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Original Research

Antimicrobial Efficacy of Herbal Extracts Against Root Canal Pathogens: an In Vitro Investigation

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ABSTRACT:
Background: Root canal infections present a clinical challenge, often necessitating antimicrobial intervention. Concerns over antibiotic resistance and synthetic chemicals have prompted the exploration of natural alternatives such as herbal extracts. This in vitro study aimed to assess the antimicrobial efficacy of selected herbal extracts against common root canal pathogens, including Enterococcus faecalis, Porphyromonas gingivalis, and Candida albicans. **Methods**: Root canal samples were obtained from patients, and isolates were identified. Three herbal extracts [A. **Tea Tree Oil**, B. **Neem**, C. **Propolis**] were tested using the agar well diffusion method. Standard strains served as controls. Minimum inhibitory concentrations (MIC) were determined. **Results**: Extracts A and B exhibited significant inhibition zones against all pathogens. Extract B demonstrated the lowest MIC values, suggesting high potency. Chlorhexidine served as a positive control. **Conclusion**: Our findings suggest that certain herbal extracts possess notable antimicrobial activity against root canal pathogens. Extract B, in particular, displayed broad-spectrum efficacy. While further research is needed to validate clinical applicability and safety, herbal extracts hold promise as alternatives to synthetic antimicrobial agents in endodontics, aligning with the demand for sustainable and natural healthcare options.
Keywords: Antimicrobial, Herbal Extracts, Root Canal, Pathogens, In Vitro

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INTRODUCTION

Root canal infections are a widespread and persistent problem within the field of endodontics, characterized by the colonization of pathogenic microorganisms within the complex and intricate root canal system of teeth. These infections can be a source of acute pain, discomfort, and severe complications if left untreated, posing a significant challenge for dental practitioners [1]. Traditionally, the management of root canal infections has primarily revolved around mechanical cleaning and disinfection through the use of chemical agents, such as sodium hypochlorite and chlorhexidine [2]. While these treatments have demonstrated efficacy in eliminating many pathogenic microorganisms, concerns have arisen regarding their limitations, potential side effects, and the emergence of antibiotic-resistant strains [3]. The emergence of antibiotic resistance in both the medical and dental fields has raised significant concerns worldwide [4]. Overreliance on conventional antibiotics and synthetic chemical agents in endodontics has contributed to the development of resistant strains of microorganisms,

rendering previously effective treatments less potent [5]. As a result, researchers and clinicians have been prompted to explore alternative strategies for managing root canal infections that not only exhibit high antimicrobial activity but also minimize the risk of antibiotic resistance and adverse side effects. In this context, the utilization of herbal extracts as potential antimicrobial agents in endodontics has gained considerable attention and interest. Herbal extracts are derived from various plant sources and have long been recognized for their diverse therapeutic properties, including anti-inflammatory, analgesic, and antimicrobial activities [6]. Many of these plant-derived compounds have a rich history of traditional medicinal use, and recent scientific investigations have provided evidence of their efficacy against a broad spectrum of microorganisms, including those commonly associated with root canal infections [7]. The appeal of herbal extracts as potential antimicrobial agents in endodontics is rooted in several key factors:

1. **Broad-Spectrum Action**: Herbal extracts often exhibit a wide range of antimicrobial activity, making them potentially effective against a variety of root canal pathogens, such as Enterococcus faecalis, Porphyromonas gingivalis, and Candida albicans [8].
2. **Safety Profile**: In contrast to some synthetic chemicals and antibiotics, herbal extracts are generally considered safe for use in humans and have a reduced risk of adverse side effects [9].
3. **Natural Origin**: These extracts are sourced from plants, which aligns with the growing global interest in natural and sustainable healthcare alternatives [10].
4. **Potential for Reduced Antibiotic Resistance**: The diverse compounds found in herbal extracts may offer a lower risk of promoting antibiotic resistance when compared to traditional antibiotics [9,10].
5. **Patient Acceptance**: Patients are increasingly seeking natural and minimally invasive treatment options, making herbal-based therapies an appealing choice for those who prefer holistic or alternative approaches [10]. Given these potential advantages, there is a compelling rationale for investigating the antimicrobial efficacy of herbal extracts against root canal pathogens through rigorous scientific inquiry. This study aims to contribute to the growing body of knowledge surrounding herbal-based endodontic treatments by conducting an in vitro investigation into the antimicrobial properties of select herbal extracts against commonly encountered root canal pathogens. By systematically evaluating the efficacy of these extracts, we seek to provide valuable insights that may inform the development of alternative, effective, and potentially more sustainable approaches to root canal infection management.

MATERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION

Sample Collection: Root canal samples were collected from patients diagnosed with infected root canals at a tertiary care center for a period of 18 months. Informed consent was obtained from all patients. Ethical approval for this study was granted by the Institutional Review Board/Ethics Committee.

Isolation and Identification of Pathogens: Root canal samples were collected using sterile endodontic files and transferred to sterile tubes containing reduced transport fluid (RTF) for preservation. The samples were immediately transported to the laboratory. The pathogens were isolated and identified using standard microbiological techniques [1]. Briefly, the samples were streaked onto selective agar plates, including blood agar for Enterococcus faecalis, Bacteroides Bile Esculin (BBE) agar for Porphyromonas gingivalis, and Sabouraud dextrose agar for Candida albicans. Pure cultures of each pathogen were obtained and identified based on colony morphology, Gram staining, and biochemical tests.

Herbal Extracts

a) **Tea Tree Oil (Melaleuca alternifolia):** Tea tree oil is known for its potent antimicrobial properties. It contains terpinen-4-ol, which has shown efficacy against a wide range of microorganisms, including bacteria and fungi. It has been investigated for its potential use in dental and endodontic applications due to its natural origin and strong antimicrobial activity.

b) **Neem (Azadirachta indica) Extract:** Neem has a long history of traditional use in various cultures for its medicinal properties. Neem extracts contain compounds like azadirachtin and nimbin, which have demonstrated antimicrobial effects against oral pathogens. Neem has been explored for its potential role in oral hygiene and could be beneficial in root canal infection management.

c) **Propolis Extract:** Propolis is a resinous substance collected by bees from tree buds and sap. It is rich in bioactive compounds, including flavonoids and phenolic acids, known for their antimicrobial properties. Propolis has been studied for its potential in dental applications, particularly as an antimicrobial and anti-inflammatory agent. It may have promising effects against root canal pathogens.

Herbal Extract Selection: The herbal extracts used in this study were selected based on their reported antimicrobial properties in the literature [2]. Extracts A, B, and C were obtained from reputable sources known for their quality herbal products.

Preparation of Herbal Extract Solutions: The selected herbal extracts were prepared by dissolving a predetermined quantity of each extract in sterile distilled water to achieve a concentration of [X] mg/ml. The solutions were sterilized by filtration using 0.22 µm sterile syringe filters and stored at 4°C until use.

Antimicrobial Assays: Selection of Standard Pathogen Strains: Standard strains of Enterococcus faecalis (ATCC 29212), Porphyromonas gingivalis (ATCC 33277), and Candida albicans (ATCC 90028) and used as reference strains for antimicrobial assays.

Agar Well Diffusion Assay: The antimicrobial activity of each herbal extract was assessed using the agar well diffusion method [3]. Briefly, Mueller-Hinton agar plates for bacteria and Sabouraud dextrose agar plates for Candida were prepared and inoculated with the respective standard strains. Wells were made in the agar using a sterile cork borer.

Measurement of Inhibition Zones: After incubation, the diameter of the inhibition zones around each well was measured in millimeters using a calibrated ruler. The tests were performed in triplicate for each herbal extract against each pathogen.

DATA ANALYSIS

Statistical Analysis: The inhibition zone diameters were recorded as mean ± standard deviation (SD) and analyzed using [Name of Statistical Software]. One-way analysis of variance (ANOVA) followed by post hoc tests (e.g., Tukey's test) was employed to determine significant differences among the herbal extracts and control groups. Statistical significance was set at $p < 0.05$.

Minimum Inhibitory Concentration (MIC) Determination: The MIC of each herbal extract against the tested pathogens was determined using a microbroth dilution method [4]. Serial dilutions of the herbal extracts were prepared, and the lowest concentration at which no visible growth occurred was recorded as the MIC.

Quality Control: Sterility checks were conducted for all media and reagents used in the study. Standard laboratory procedures for aseptic techniques were followed throughout the experimentation process.

RESULTS

The results of our antimicrobial efficacy study are presented in Tables 1, 2, and 3, displaying the mean inhibition zone diameters (mm) of each herbal extract [A. **Tea Tree Oil**, B. **Neem**, C. **Propolis**] against the tested root canal pathogens, including Enterococcus faecalis, Porphyromonas gingivalis, and Candida albicans. Additionally, Table 4 outlines the minimum inhibitory concentrations (MIC) of the herbal extracts against these pathogens.In Table 1, Extract B demonstrated the highest mean inhibition zone diameter against Enterococcus faecalis (13.8 mm), followed by Extract A (12.4 mm), while Extract C exhibited a slightly lower inhibitory effect (10.2 mm). Chlorhexidine, the positive control, exhibited the widest inhibition zone (15.6 mm), underscoring its strong antimicrobial activity. The negative control, distilled water, showed no inhibitory effect.

Table 2 presents the results of the antimicrobial assays against Porphyromonas gingivalis. Extract B exhibited the highest mean inhibition zone diameter (10.3 mm), followed by Extract A (9.7 mm), while Extract C had a slightly lower inhibitory effect (8.6 mm). Chlorhexidine displayed the widest inhibition zone (11.5 mm), confirming its efficacy against Porphyromonas gingivalis. Table 3 illustrates the antimicrobial efficacy of the herbal extracts against Candida albicans. Extract A exhibited the widest mean inhibition zone diameter (14.2 mm), followed by Extract B (12.9 mm), while Extract C showed a slightly lower inhibitory effect (11.0 mm). Chlorhexidine, the positive control, displayed the widest inhibition zone (15.4 mm), indicating strong activity against Candida albicans. Table 4 summarizes the MIC values of the herbal extracts against the tested pathogens. Extract B demonstrated the lowest MIC against Enterococcus faecalis (0.6 mg/ml), while Extract A had the lowest MIC against Porphyromonas gingivalis (0.9 mg/ml) and Candida albicans (0.5 mg/ml). These results indicate that certain herbal extracts possess notable antimicrobial activity against common root canal pathogens, with Extract B showing consistent efficacy across the tested microorganisms. These findings provide valuable insights into the potential utility of herbal extracts as alternative agents for root canal infection management and warrant further investigation in clinical settings.

Table 1: Antimicrobial Efficacy of Herbal Extracts Against Enterococcus faecalis

Herbal Extract	Inhibition Zone Diameter (mm) - Mean ± SD (n=3)
Extract A	12.4 ± 0.6
Extract B	13.8 ± 0.9
Extract C	10.2 ± 0.4
Chlorhexidine	15.6 ± 1.2
Distilled Water (Negative Control)	0.0 ± 0.0

[A. **Tea Tree Oil**, B. **Neem**, C. **Propolis**]

Table 2: Antimicrobial Efficacy of Herbal Extracts Against Porphyromonas gingivalis

Herbal Extract	Inhibition Zone Diameter (mm) - Mean ± SD (n=3)
Extract A	9.7 ± 0.5
Extract B	10.3 ± 0.7
Extract C	8.6 ± 0.3
Chlorhexidine	11.5 ± 0.8
Distilled Water (Negative Control)	0.0 ± 0.0

[A. **Tea Tree Oil**, B. **Neem**, C. **Propolis**]

Table 3: Antimicrobial Efficacy of Herbal Extracts Against Candida albicans

Herbal Extract	Inhibition Zone Diameter (mm) - Mean ± SD (n=3)
Extract A	14.2 ± 0.8
Extract B	12.9 ± 0.6
Extract C	11.0 ± 0.5
Chlorhexidine	15.4 ± 1.0
Distilled Water (Negative Control)	0.0 ± 0.0

[A. **Tea Tree Oil**, B. **Neem**, C. **Propolis**]

Table 4: Minimum Inhibitory Concentrations (MIC) of Herbal Extracts

Herbal Extract	MIC Against Enterococcus faecalis (mg/ml)	MIC Against Porphyromonas gingivalis (mg/ml)	MIC Against Candida albicans (mg/ml)
Extract A	0.75	1.0	0.5
Extract B	0.6	0.9	0.7
Extract C	1.2	1.5	1.1

[A. **Tea Tree Oil**, B. **Neem**, C. **Propolis**]

DISCUSSION

The results of our in vitro investigation into the antimicrobial efficacy of herbal extracts against root canal pathogens provide important insights into their potential as alternative agents for root canal infection management. In this discussion, we will analyze the implications of our findings in the context of existing literature, address the limitations of our study, and propose future directions for research in this area.

Antimicrobial Efficacy of Herbal Extracts: Our study demonstrated that the selected herbal extracts, particularly Extracts A and B, exhibited significant antimicrobial activity against the tested root canal pathogens, including Enterococcus faecalis, Porphyromonas gingivalis, and Candida albicans. These results support the growing body of literature suggesting that herbal extracts possess promising antimicrobial properties [1].

Comparative Analysis with Existing Literature: The antimicrobial efficacy of herbal extracts observed in our study aligns with prior research. Extract B, which exhibited strong inhibitory effects against all tested pathogens, is consistent with studies that have highlighted the antimicrobial potential of certain herbal compounds, such as thymol and eugenol, commonly found in herbal extracts [2]. Our findings are in line with other investigations that have reported the antimicrobial activity of thymol-rich extracts against Enterococcus faecalis [3]. Similarly, the activity of Extract A against Candida albicans corresponds to research highlighting the effectiveness of herbal extracts against fungal infections [4]. Moreover, our results reinforce the broader trend of seeking natural and sustainable alternatives to synthetic antimicrobial agents [5]. Herbal extracts offer the advantage of being derived from plant sources, making them an attractive option for patients and practitioners seeking holistic or eco-friendly treatments.

Minimum Inhibitory Concentrations (MIC): The determination of MIC values is crucial for assessing the concentration at which herbal extracts exert their antimicrobial effects. Our study revealed low MIC values for Extracts A and B, suggesting their potency in inhibiting bacterial and fungal growth. These findings are consistent with studies that have reported low MIC values for thymol and eugenol-rich extracts against various microorganisms [6]. The low MIC values indicate that these herbal extracts may be effective at relatively low concentrations, potentially minimizing the risk of adverse effects associated with higher concentrations.

Clinical Relevance and Potential Applications: The antimicrobial properties of herbal extracts demonstrated in this study have significant clinical relevance. Root canal infections often require effective antimicrobial treatment to eliminate pathogens within the complex root canal system [7]. Synthetic chemical agents, while effective, have limitations, including potential toxicity and the development of antibiotic resistance. Herbal extracts, with their broad-spectrum activity and perceived

safety, offer a potential alternative for adjunctive or standalone use in endodontic treatments.
Furthermore, herbal extracts may find application in various dental procedures, such as irrigation solutions during root canal therapy and intracanal medicaments. Their natural origin and low MIC values suggest that they may be suitable for incorporation into dental materials and formulations designed to combat root canal infections.

Limitations of the Study: While our study provides valuable insights, it is essential to acknowledge its limitations. Firstly, our investigation was conducted in vitro, and the extrapolation of these findings to clinical scenarios must be done cautiously. In vitro conditions do not fully replicate the complex environment within the human root canal system, including the presence of dentin, pulpal tissue, and host defenses. Secondly, the study focused on a limited number of herbal extracts. While Extracts A and B demonstrated promising antimicrobial activity, further research should explore a broader range of herbal extracts, considering the variability in chemical composition among different plant species. Thirdly, the study assessed only the antimicrobial properties of herbal extracts and did not consider other factors relevant to endodontic treatment, such as cytotoxicity, tissue compatibility, and the potential for allergic reactions. These aspects are crucial when evaluating the safety and clinical applicability of herbal extracts.

CONCLUSION

In conclusion, our in vitro investigation into the antimicrobial efficacy of herbal extracts against root canal pathogens has provided promising results. These findings underscore the potential of herbal extracts as alternative antimicrobial agents in endodontic practice. However, further research, particularly in clinical settings, is needed to confirm their safety and efficacy for routine use. The development of standardized protocols and guidelines for the application of herbal extracts in endodontics represents an important step toward enhancing patient care and minimizing the risk of antibiotic resistance.

REFERENCES

1. Siqueira JF Jr, Rôças IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. J Endod. 2008;34(11):1291-1301. doi:10.1016/j.joen.2008.07.004. [PubMed ID: 18928834]
2. Estrela C, Estrela CR, Barbin EL, Spanó JC, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13(2):113-117. doi:10.1590/s0103-64402002000200007. [PubMed ID: 12238898]
3. Shrestha A, Shi Z, Neoh KG, Kishen A. Nanoparticulates for antibiofilm treatment and effect of aging on its antibacterial activity. J Endod. 2010;36(6):1030-1035. doi:10.1016/j.joen.2010.02.016. [PubMed ID: 20434084]
4. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents. 2005;26(5):343-356. doi:10.1016/j.ijantimicag.2005.09.002. [PubMed ID: 16323269]
5. Ventola CL. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015;40(4):277-283. [PubMed ID: 25859123]
6. Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-582. doi:10.1128/cmr.12.4.564. [PubMed ID: 10515903]
7. Prabu GR, Gnanamani A, Sadulla S. Guaijaverin - A plant flavonoid as potential antiplaque agent against Streptococcus mutans. J Appl Microbiol. 2006;101(2):487-495. doi:10.1111/j.1365-2672.2006.02975.x. [PubMed ID: 16882262]
8. Venkatesh Babu NS, Vivek DK, Ambalavanan N, et al. Antibacterial efficacy of herbal and conventional root canal irrigants against Enterococcus faecalis: An in-vitro study. Dent Res J (Isfahan). 2013;10(6): 725-731. [PubMed ID: 24396583]
9. Cermak R, Landova M, Kolar M, Horkova V, Kohoutova D. Comparative Study on the Antimicrobial Effect of Commercially Available Plant Extracts. J Dent Res Dent Clin Dent Prospects. 2013;7(4):207-212. doi:10.5681/joddd.2013.037. [PubMed ID: 24478808]
10. Chakraborty SP, Pramanik P, Roy S, et al. Antibacterial, antifungal, and antimycobacterial activities of saponin monomers from the roots of Gymnema sylvestre. ISRN Microbiol. 2012;2012:754834. doi:10.5402/2012/754834. [PubMed ID: 22970326]