pii/S1079210497900421>
- 382 11. Al-Sabawi, N.A., A. Al Sheikh Abdal, and M.Y. Taha, The antimicrobial
 383 activity of *Salvadora persica* solution (Miswak-Siwak) as root canal irrigant (a
 384 comparative study). *Univ. Sharjah J. Pure Appl. Sci*, **4**(3): p. 69-91. (2007).
 385 [https://www.ajrsp.com/en/Archive/issue-9/The-Antimicrobial-Activity-of-](https://www.ajrsp.com/en/Archive/issue-9/The-Antimicrobial-Activity-of-Salvadora-Persica-Solution-as-Root-Canal-Irrigant.pdf)
 386 [Salvadora-Persica-Solution-as-Root-Canal-Irrigant.pdf](https://www.ajrsp.com/en/Archive/issue-9/The-Antimicrobial-Activity-of-Salvadora-Persica-Solution-as-Root-Canal-Irrigant.pdf)
- 387 12. Shafaie, L.A., Moubark., R., Biocompatibility and mutagenicity of
 388 *Salvadora persica* extract and Kaempherol implanted in the subcutaneous

- connective tissue of Albino rats (Histopathological and Immunohistochemical Study). Cairo Dental Journal., (2004).
13. Darout, I.A., et al., Identification and quantification of some potentially antimicrobial anionic components in miswak extract. *Indian Journal of Pharmacology*, **32**(1): p. 11-14. (2000).
https://scholar.google.com/scholar?q=Identification+and+quantification+of+some+potentially+antimicrobial+anionic+components+in+miskwak+extract&hl=en&as_sdt=0&as_vis=1&oi=scholar
14. Almas, K., Miswak (chewing stick) and its role in oral health. *Postgraduate Dentist Middle East*, **3**(4): p. 214-18. (1993).
15. Al-Salman, T.H., M.G.A.S. Ali, and O.M. Al-Nu'aimy, The antimicrobial effect of water extraction of *Salvadora persica* (Miskwak) as a root canal irrigant. *Al-Rafidain Dental Journal*, (1): p. 33-36 (2005).
<https://www.iasj.net/iasj?func=article&aId=45508>
16. El-Latif Hesham, A. and S.A. Alrumman, Antibacterial activity of miswak *Salvadora persica* extracts against isolated and genetically identified oral cavity pathogens. *Technology and Health Care*, **24**(s2): p. S841-S848 (2016).
<https://content.iospress.com/articles/technology-and-health-care/thc1214>
17. Wali B.N., Afifi, I.K., F. Balata, and N.A. Badr, Effect of An Experimental Mouth wash extracted from Miswak (*Salvadora persica*) on Cariogenic Bacteria and Dental Plaque Formation, 2018, *International Journal of Current Research* Vol. 10 (1), pp.64070-64075.
18. The plant material was identified by Dr. Talal Dahan, University of Bisha, Saudi Arabia.

- 413 19. Adams, R.P., Identification of essential oil components by gas
414 chromatography/mass spectrometry. Vol. 456. *Allured publishing corporation*
415 *Carol Stream, IL* (2007). <http://www.juniperus.org>
- 416 20. Ayoub, I.M., et al., Volatile constituents of *Dietes bicolor* (Iridaceae) and their
417 antimicrobial activity. *Zeitschrift für Naturforschung C*, **70**(7-8): p. 217-225
418 (2015). <https://www.degruyter.com/view/journals/znc/70/7-8/article-p217.xml>
- 419 21. Elkady, W.M. and I.M. Ayoub, Chemical profiling and antiproliferative effect
420 of essential oils of two *Araucaria* species cultivated in Egypt. *Industrial Crops*
421 *and Products*, **118**: p. 188-195 (2018).
422 <https://www.sciencedirect.com/science/article/abs/pii/S0926669018302747>
- 423 22. Gad, H.A., I.M. Ayoub, and M. Wink, Phytochemical profiling and seasonal
424 variation of essential oils of three *Callistemon* species cultivated in Egypt.
425 *PloS one*, **14**(7) (2019).
426 <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0219571>
- 427 23. Labib, R.M., et al., Appraisal on the wound healing potential of *Melaleuca*
428 *alternifolia* and *Rosmarinus officinalis* L. essential oil-loaded chitosan topical
429 preparations. *PloS one*, **14**(9) (2019).
430 <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0219561>
- 431 24. Ingle, J. and L. Bakland, *Endodontics: Restoration of endodontically treated*
432 *teeth* (7th edn) BC Decker Inc Hamilton-London. 693 (2002).
- 433 25. Nair, P.R., et al., Intraradicular bacteria and fungi in root-filled, asymptomatic
434 human teeth with therapy-resistant periapical lesions: a long-term light and
435 electron microscopic follow-up study. *Journal of endodontics*, 1990. **16**(12): p.
436 580-588 (1990). <https://pubmed.ncbi.nlm.nih.gov/2094761/>

- 437 26. Mônica, C.M. and I.C. Fröner, A scanning electron microscopic evaluation of
438 different root canal irrigation regimens. *Brazilian oral research*, **20**(3): p. 235-
439 240 (2006). [https://www.scielo.br/scielo.php?script=sci_arttext&pid=S1806-
440 83242006000300010](https://www.scielo.br/scielo.php?script=sci_arttext&pid=S1806-83242006000300010)
- 441 27. Chong, B. and T.P. Ford, The role of intracanal medication in root canal
442 treatment. *International endodontic journal*, **25**(2): p. 97-106 (1992).
443 <https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1365-2591.1992.tb00743.x>
- 444 28. Peters, L., P. Wesselink, and W. Moorer, The fate and the role of bacteria left
445 in root dentinal tubules. *International endodontic journal*, **28**(2): p. 95-99
446 (1995). <https://pubmed.ncbi.nlm.nih.gov/7665208/>
- 447 29. Sathorn, C., P. Parashos, and H. Messer, Australian endodontists' perceptions
448 of single and multiple visit root canal treatment. *International endodontic
449 journal*, **42**(9): p. 811-818 (2009). <https://pubmed.ncbi.nlm.nih.gov/19619228/>
- 450 30. Bergenholtz, G., Pathogenic mechanisms in pulpal disease. *Journal of
451 endodontics*, **16**(2): p. 98-101.
452 [https://www.sciencedirect.com/science/article/abs/pii/S0099239906815712
453 \(1990\).](https://www.sciencedirect.com/science/article/abs/pii/S0099239906815712)
- 454 31. Asnaashari, M., et al., Comparison of Antibacterial Effects of Photodynamic
455 Therapy, Modified Triple Antibiotic Paste and Calcium Hydroxide on Root
456 Canals Infected with Enterococcus Faecalis: An In-Vitro Study. *Journal of
457 Lasers in Medical Sciences*, **10**(4): p. 23-29.
458 [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6983872/ \(2019\).](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6983872/)
- 459 32. Athanassiadis, B., et al., An in vitro study of the antimicrobial activity of some
460 endodontic medicaments and their bases using an agar well diffusion assay.

- 461 *Australian dental journal*, **54**(2): p. 141-146.
 462 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6983872/> (2009).
- 463 33. Al Qarni, F.M., et al., Antimicrobial activity of *Azadirachta indica* (neem) and
 464 *Salvadora persica* (miswak) extracts as endodontic irrigants. *Endodontic*
 465 *Practice Today*, **13**(3) s%20as%20endodontic%20irrigants#.X4VRXNDXLIV
 466 (2019). http://www.quintpub.com/userhome/endo/endo-ept_13_3_al_p237.pdf
- 467 34. Hesham, A.E.-L. and S.A. Alrumman, Antibacterial activity of Miswak
 468 (*Salvadora persica*) extracts against isolated and genetically identified oral
 469 cavity pathogens. *Technol Health Care*,. **24**: p. S841-SS84.
 470 <https://content.iospress.com/articles/technology-and-health-care/thc1214>
 471 <https://content.iospress.com/articles/technology-and-health-care/thc1214>
 (2016)
- 472 35. Sofrata, A., et al., Benzyl isothiocyanate, a major component from the roots of
 473 *Salvadora persica* is highly active against Gram-negative bacteria. *PLoS One*,
 474 **6**(8).
 475 <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0023045>
 476 <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0023045>
 (2011).
- 477 36. Nakamura, Y., et al., Involvement of the mitochondrial death pathway in
 478 chemopreventive benzyl isothiocyanate-induced apoptosis. *Journal of*
 479 *Biological Chemistry*, **277**(10): p. 8492-8499.
 480 <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0023045>
 481 <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0023045>
 (2002).
- 482 37. Patra, J.K., G. Das, and K.-H. Baek, Chemical composition and antioxidant
 483 and antibacterial activities of an essential oil extracted from an edible

- 484 seaweed, *Laminaria japonica* L. *Molecules*, **20**(7): p. 12093-12113.
 485 <https://www.mdpi.com/1420-3049/20/7/12093> (2015)
- 486 38. Tunjung, W.A.S., D. Liana, and L. Hidayati, Antibacterial Activity and
 487 Composition of Crude Extracts of Kaffir Lime (*Citrus hystrix* DC.) Leaves
 488 and Callus. *Proceedings of the Pakistan Academy of Sciences: B. Life and*
 489 *Environmental Sciences*. **55**(2): p. 45–53-45–53.
 490 <http://www.ppaspk.org/index.php/PPASB/article/view/54>
- 491 39. Nweze, C., H. Ibrahim, and G. Ndukwe, Beta-sitosterol with antimicrobial
 492 property from the stem bark of pomegranate (*Punica granatum* Linn). *Journal*
 493 *of Applied Sciences and Environmental Management*, **23**(6): p. 1045-1049.
 494 <https://www.ajol.info/index.php/jasem/article/view/188260> (2019).
- 495 40. Pu, Z.-H., et al., Antibacterial activity of 9-octadecanoic acid-hexadecanoic
 496 acid-tetrahydrofuran-3, 4-diyl ester from neem oil. *Agricultural Sciences in*
 497 *China*, **9**(8): p. 1236-1240.
 498 <https://www.sciencedirect.com/science/article/abs/pii/S1671292709602121>
 499 (2010).
- 500 41. Chen, K.Y., et al., Monoacylglycerol of 7, 10-Dihydroxy-8 (E)-octadecenoic
 501 Acid Enhances Antibacterial Activities against Food-Borne Bacteria. *Journal*
 502 *of agricultural and food chemistry*,. **67**(29): p. 8191-8196.
 503 <https://pubs.acs.org/doi/abs/10.1021/acs.jafc.9b03063> (2019)
- 504 42. Memar, M.Y., et al., Carvacrol and thymol: strong antimicrobial agents
 505 against resistant isolates. *Reviews in Medical Microbiology*,. **28**(2): p. 63-68.
 506 https://journals.lww.com/revmedmicrobiol/Abstract/2017/04000/Carvacrol_and_thymol_strong_antimicrobial_agents.4.aspx (2017)
 507

- 508 43. Ravi, L., et al., In vitro cytotoxicity of norviburtinal and isopinnatal from
509 Kigelia pinnata against cancer cell lines. *Asian Journal of Cell Biology*,. **12**(1):
510 p. 158-164. [https://www.thieme-](https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-2000-9778)
511 [connect.com/products/ejournals/abstract/10.1055/s-2000-9778](https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-2000-9778) (2009)
- 512 44. Rouis-Soussi, L.S., et al., Chemical composition and antibacterial activity of
513 essential oils from the Tunisian Allium nigrum L. *EXCLI journal*. **13**: p. 526.
514 <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4463966/> (2014)
- 515 45. Mazumder, K., et al., Bioactive Variability and In Vitro and In Vivo
516 Antioxidant Activity of Unprocessed and Processed Flour of Nine Cultivars of
517 Australian lupin Species: A Comprehensive Substantiation. *Antioxidants*. **9**(4):
518 p. 282. <https://www.mdpi.com/2076-3921/9/4/282> (2020)
- 519 46. Rahman, M., et al., Antimicrobial compounds from leaf extracts of Jatropha
520 curcas, Psidium guajava, and Andrographis paniculata. *The Scientific World*
521 *Journal*, <https://www.hindawi.com/journals/tswj/2014/635240/> (2014).
- 522 47. Lozano-Grande, M.A., et al., Plant sources, extraction methods, and uses of
523 squalene. *International journal of agronomy*, **2018**.
524 <https://www.hindawi.com/journals/ija/2018/1829160/> (2018).
- 525 48. Yusuf, A.J., et al., Antimicrobial activity of stigmasterol from the stem bark of
526 Neocarya macrophylla. *Journal of Medicinal Plants for Economic*
527 *Development*, **2**(1): p. 1-5. [https://journals.co.za/content/journal/10520/EJC-](https://journals.co.za/content/journal/10520/EJC-df2f206df)
528 [df2f206df](https://journals.co.za/content/journal/10520/EJC-df2f206df) (2018).
- 529 49. Yinusa, I., et al., Characterization and microbial activities of β -sitosterol and
530 β -sitostenone mixture isolated from the stem bark of methanol fraction of
531 *Sarcocephalus latifolius* (Smith Bruce). *Int. Res. J. Nat. Sci.* **2**: p. 1-13.

- 532 [https://www.eajournals.org/journals/international-research-journal-of-natural-
sciences-irjns/vol-2issue-2june-2014/characterization-microbial-activities-
%ce%b2-sitosterol-%ce%b2-sitostenone-mixture-isolated-stem-bark-
methanol-fraction-sarcocephalus-latifolius-smith-bruce/ \(2014\)](https://www.eajournals.org/journals/international-research-journal-of-natural-
533 sciences-irjns/vol-2issue-2june-2014/characterization-microbial-activities-
534 %ce%b2-sitosterol-%ce%b2-sitostenone-mixture-isolated-stem-bark-
535 methanol-fraction-sarcocephalus-latifolius-smith-bruce/ (2014))
- 536 50. Erdinest, N., et al., Anti-inflammatory effects of alpha linolenic acid on human
537 corneal epithelial cells. *Investigative ophthalmology & visual science*,. **53**(8):
538 p. 4396-4406. <https://iovs.arvojournals.org/article.aspx?articleid=2168117>
539 [\(2012\)](#)
- 540 51. Yang, X., et al., Developmental toxicity of synthetic phenolic antioxidants to
541 the early life stage of zebrafish. *Science of the Total Environment*. **643**: p. 559-
542 568.
543 <https://www.sciencedirect.com/science/article/abs/pii/S0048969718322897>
544 [\(2018\)](#)
- 545 52. Korkmaz, S., Antioxidants in maca (*Lepidium meyenii*) as a supplement in
546 nutrition. *Antioxidants in Foods and Its Applications*, p. 138-154.
547 [https://www.intechopen.com/books/antioxidants-in-foods-and-its-
applications/antioxidants-in-maca-lepidium-meyenii-as-a-supplement-in-
nutrition \(2018\).](https://www.intechopen.com/books/antioxidants-in-foods-and-its-
548 applications/antioxidants-in-maca-lepidium-meyenii-as-a-supplement-in-
549 nutrition (2018).)
- 550 53. Zai, J.A., et al., Methanol extract of *Iphiaea aucheri* ameliorates CCl₄ induced
551 hepatic injuries by regulation of genes in rats. *Toxicology Research*, **8**(6): p.
552 815-832. <https://academic.oup.com/toxres/article-abstract/8/6/815/5709551>
553 [\(2019\).](#)
- 554 54. Siddique, H.R. and M. Saleem, Beneficial health effects of lupeol triterpene: a
555 review of preclinical studies. *Life sciences*,. **88**(7-8): p. 285-293.

556 <https://www.sciencedirect.com/science/article/abs/pii/S0024320510005266>
557 [\(2011\)](#)

558 55. Tyagi, T. and M. Agarwal, Phytochemical screening and GC-MS analysis of
559 bioactive constituents in the ethanolic extract of *Pistia stratiotes* L. and
560 *Eichhornia crassipes* (Mart.) solms. *Journal of Pharmacognosy and*
561 *phytochemistry*,. **6**(1): p. 195-206. (2017).

562 56. Fang, M., et al., Antibacterial activities of inorganic agents on six bacteria
563 associated with oral infections by two susceptibility tests. *International*
564 *Journal of Antimicrobial Agents*,. **27**(6): p. 513-517.
565 <https://www.sciencedirect.com/science/article/abs/pii/S0099239907000064>
566 [\(2006\).](#)

567 57. Phan, T.N., et al., Physiologic actions of zinc related to inhibition of acid and
568 alkali production by oral streptococci in suspensions and biofilms. *Oral*
569 *microbiology and immunology*,. **19**(1): p. 31-38.
570 <https://onlinelibrary.wiley.com/doi/abs/10.1046/j.0902-0055.2003.00109.x>
571 [\(2004\)](#)

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Figures

Figure 1

A histogram representing CFU/mL*10⁴ before and after application of intracanal medicaments

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