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IN VITRO ANTIBACTERIAL ACTION OF *ZINGIBER OFFICINALE* ROSCOE CRUDE EXTRACT IRRIGATION AGAINST *STAPHYLOCOCCUS AUREUS* ISOLATED FROM INFECTED HUMAN ROOT CANALS

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ABSTRACT

Root canal irrigating solutions with strong antibacterial properties are crucial for effective endodontic therapy by preventing recurrent and persistent infections. The question now is not so much whether microbes play a role in the pathophysiology of pulpitis and apical periodontitis, but rather whether species of microbes play a role. The list of microorganisms linked to periodontitis is still growing and could get more precise in future years. Conventional antibacterial agents such as sodium hypochlorite (NaOCl) have failed to overcome a variety of *Staphylococcus aureus* moreover its toxicity and the occasional report of pain when higher concentrations are used. 7 *S. aureus* strains were isolated from infected root canals and identified using the VITEK® 2 compact system. An aqueous crude extract of *Zingiber officinale* Roscoe was prepared and used as a safe and effective antibacterial agent against the *S. aureus* isolates and *S. aureus* ATCC 25923. The gas chromatography-mass spectrometry (GC-MS) analysis of *Z. officinale* Roscoe extract exhibits 14 different biochemical compounds including gingerol (46.11%), zingiberene (17.12%), and thymol (11.20%) as the main constituents. The antibacterial activity of *Z. officinale* Roscoe extract and NaOCl was investigated against *S. aureus* strains compared to ciprofloxacin as a standard drug using agar well diffusion method, minimum inhibition zone concentration (MIC), and minimum bactericidal concentration (MBC). The *Z. officinale* Roscoe extract revealed higher antibacterial activity than NaOCl and ciprofloxacin with inhibition zones averages of 18 ± 0.03, 16 ± 0.06, and 15 ± 0.14 mm, respectively. The MIC and MBC values of *Z. officinale* Roscoe extract were 40 and 40 µg/mL compared to NaOCl (55 and 65 µg/mL, respectively). This study provides an alternate antibacterial irrigation root canal solution, *Z. officinale* Roscoe extract against human pathogenic *S. aureus*.

Keywords: *Zingiber officinale* Roscoe, sodium hypochlorite, root canal, irrigation, GC-MS, antibacterial

INTRODUCTION

Since the mouth is the gateway to the rest of the body's health, maintaining good oral health is essential for improving life quality. There is a need for dependable, effective, and affordable alternative solutions for the prevention and treatment of periodontal diseases due to the drawbacks of several antimicrobial drugs frequently used in dentistry, the lack of resources in developing nations, the prevalence of oral inflammatory conditions, and the rise in bacterial antibiotic resistance **(Mosaddad et al., 2023)**. Operative dental therapy is addressed in endodontic treatment.
Endodontics is the area of dentistry that deals with dental pulp and the tissues that surround a tooth's roots. The procedure known as root canal therapy involves extracting infected tooth pulp and replacing it with filling material. It is carried out when the dental pulp has an irreversible inflammatory process, becomes necrotic, or when an earlier root canal procedure fails. Numerous reasons, like dental trauma, ongoing irritation, and deep cavities, can lead to pulp inflammation **(Raducka et al., 2023)**. Apical periodontitis, which is essentially an infectious and inflammatory disease with a microbial etiology, is primarily brought on by a root canal infection. Numerous pathways exist for bacteria to enter endodontic tissues, including tooth tubules, exposed cavities, periodontal membrane, bloodstream via injured tissues, and contamination by infected tissues.
The mouth cavity contains almost 700 different bacterial species, with every single individual containing 100–200 of them **(Aas et al., 2005)**. There is an average of 100 million bacteria per milliliter of saliva. 65% of the microflora is made up of bacteria, 30% of it of fungus, and 5% of other organisms. Endodontic infections are polymicrobial where a lack of microbiological specificity may alter how well a treatment works because there is no longer any reliable data **(Siqueira et al., 2022)**. The high prevalence of *S. aureus* and its multidrug-resistant strains in dental infections was previously reported **(Al-Akwa et al., 2020)**. It plays a major role in the etiology of primary and persistent endodontic infections. *S. aureus* is one of the important resistant microorganisms frequently isolated from recurrent root canal treatments **(Molander et al., 1998; Siren et al., 1997; Zan et al., 2015)**.
Currently, no method can ensure the complete cleaning, sterilization, and sealing of the whole canal system of a dead tooth because the healthy tooth's root is formed of a highly porous substance that constantly exudes fluids when the tooth is alive. The tremendous complexity and variability of the root canal system's structure also make thorough cleaning and disinfection not always achievable. Accordingly, root canal therapy aims to eliminate all germs, microbial by-products, and necrotic and vital tissues from the root canal system as possible through mechanical and chemical means **(Jhajharia, 2019)**. Typically, hand tools and rotating equipment are used to form root canals while they are continuously irrigated. Emphasis has been placed on the significance of irrigation and thorough root canal disinfection. Irrigation solutions should also help in removing the smear layer. For safe and efficient irrigation, a combination of two or more solutions is needed, as no single solution can have all the necessary qualities **(Topbas and Adiguzel, 2017)**.
The most used irrigation solution is sodium hypochlorite (NaOCl). When compared to alternative irrigation solutions, it is the best option because it is the only one having most of the necessary characteristics. NaOCl is viricidal and sporicidal with a broad antibacterial spectrum. Compared to vital tissue, necrotic tissue is more susceptible to its tissue-dissolving properties **(Haapasalo et al., 2005)**. Since the early 1920s, liquid NaOCl has been used as a basic irrigation solution in endodontics due to its beneficial qualities **(Mohammadi, 2008)**. Chloramine reactions, neutralization of amino acids, and saponification are the outcomes of NaOCl's reaction with organic tissue. NaOCl is now the most often utilized irrigation solution in endodontics because of its solvent impact on necrotic tissues. There is much disagreement, nevertheless, regarding the ideal concentration for NaOCl solutions used in endodontics **(Zehnder, 2006)**. In root canals, organic matter (microbial mass, tissue residue, and inflammatory exudate) lessens the impact of NaOCl. Higher NaOCl concentrations have more effective

tissue-dissolving properties. The potency of low concentrations applied in large quantities is equal to that of high concentrations, however, such greater amounts of NaOCl are more hazardous and toxic **(Martinho and Gomes, 2008)**.

The capacity of a wide variety of plant species to sanitize the root canal system during root canal therapy has been investigated **(Spangberg et al., 1973)**. It has been tried to disinfect root canals using propolis, miswak, neem tree, *Morinda citrifolia*, *Myrtus communis*, *Myristica scent*, *Aloe vera*, turmeric, chamomile, garlic, and other terrestrial plant products **(Mohamed et al., 2024; Shingare and Chaugule, 2011; Topbas and Adiguzel, 2017)**. The primary benefits of using herbal alternatives in root canal therapy are that the items are low-toxicity, long-lasting, and easily obtained; also, they don't create germ resistance **(Ganesh et al., 2023)**. Unfortunately, **Borzini et al. (2016)** and **Attavar (2022)** reported the quickly developed resistance of different bacterial strains to both chemical and herbal irrigation solutions. However, *Zingiber officinale* was not fully investigated as an antibacterial irrigation solution. *Z. officinale* was reported to have bactericidal activity against Gram-positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and *S. epidermidis* as well as Gram-negative such as *Klebsiella pneumonia*, *Escherichia coli*, and *Pseudomonas aeruginosa* **(Alfuraydi et al., 2024)**. The rhizome inhibits the production of free radicals in human cells and possesses antioxidant activities. Its actions are insecticidal, antifungal, antibacterial, and antiprotozoal. The extracted phytochemicals from *Z. officinale*'s rhizome are reported as safe and effective agents **(Hosseinzadeh et al., 2017)**. The crude extract from the rhizomes primarily included monoterpenoid, with α-pinene, 1,8-cineole, geraniol, geranyl acetate, β-caryophyllene, and camphene as the most abundant compounds that are known to possess antibacterial activity **(Sivasothy et al., 2011)**. Accordingly, the current study aimed to test the antibacterial effect of *Z. officinale* Roscoe extract as a new alternative antibacterial root canal irrigation against the clinically isolated *S. aureus* from endodontic infections. The null hypothesis was the absence of a significant difference between the tested extract and conventional antibacterial agents regarding its anti-bacterial effect against *S. aureus*.

MATERIALS AND METHODS

Preparation of *Z. officinale* Roscoe extract

Aqueous ginger (*Z. officinale* Roscoe) extract was prepared according to **Adetunde et al. (2014)**. In brief, the plant materials were washed with clean water, chopped, and partially allowed to air-dry in the shade at room temperature for three days to remove excess moisture, then 20 g of dried fine grounded powder of rhizome of ginger were weighed on an electronic weighing balance and dispensed into a beaker containing 80 mL of distilled water. These were soaked for 72 hrs, after which the solution was carefully filtered with muslin cloth into a sterilized conical flask, and the filtrates obtained were stored in the refrigerator at a temperature of 4°C for further use.

Gas chromatography-mass spectrometry (GC-MS) analysis of *Z. officinale* Roscoe extract

The identification of phytochemicals that constitute *Z. officinale* Roscoe extract was investigated by GC-MS studies according to **Elbestawy et al. (2023)**. The chemical composition was determined using the GC–MS system (GC–MS QP-2010 Plus, Shimadzu, Kyoto, Japan) with an Rtx-5 MS column with dimensions of 30 m × 0.25 mm (0.25 μm film thickness). Helium gas was applied as a carrier gas at a constant flow of 1.2 mL/min and an injection volume of 1.0 μL (split ratio of 10:1). The ionization mass of the spectroscopic analysis was performed at 70 eV. The injector temperature is 250°C, and the ion-source temperature is 280°C. Programming for the oven temperature included an initial isothermal setting of 110°C for two minutes, followed by increases of 10°C/min to 200°C, 5°C/min to 280°C, and a 9°C isothermal at 280°C. The data were obtained using GC–MS post-run software.

Sample (patient) selection

Twenty-four permanent incisors in 24 patients from the endodontic department's outpatient clinics (Faculty of Dentistry, Suez Canal University) were selected according to the following inclusion criteria: cooperative, healthy patients, aged 30 to 50 years. The clinical diagnostic for the chosen patients should demonstrate the following: one maxillary incisor should have asymptomatic pulp necrosis, which was verified by electrical (DY310, Denjoy Dental Co.) and thermal pulp sensitivity testing. There should be less than 3 mm of pocket depth.

Prior to endodontic treatment, digital periapical radiographs were used to assess the study's teeth. According to **Vertucci et al. (1974)**, the teeth should have had a single root with a single canal type I, closed apices, periapical lesions of endodontic origin with a diameter ranging from 2 to 5 mm, a periapical index score of 3 or 4 **(Ørstavik et al., 1986)**, and no root canal filling. In the teeth that were enrolled, pulp exposure to the oral cavity due to caries should have resulted in pulp necrosis and accompanying apical periodontitis. Patients who had immune system compromises, complex systemic diseases, physical disabilities, or psychological issues were excluded from the current study. Throughout the previous three months, some patients underwent antibiotic medication. The following teeth were excluded also if the teeth are not repairable due to extensive carious diseases, periodontal fractures, or cracks, exhibiting resorption or calcifications inside the root canal, teeth with or without concurrent or simultaneous endo-perio-communication, and periodontal pockets larger than 4 mm, and teeth with a history of endodontic therapy or those connected to bone expansion.

Sample randomization, allocation concealment and blinding

The patients were numbered based on when they arrived at the faculty's diagnostic clinic by the first allocator (author DMF), who also completed the clinical and radiological diagnosis for each case. The online application (http://www.random.org/) was used to randomly assign these numbers (1–24) into the two study groups (n = 12 each) based on the irrigation placement (I: NaOCl, II: *Zingiber officinale* Roscoe crude extract). The endodontist (author DAM) carried out all root canal treatment treatments blindly, therefore random codes (such as I-1A and II-14A) were created and kept a secret from them. The irrigation syringes were tagged as I or II and concealed in closed envelopes by the second allocator (Author HME). When the irrigations were applied, they were set up using the random codes that the first allocator provided. As a result, the patient's group and the method of irrigation were unknown to the operator (Author DAM). Additionally, the irrigation intervention was double-blinded for the patients.

Root canal treatment and microbiological sampling procedures

Patients in the study had their teeth scaled and polished before receiving treatment **(Teles et al., 2013)**. Chlorhexidine digluconate (CHX, DEXA firm for chemicals) was instructed to be used as a mouthwash for the patients. Before beginning the treatment, each tooth was topically anesthetized with 20% benzocaine gel (Prime-Dent). This was followed by a local anesthetic injection using the maxillary buccal infiltration technique, which involved injecting 0.9 ml of a solution containing 2% Lidocaine HCL and 1:100 000 epinephrine (Lignospan standard). A rubber dam and aseptic method were employed throughout the endodontic operation. 30% hydrogen peroxide (H_2O_2, LUNA pharmaceutical Co.) and 2.5% sodium hypochlorite (NaOCl, DEXA company for chemicals, Egypt) were used to disinfect the operative field, which included the tooth, clamp, sheet (Coltène/Whaledent), and adjacent areas. Using a sterile round bur (Mani, Inc.) and sterile saline solution (0.9% v/w, Otsuka Pharmaceutical Co.), decayed coronal structures were removed while the area was cooled. The crown surface and the surrounding structures (the clamp and dam) were re-cleaned for thirty seconds, as previously mentioned. After that, the impact of the NaOCl was neutralized using 5% sodium thiosulfate (El Gomhorya Co., Egypt).

Sterile high-speed round and fissure burs were used to create the access cavity in an aseptic environment (Mani, Inc.). Using tapered low-speed stone (Mani, Inc.) in an aseptic setting, the access cavity was smoothed. Following the completion of access cavity preparation, the disinfection treatment was carried out once more, as previously mentioned. As previously mentioned, sterile Teflon tape and cotton pellets were employed to seal the pulp chamber's floor and roof in order to stop disinfectants from entering the coronal and radicular pulp spaces **(Karataş et al., 2020)**. Sterile Omni Swabs (Whatman FTA; Sigma-Aldrich) with an ejectable head were used to collect sterility control samples (SR) from the coronal surface of the tooth, rubber dam, and clamp after the effect of NaOCl was neutralized with sodium thiosulfate **(Karataş et al., 2020; Zandi et al., 2016)**.

After the swab was placed in a cryotube filled with Tris-EDTA buffer (10 mmol/L Tris–HCL, 1 mmol/L EDTA, pH = 7.6) (Sigma–Aldrich), it was either put straight into a freezer at -80°C or into an ice box filled with ice. Afterwards, the samples in the ice box were moved to a freezer. Sterility control samples were obtained at each visit in order to include a tooth in the study.

Working length (WL) was measured with a sterile #20 K-file (Micromega) and validated by periapical radiography method (WL was about 1 mm short of the radiographic root apex) using an electronic apex locator (E-connect, Eighteeth Medical). Using sterile disposable syringes (AMECO business), sterile saline was poured into the pulp chamber and canal, and a 23-G needle (Max-i-Probe, Dentsply Maillefer) was fastened to it. The bacterial suspension from the main pulpal space was then collected from the intracanal contents using three sterile paper points # 20 (Meta Biomed) that were inserted into the root canals up to the level of WL and left inside for 60 seconds while being pumped. In addition, aspiration was done under negative pressure using a plastic syringe. Paper points and other collected materials were put in an Eppendorf tube with a 1 mL solution of 0.84% NaCl that was tightly sealed. The initial microbiological sample (A) was the label applied to this specimen.

The root canal system was subsequently prepared using Fanta (AF) nickel-titanium rotary files (Fanta) and an E-connect endo motor (Eighteenth Medical) in rotation mode, set at 325 rpm and 1.8 N cm torque, as per the manufacturer's recommendation, till AF4 35/04. Up until clean, white dentine chips were clearly visible at the apical 3 mm of the master file, additional shaping was performed with hand file #45 (Micromega) in accordance with each canal's anatomy. After every file use, irrigation was carried out with 2 ml of the matching irrigation (a total of ~10 ml/canal through ~10 min). Side-vented needles with size #30 G (Steri irrigation tips, DiaDent Group International) were positioned 1 mm short of the

WL (by the second allocator, discussed before). Three sterile absorbent paper points that matched the MAF size were used to collect and dry the canal content before aspirating to obtain the second post-operative microbiological sample (P). Following that, the irrigation protocol recommended by **Basrani and Haapasalo (2012)** with assistance from PUI was applied to the canals using 10 ml of 2.5% NaOCl, 10 ml of saline, 3 ml of EDTA 17% (Prevest Denpro Limited Company) for 1 minute, 3 ml of saline, and 2 ml of CHX 2% (DEXA company for chemicals) for 1 minute, respectively. Using the cold lateral compaction method, canals were obturated with gutta-percha cones (Meta Biomed) and AH Plus sealant (Dentsply/Maillefer) after being dried with sterile paper tips. A composite restoration was placed on the tooth right away, and a follow-up radiograph was obtained to assess the level of obturation.

Isolation, purification, and identification of *S. aureus* isolates

The bacteria were isolated by plating dilutions in 2 mL sterile saline solution, vortexed well and these suspensions were considered as 10^{-1} dilution. Then 1 mL of supernatant was transferred to another test tube containing 9 mL of sterile distilled water by using a single pipette for each time to obtain dilution 1/100. The previous step was repeated to get the serial dilutions 1/1000. The blood agar medium (Oxoid, UK) was prepared, and the bacteria were isolated using the pour plate technique, it was poured into Petri dishes and mixed well with 1 mL of the sample. After solidification of the agar in the dishes, they were inverted and incubated at 37°C for 24 hrs. in a static incubator. After the incubation period, the bacterial total count was done for each sample and each developed single colony of bacteria which varied in shape and color was picked up and purified by streaking on fresh nutrient agar plates. The purified bacterial isolates were stained using Gram stain. The staphylococcus-shaped isolates were selected, purified, and stored on nutrient agar slants at 4°C for further use. Isolates were cultivated on nutrient agar plates and species-level identification was performed according to the standard recommended guidelines **(Koneman et al., 1997)**. Furthermore, the bacterial isolates were sub-cultured on blood agar plates and examined with the VITEK® 2 Compact system (BioMerieux, Marcy-l'Étoile, France) to confirm the identification results **(Lupetti et al., 2010)**.

Antibiotic susceptibility test of *S. aureus* clinical isolates

Mueller-Hinton broth (MHB) medium (Oxoid, UK) supplemented with 5% horse blood was used to culture 7 previously reported *S. aureus* isolates and the reference strain ATCC 25923 until they reached the exponential phase at 37°C. Every 30 min., the turbidity of the bacterial growth was assessed to determine the exponential phase using a spectrophotometer calibrated at 600 nm. Then, in sterile saline (0.84% NaCl), the inoculum density in each bacterial solution was adjusted to 0.5 McFarland Standard (1.5×10^8 CFU/mL). The disc diffusion technique was used to test the antibiotic susceptibility of *S. aureus* strains according to the guidelines provided in the Clinical and Laboratory Guidelines (M7-A5) from the Clinical and Laboratory Standards Institute **(CLSI, 2019)**. Briefly, 50µl of the bacterial suspension at the turbidity concentration indicated above was added to Mueller-Hinton agar (MHA) medium that was supplemented with 5% horse blood. Sterile forceps were used to carefully load antibiotic discs representing various antibiotic classes (the antibiotic panel included levofloxacin 5 µg/mL, cotrimoxazole 25µg/mL, ciprofloxacin 5 µg/mL, gentamicin 10 µg/mL, erythromycin 15 µg/mL, ofloxacin 5 µg/mL, tetracycline 30 µg/mL, cloxacillin 5 µg/mL, ceftriaxone 30 µg/mL, amoxicillin/clavulanate 30/10 µg/mL, streptomycin 30 µg/mL, penicillin 10 IU, amoxicillin 30 µg/mL, and chloramphenicol 30 µg/mL). After that, they were incubated at 37°C for 24 hrs. The inhibition zone's diameter was measured in millimeters (mm) and compared to the procedure chart's standard zone dimension.

Antibacterial activity using agar well diffusion method

The bactericidal action of *Z. officinale* Roscoe extract against the isolated different *S. aureus* stains in addition to *S. aureus* ATCC 25923 was investigated *in vitro* using the agar well diffusion test according to the guidelines of the **CLSI (2017)**. MHA plates were prepared and inoculated by 0.5 McFarland standard from *S. aureus* isolates. About 150 µL from *Z. officinale* Roscoe extract, NaOCl, and ciprofloxacin were prepared with a concentration of 50, 100, and 150 µg/mL and added separately into wells (5 mm diameter) in the inoculated MHA plates under aseptic conditions. Plates were incubated at 37°C for 24 hrs and then the zones of inhibition were measured in mm.

Determination of minimum inhibition concentration (MIC)

MHB medium was used to determine the MIC for *Z. officinale* Roscoe extract and NaOCl by the broth dilution method **(CLSI, 2000)**. After preparing various concentrations (0-100 µg/mL) of *Z. officinale* Roscoe extract and NaOCl, they were introduced into MHB flasks (50 mL) that had been inoculated with 0.5 McFarland Standard of the tested bacteria. For 24 hrs., flasks were incubated at 150 rpm and 37°C. Using a UV-visible spectrophotometer (UV1100, Shanghai Yoke Instrument Company Co., Ltd., Shanghai, China) at 600 nm, the MIC values of the *Z. officinale* Roscoe extract and NaOCl were determined. There were no obvious growing flasks that mentioned the MIC values.

Determination of minimum bactericidal concentration (MBC)

The results of the MIC test were used to calculate the MBC values. MHA medium was melted, cooled, and then filled into sterile Petri dishes with approximately 0.1 mL of inoculant from each MIC bacterial flask. The total count of each bacterial colony was measured in colony-forming units per milliliter (CFU/mL) during a 24-hrs. incubation period at 37°C **(El-Zahed et al., 2024)**.

Statistical analysis

Using SPSS version 18, the ANOVA test was used to analyse the results. A significance threshold of 0.05 was chosen. Three repetitions of the experiments were conducted. Each result's mean and standard deviation (SD) were given **(O'Connor, 2000)**.

RESULTS

Chemical composition of *Z. officinale* roscoe extract using GC-MS analysis

About 14 different compounds were found during the GC-MS screening of the aqueous extraction of *Z. officinale* Roscoe (Figure 1 & Table 1). Gingerol (46.11%), zingiberene (17.12%), and thymol (11.20%) were the main constituents.

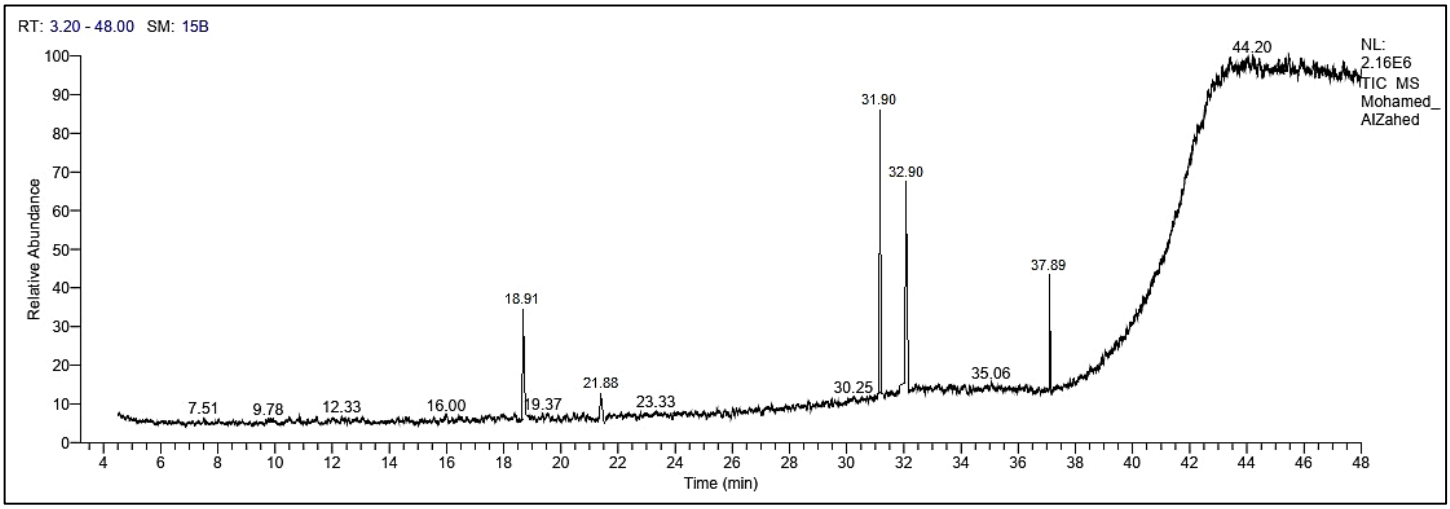

Figure 1 GC-MS analysis of Z. officinale Roscoe extract

Table 1 Chemical profile of Z. officinale Roscoe extract by GC-MS

Peak	Retention time	Contents%	Compound name	Molecular formula	Molecular weight
1	7.51	0.08	3-(3-Pyridyl)-5-phenylisoxazoline	$C_{14}H_{12}N_2O$	224
2	9.78	0.14	Decanal	$C_{10}H_{20}O$	156
3	12.33	0.18	Benzoic acid 2-methoxy-3-(4-mehoxy-2-methyl-4-oxobutanoyl)-6-methyl-,methyl ester	$C_{16}H_{20}O_6$	308
4	16.00	0.32	Dodecanamine, N, N-dimethyl-	$C_{14}H_{31}N$	213
5	18.91	11.20	Thymol	$C_{10}H_{14}O$	150
6	19.37	0.24	4-Benzylidene-3-phenethyl-4H-isoxazol-5-one	$C_{18}H_{15}NO_2$	277
7	21.88	0.10	psi.-Carotene,3,4-didehydro-1,2-dihydro-1-methoxy-	$C_{41}H_{58}O$	566
8	23.33	0.25	Alpha-terpineol	$C_{10}H_{20}O$	156
9	30.25	0.20	Shogaol	$C_{19}H_{28}O_3$	304
10	31.90	46.11	Gingerol	$C_{17}H_{28}O_4$	246
11	32.90	17.12	Zingiberene	$C_{15}H_{27}$	207
12	35.06	0.46	Beta-bisabolene	$C_{15}H_{22}$	200
13	37.89	6.72	Cyclohexane,3-(1,5-dimethyl-4-hexenyl)-	$C_{15}H_{24}$	204
14	44.20	26.09	1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-Hexadecamethyloctasiloxane	$C_{16}H_{50}O_7Si_8$	578

Isolation, total count, and identification of bacterial isolates from infected root canal system

48 samples were collected from the Suez Canal University outpatient clinic. Most subjects were 35 ± 10 years of age (Table 2). A microbiological sample was collected from a single root canal with primary endodontic infection. A total of 321 bacterial isolates were isolated from the microbiological samples. The bacterial isolates were picked, purified, and stained with Gram stain. The results indicated that Gram-positive bacteria were common in the samples rather than Gram-negative bacteria (Figure 2). 295 (91.9%) Gram-positive (rods, diplococci, and cocci clusters) bacteria were recorded, while only 26 (8.09%) Gram-negative rods were observed. The used protocol succeeded in the isolation of 7 Gram-positive, spherical, and in clusters, golden yellow on mannitol salt agar and non-spore-forming bacterial isolates. According to the standard guidelines and VITEK® 2 compact system test, the 7 bacterial isolates (SADM1, SADM2, SADF13, SADF8, SANM6, SANF2, and SAKF5) were identified and designated as *S. aureus* strains.

Table 2 Total count of microbial isolates for the dental samples before and after treatment with the irrigation solutions

Treatment	Patient No.	Sample No.	ID	Total count (CFU/mL)	Treatment	Patient No.	Sample No.	ID	Total count (CFU/mL)
NaOCl treatment	1	1	1A	123×10^3	*Z. officinale* roscoe extract treatment	13	25	13A	602×10^3
		2	1P	2×10			26	13P	444×10
	2	3	2A	924×10^3		14	27	14A	568×10^3
		4	2p	13×10			28	14P	14×10
	3	5	3A	276×10^3		15	29	15A	775×10^3
		6	3P	108×10			30	15P	18×10
	4	7	4A	300×10^3		16	31	16A	412×10^3
		8	4P	128×10			32	16P	155×10
	5	9	5A	412×10^3		17	33	17A	332×10^3
		10	5P	124×10			34	17P	5×10
	6	11	6A	615×10^3		18	35	18A	900×10^3
		12	6P	210×10			36	18P	100×10
	7	13	7A	350×10^3		19	37	19A	683×10^3
		14	7P	14×10			38	19P	82×10
	8	15	8A	910×10^3		20	39	20A	223×10^3
		16	8P	214×10			40	20P	128×10
	9	17	9A	517×10^3		21	41	21A	177×10^3
		18	9P	125×10			42	21P	12×10
	1	19	10A	222×10^3		22	43	22A	632×10^3
	0	20	10P	14×10			44	22P	198×10
	1	21	11A	661×10^3		23	45	23A	912×10^3
	1	22	11P	290×10			46	23P	111×10
	1	23	12A	898×10^3		24	47	24A	120×10^3
	2	24	12P	370×10			48	24P	21×10

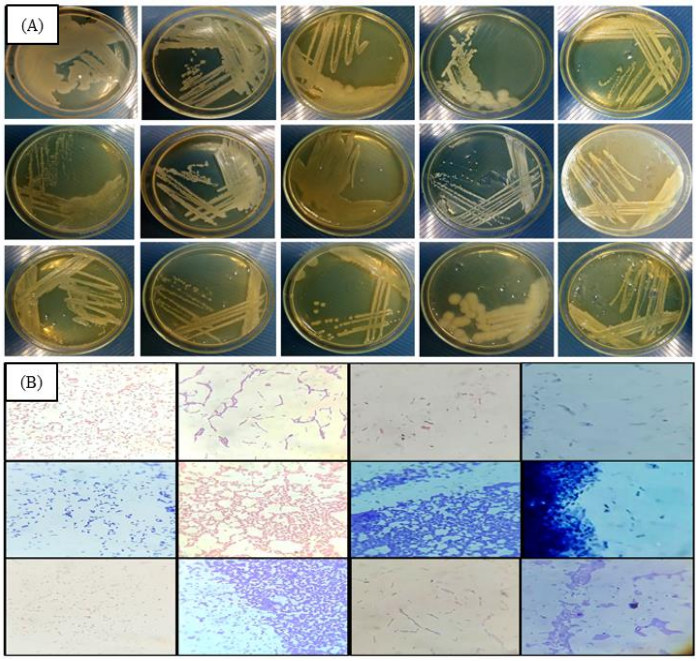

Figure 2 Isolation, purification; (A), and Gram stain; (B), of different bacterial isolates

Antibiotic sensitivity pattern of *S. aureus*

Disc diffusion techniques were used to study the antibiotic susceptibility of the 7 previously reported *S. aureus* isolates and the reference strain ATCC 25923. The results show that *S. aureus* strains have resistance percentages of 85.7%, 71.4%, 57.1, 42.9, 28.6, and 14.3%, respectively, to penicillin, cotrimoxazole, amoxicillin, tetracycline, cloxacillin, and streptomycin. Levofloxacin showed the strongest activity against those strains, with ratio sensitivity values of 100%. Table 3 displays the strain's susceptibility to all tested antibiotics. It is necessary to discover a new, potent antibacterial agent for *S. aureus* since it quickly becomes resistant to several antibiotics.

Table 3 Antibiotic sensitivity and resistance pattern of *S. aureus* strains

Antibiotic	Antibiotics susceptibility	
	No. sensitive (%)	No. resistant (%)
Gentamicin	85.7	14.3
Amoxycillin/clavulanate	57.1	42.9
Streptomycin	14.3	85.7
Cloxacillin	28.6	71.4
Erythromycin	57.1	42.9
Chloramphenicol	71.4	28.6
Cotrimoxazole	71.4	28.6
Tetracycline	42.9	57.1
Penicillin	85.7	14.3
Ciprofloxacin	71.4	28.6
Ofloxacin	71.4	28.6
Levofloxacin	100	0
Ceftriaxone	85.7	14.3
Amoxycillin	57.1	42.9

Antibacterial activity *Z. officinale* Roscoe extract when used as root canal irrigation

The antibacterial activity of *Z. officinale* Roscoe extract was investigated using the agar well diffusion technique, MIC, and MBC tests against *S. aureus* isolates and the ATCC 25923 strain. The results are displayed in Table 4 and Figure 3. The antibacterial activity of *Z. officinale* Roscoe extract in the current study rose as their concentration increased, suggesting a dose-related inhibitory impact. *Z. officinale* Roscoe extract and NaOCl had MIC means of 40 and 55 µg/mL, respectively, against strains of *S. aureus* (Figure 3). In contrast to NaOCl, whose MBC values were greater than its MIC values, *Z. officinale* Roscoe extract's MBC values matched its MIC values, showing its significant potential as a strong antibacterial agent (Figure 4). The mean MBC values of *Z. officinale* Roscoe extract and NaOCl were 40 and 65 µg/mL, respectively.

Table 4 Antimicrobial activity of *Z. officinale* Roscoe extract compared to NaOCl and ciprofloxacin against *S. aureus* strains

Antibacterial agent	Concentration, µg/mL	SADM1	SADM2	SADF13	SADF8	SANM6	SANF2	SAKF5	ATCC 25923
Z. officinale Roscoe extract	50	15 ± 0	12 ± 0.03	12 ± 0	14 ± 0	11 ± 0.03	16 ± 0	14 ± 0.03	14 ± 0
	100	17 ± 0	14 ± 0.03	15 ± 0	16 ± 0	13 ± 0.03	18 ± 0	16 ± 0.03	16 ± 0
	150	19 ± 0	16 ± 0.03	18 ± 0	19 ± 0	15 ± 0.03	20 ± 0	18 ± 0.03	18 ± 0
NaOCl	50	7 ± 0.14	6 ± 0.14	7 ± 0.06	12 ± 0	9 ± 0.03	11 ± 0.03	9 ± 0.14	8 ± 0.03
	100	10 ± 0.14	8 ± 0.14	10 ± 0.03	14 ± 0	11 ± 0.03	13 ± 0.03	11 ± 0.03	12 ± 0.03
	150	13 ± 0.14	10 ± 0.06	13 ± 0.03	16 ± 0	13 ± 0.03	15 ± 0	14 ± 0.03	16 ± 0.03
Ciprofloxacin	50	8 ± 0.14	6 ± 0.14	10 ± 0.14	10 ± 0.03	8 ± 0.06	14 ± 0.03	11 ± 0.14	10 ± 0.03
	100	12 ± 0.03	10 ± 0.06	13 ± 0.14	13 ± 0.03	10 ± 0.06	16 ± 0	13 ± 0.03	13 ± 0.03
	150	15 ± 0.06	13 ± 0.03	15 ± 0	16 ± 0.03	12 ± 0.06	17 ± 0	15 ± 0.06	17 ± 0.03

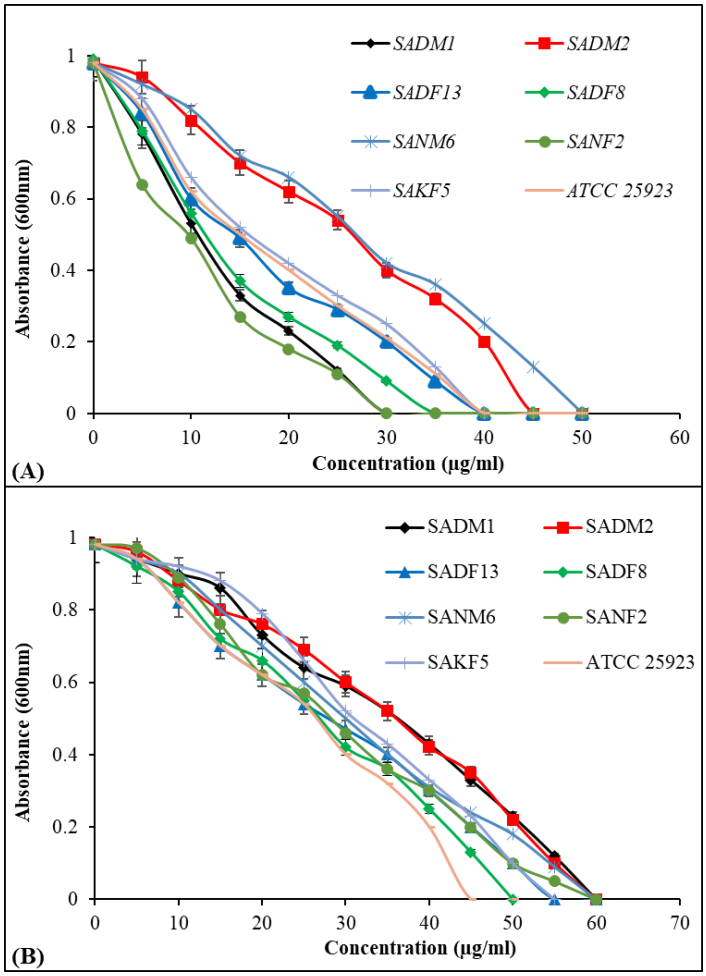

Figure 3 Minimum inhibition concentration of *Z. officinale* Roscoe extract; (A), and NaOCl; (B) against *S. aureus* strains.

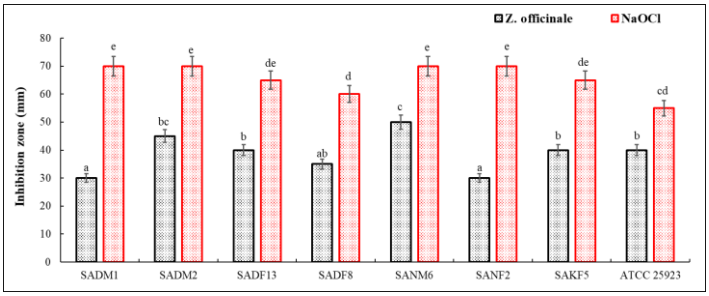

Figure 4 Minimum bactericidal concentration of *Z. officinale* Roscoe extract and NaOCl.

DISCUSSION

Developing bacterial resistance to currently available antibiotics has emerged as an urgent global health concern **(Abou-Dobara et al., 2024; Mohamed and El-Zahed, 2024)**. Consequently, the fabrication of novel antibacterial drugs is a topic of significant interest for several investigations. Over the past few years, there has been an increase in drug-resistant *S. aureus* that causes treatment failures and decreases the effectiveness of traditional first-line treatment regimens due to β-lactam antibiotic resistance on a global scale **(Lupetti et al., 2010)**. It is necessary to discover a new, potent antibacterial agent for *S. aureus* since it quickly becomes resistant to several antibiotics. In this work, the antibacterial activity of *Z. officinale* Roscoe extract was evaluated against strains of *S. aureus* obtained from a single root canal containing a primary endodontic infection.

Different microbiological samples were collected from a single root canal with primary endodontic infection and used to isolate and identify 7 *S. aureus* strains using the standard guidelines and VITEK® 2 compact system test. All *S. aureus* strains were tested to study their antibiotic susceptibility compared to the reference strain ATCC 25923. *S. aureus* strains revealed varied resistance percentages to penicillin, cotrimoxazole, amoxicillin, tetracycline, cloxacillin, and streptomycin. According to **Onwubiko & Sadiq (2011)**, the antimicrobial agent levofloxacin (100%) had the highest incidence of *S. aureus* occurrences, followed by ciprofloxacin (78.9%) and penicillin (7.1%). Furthermore, **Akanbi et al. (2017)** study on antibiotic susceptibility found that *S. aureus* had varying degrees of resistance to various antibiotics, with the highest percentages of resistance to ampicillin and penicillin (96.7%), rifampicin and clindamycin (80%), oxacillin (73.3%), and erythromycin (70%).

The current study succeeded in the isolation of 14 different compounds from the aqueous extraction of *Z. officinale* Roscoe at which Gingerol, zingiberene, and thymol were recorded as the major constituents. These results were similarly matched with the **Elbestawy et al. (2023)** study which reported the presence of gingerol (45.05%), zingiberene (16.05%), and thymol (10.50%). Also, **Elbashir et al. (2021)** recorded gingerol by 43%, followed by zingiberene (14%) as the major component found in the *Z. officinale* extract. The methanol extract of *Z. officinale* from India was screened by GC-MS, and the results showed the presence of zingiberene, α-bergamotene, gingerol, zingerone, caryophyllene, and δ-elemene. Gingerol and thymol are known to inhibit the growth of Gram-positive and Gram-negative bacteria **(Kachur et al., 2020; Marchese et al., 2016; Park et al., 2008)**. **Kumar and Geetha (2018)** documented the antibacterial activity of (10)-gingerol and (12)-gingerol isolated from dry ginger extract against methicillin-resistant *S.*

aureus (MRSA) that showed zones of inhibition of 24 mm at a concentration of 2000 µg/mL. While **Das et al. (2019)** investigated the antibacterial and antibiofilm activity of terpenes such as α-zingiberene, β-sesquiphellandrene, α-farnesene, neral, and ar-curcumene extracted from *Z. officinale* against multi-drug resistant *S. aureus* (1.56 µL/mL), *K. pneumonia* (3.125 µL/mL), *E. coli* (3.125 µL/mL), and *Enterococcus faecalis* (0.78 µL/mL) bacteria.

The antibacterial activity of *Z. officinale* Roscoe extract was studied using the agar well diffusion technique, MIC, and MBC tests against *S. aureus* strains. The *Z. officinale* Roscoe extract at 150 µg/mL caused inhibitory zones of 19 against isolates of SADM1, SADF8, 20 mm against isolates of SANF2, and 18 mm against isolates of SADF13, SAKF5, and ATCC 25923. *Z. officinale* Roscoe extract showed enhanced antibacterial activity against *S. aureus* strains in comparison to the conventional drug ciprofloxacin. **Njobdi et al. (2018)** also observed that 100 µg/mL of *Z. officinale* induced an inhibitory zone against *S. aureus* of 17.5 ± 0.87 mm. On the other hand, **Zainal et al. (2022)** showed that a concentration of 80 to 100 mg/mL *Z. officinale* was able to suppress *S. aureus* with a mean zone of inhibition varied from 11 to 15 mm at a ginger extract.

In the current investigation, *Z. officinale* Roscoe extract's antibacterial activity increased as the concentration increased, indicating a dose-related inhibitory effect. MIC values for Z. officinale Roscoe extract and NaOCl against *S. aureus* strains were 40 and 55 µg/mL, respectively. According to **Njobdi et al. (2018)**, *Z. officinale* Roscoe extract had MIC values of 2.5 mg/mL against *S. aureus*. At the same time, *Z. officinale* Roscoe ethanolic extract demonstrated stronger antibacterial activity against *S. aureus* at a concentration of 1000 µg/mL than at lower concentrations **(Yassen and Ibrahim, 2016)**. Also, *Z. officinale*'s MIC against *S. aureus* (ATCC 6538) was found to be 50 mg/mL as reported by **Silva et al. (2022)**. *Z. officinale* oils had a mean MIC value of 10.8 µl against *S. aureus*, according to **Raja et al. (2016)**. In contrast to NaOCl, whose MBC values were greater than its MIC values, *Z. officinale* Roscoe extract's MBC values matched its MIC values, showing its significant potential as a strong antibacterial agent. The mean MBC values of *Z. officinale* Roscoe extract and NaOCl were 40 and 65 µg/mL, respectively. **Nikolić et al. (2014)** study found that the MBC values of *Z. officinale* Roscoe ethanolic extract were in the range from 0.0024 to > 20 mg/mL. While 0.5 mg/mL essential oil of *Z. officinale* Roscoe as MBC value was enough to cause the complete killing of *S. aureus* ATCC 29213 as documented **by López et al. (2017)**. Despite these results, to reveal the actual effect of *Z. officinale* Roscoe extract as an endodontic irrigation, *Z. officinale* Roscoe extract required to be evaluated on other endodontic multidrug-resistant pathogenic microorganisms using a wider sample size, since the endodontic infection is polymicrobial. Also, testing the antibacterial effect of this extract using genome analysis, identification of toxin genes and other antibiotic-resistant genes, and using more advanced molecular identification methods like next-generation sequencing is highly recommended.

CONCLUSIONS

Different *S. aureus* strains were isolated, identified, and selected for studying the antibacterial activity of aqueous crude extract of *Z. officinale* Roscoe. The provided method was simple, cheap, and eco-friendly with high antibacterial activity. The chemical profile of *Z. officinale* Roscoe extract by GC-MS confirmed the presence of different antibacterial phytochemical compounds such as gingerol, zingiberene, and thymol. This study showed that *Z. officinale* Roscoe extract can be a source of the plants possessing antimicrobial activities that may be useful plant may potentially be used as antibacterial agents in new drugs for the treatment of endodontic diseases caused by bacterial pathogens, especially *S. aureus*.

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Author contributions: A.A.A, M.M.A, D.M.F, H.M.E, and M.M.E contributed to the idea and the design of the study. H.M.E and M.M.E performed the practical work. A.A.A, M.M.A, D.M.F, H.M.E, M.M.E, D.A.A and M.A.KH analysed and interpreted the data. A.A.A., M.M.A., and D.M.F supervised the overall work. H.M.E, M.M.E, and M.A.KH wrote the original draft. All authors reviewed and edited the manuscript. All authors read and approved the final manuscript.

Data availability: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Ethics approval and consent to participate: The study was carried out in accordance with the guidelines and regulations of the scientific research ethics committee, Faculty of Pharmacy, Suez Canal University, Egypt (Reference Number 201810MH1). Written informed consent for enrolment, screening, and specimen collection was obtained from each patient. Moreover, the data of the patients were not exposed.

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