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Assessment Of Synergistic Antioxidant Potential Of A Polyherbal Formulation Containing Ocimum Sanctum And Cinnamomum Zeylanicum

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Background: Oxidative stress, characterized by the excessive production of reactive oxygen species (ROS), is a key factor in the progression of periapical inflammation and impaired tissue healing in endodontic infections. Conventional irrigants and medicaments, while effective, may cause cytotoxicity and adverse reactions. Polyherbal formulations, which combine multiple herbal extracts, can enhance therapeutic efficacy through synergistic effects, providing natural alternatives that may improve healing and reduce complications in endodontic therapy. The study aimed to evaluate the antioxidant potential of a polyherbal formulation combining Ocimum sanctum (Tulsi) and Cinnamomum zeylanicum (Cinnamon) extracts.

Methods: Fresh leaves of Ocimum sanctum and bark of Cinnamomum zeylanicum were collected, processed, and subjected to aqueous extraction. The antioxidant activity of the polyherbal formulation was assessed using three in vitro assays – DPPH radical scavenging, H_2O_2 scavenging and FRAP assay.

Results: The polyherbal formulation demonstrated significant antioxidant activity in all assays. The DPPH and H_2O_2 assays confirmed enhanced free radical neutralization and oxidative stress mitigation respectively, while the FRAP assay indicated superior reducing capacity.

Conclusion: The polyherbal combination exhibits substantial antioxidant potential, making it a promising biocompatible alternative or adjuvant to conventional chemical agents used in endodontic treatment protocols. Further clinical studies are needed to validate its efficacy and safety in endodontic therapy.

Keywords: Ocimum sanctum, Cinnamomum zeylanicum, antioxidant, polyherbal, endodontics, oxidative stress, reactive oxygen species, irrigant, intracanal medicament.

Introduction:

Endodontic infections, characterized by microbial invasion of the pulp and periapical tissues, are a significant concern in dental practice due to their complex nature and potential to cause extensive tissue damage [1,2]. Effective management of these infections is crucial to ensure successful root canal treatment, prevent post-treatment complications, and promote tissue healing.[3,4] Traditionally, endodontic therapy has focused on the mechanical and chemical removal of microorganisms from the root canal system.[5] However, recent research has highlighted the critical role of oxidative stress in the pathogenesis of endodontic infections and the subsequent impact on tissue healing, emphasizing the potential therapeutic benefits of antioxidants in endodontics.[6,7]

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, leading to cellular and tissue damage. [8,9] In the context of endodontic infections, oxidative stress is primarily driven by the host's immune response to microbial invasion.[10] During infection, immune cells such as neutrophils and macrophages are recruited to the site of infection, where they generate ROS as part of the respiratory burst aimed at destroying pathogens.[11] While ROS play an essential role in microbial killing, excessive ROS production can lead to collateral damage to the surrounding tissues, exacerbating inflammation and contributing to the destruction of periapical tissues. [12,13] This oxidative damage is particularly concerning in chronic endodontic infections, where persistent inflammation can result in the formation of periapical lesions, characterized by bone resorption, tissue necrosis, and impaired healing. [14] Studies have shown that high levels of oxidative stress markers are present in periapical lesions, indicating that oxidative damage plays a significant role in the progression of these lesions.[14] Patients with chronic apical periodontitis exhibited elevated levels of malondialdehyde (MDA), a marker of lipid peroxidation, in periapical tissues, suggesting ongoing oxidative damage.[15]

In endodontics, use of antioxidants in clinical protocols can mitigate oxidative damage to dentinal tissues, enhance the healing of periapical lesions, and modulate the host's immune response.[6,16] Traditional irrigants and intracanal medicaments, such as sodium hypochlorite and calcium hydroxide, are widely used for their potent antimicrobial properties and ability to

dissolve organic tissue. However, these agents are not without limitations, including cytotoxicity to periapical tissues, potential for severe inflammatory responses, and risks associated with their extrusion beyond the apex. [5,17] In recent years, there has been a growing interest in the use of natural products that often exhibit a broad spectrum of biological activities, including antioxidant and anti-inflammatory properties, which can be beneficial in clinical settings. [18,19] Research has shown that antioxidants derived from natural sources, such as herbal extracts, offer significant benefits in reducing oxidative stress and promoting tissue regeneration.[20,21] Two such promising natural agents are Ocimum sanctum and Cinnamomum zeylanicum, which have a long history of use in traditional medicine and have notable antioxidant properties. Ocimum sanctum contains bioactive compounds which have demonstrated potent antioxidant and anti-inflammatory effects.[22] Similarly, Cinnamomum zeylanicum is rich in cinnamaldehyde, a compound with strong antioxidant activity.[23] These extracts not only offer antioxidant protection but also possess antimicrobial properties, making them suitable candidates for use as irrigants and intracanal medicaments in endodontics.[21,24]

Despite the extensive research on the individual properties of these botanicals, there is a paucity of studies investigating their combined effects. The combination of Ocimum sanctum and Cinnamomum zeylanicum offers a synergistic approach to enhancing antioxidant defenses and antimicrobial efficacy. Combination or synergy in botanical formulations can lead to increased efficacy, where the combined effect of the constituents surpasses the sum of their individual effects.[25] This synergy is particularly advantageous in mitigating oxidative stress and reducing microbial load, both of which are critical in endodontic treatments. This study aims to fill this gap by evaluating the synergistic antioxidant potential of a combined/polyherbal Ocimum sanctum and Cinnamomum zeylanicum formulation through a series of in vitro assays —DPAP, H_2O_2, and FRAP.

Materials and methods:

Collection of plant materials:

Fresh leaves of Ocimum sanctum and Cinnamomum zeylanicum bark sticks were procured from a local market in Chennai. The authenticity of the plant materials was confirmed by a botanist.

Preparation of polyherbal extract:

The collected plant materials were thoroughly cleaned twice with distilled water to remove any impurities and dirt. The cleaned Ocimum sanctum leaves and Cinnamomum zeylanicum bark sticks were then air-dried at room temperature for 48 hours to remove excess moisture. The dried plant materials were ground into a fine powder using a mechanical grinder. Five grams of Ocimum sanctum leaf powder and five grams of Cinnamomum zeylanicum bark powder were accurately weighed using an analytical balance (Figure 1A and 1B). The weighted powders were mixed with 50 ml of distilled water in a clean beaker. The mixture was heated using a heating mantle at 70°C for 10 minutes, ensuring continuous stirring to

facilitate the extraction process. After heating, the mixture was allowed to cool to room temperature. The cooled mixture was filtered through Whatman No.1 filter paper to remove any solid residues.

Concentration of the extract and storage:

The filtered extract was further concentrated by placing it in a heating mantle, allowing the water to evaporate until the volume was reduced to approximately 5 ml (Figure 1C). The concentrated extract was then transferred to a clean container and subjected to drying under controlled conditions to preserve the therapeutic constituents of the plants. The dried extract was reconstituted with a small volume of distilled water to ensure homogeneity. The prepared extract was placed in an orbital shaker set at 100 rpm and maintained at room temperature for 24 hours. After incubation, the extract was stored in a sterile, airtight container at 4°C until further use in the experimental assays.

Evaluation of antioxidant activity:

To evaluate the antioxidant properties of the polyherbal extract, three in vitro assays were performed: DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging assay, hydrogen peroxide (H_2O_2) scavenging assay, and ferric reducing antioxidant power (FRAP) assay.

DPPH Radical Scavenging Assay: To assess the DPPH radical scavenging activity, a 0.1 mM DPPH stock solution was prepared using methanol. For each test, a fresh working solution of 20 µM DPPH was created. Various concentrations of the polyherbal extract (ranging from 10 µg/mL to 50 µg/mL) were added to 200 µL of the DPPH solution in a 96-well plate. The mixture was then incubated at room temperature for 30 minutes, shielded from light. The absorbance was recorded at 517 nm using a microplate reader. Methanol was used as a blank control. Ascorbic acid at 1 mg/mL was used as the reference standard. The scavenging activity of the polyherbal extract was calculated with the following equation:

$$\% \text{ DPPH Scavenging Activity} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

where $A_{control}$ represents the absorbance of the DPPH solution without the sample and A_{sample} is the absorbance of the DPPH solution mixed with the polyherbal extract.

Hydrogen Peroxide (H_2O_2) Scavenging Assay: The hydrogen peroxide scavenging activity was tested following a modified Halliwell method. A reaction mixture containing 1 mL of 100 µL of 28 mM 2-deoxy-2-ribose was prepared. The polyherbal extract was added in varying concentrations (10-50 µg/mL) alongside 200 µL of 200 µM ferric chloride, 200 µL of EDTA, and 100 µL of ascorbic acid. After incubation at 37°C for 1 hour, the absorbance was measured at 532 nm using a UV spectrophotometer. Vitamin E was employed as the positive control. Scavenging activity was determined using the formula:

$$\% \text{ Hydroxyl radical scavenging activity} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

Where $A_{control}$ is the is the absorbance without the test sample, and A_{sample} includes the polyherbal extract.

Ferric Reducing Antioxidant Power (FRAP) Assay:

To determine the ferric reducing antioxidant power (FRAP), several reagents were prepared, including 300 mM acetate buffer at pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3.6H_2O$. The FRAP reagent was freshly mixed in a ratio of 10:1:1 (acetate buffer: TPTZ: $FeCl_3.6H_2O$). 3.6 mL of the FRAP reagent was mixed with 0.4 mL of distilled water and incubated at 37°C for 5 minutes. Various concentrations of the polyherbal extract (10-50 µg/mL) were added to the solution, incubated for 10 minutes at 37°C, and absorbance was measured at 593 nm. A standard curve was constructed using ferrous sulfate ($FeSO_4.7H_2O$) solutions to quantify the reducing power of the extract.

Statistical Analysis

The antioxidant activity of the polyherbal extract was evaluated using SPSS version 2.0. Results are presented as mean ± standard deviation (SD), derived from three independent experiments. A one-way ANOVA was applied to assess statistical differences between groups, with a significance level of $p < 0.05$.

Results:

The combined polyherbal extract Ocimum sanctum and Cinnamomum zeylanicum consistently exhibited significant antioxidant activity across all three assays (Figure 2).The assay results exhibited a clear dose-response effect, where higher extract concentrations were associated with progressively stronger antioxidant activity.. The statistically significant findings across all assays ($p < 0.05$) exhibit the polyherbal extract's potential as a powerful antioxidant agent.

The DPPH assay highlights the extract's capacity to neutralize free radicals, an essential function in preventing the initiation of oxidative chain reactions that can lead to cellular damage. The polyherbal extract demonstrated a strong antioxidant capacity in the DPPH assay, with results showing a concentration-dependent increase in free radical scavenging activity (Figure 2A). The results show that 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml of the polyherbal extract has higher inhibition of DPPH free radicals than compared with ascorbic acid (standard).

The H_2O_2 assay highlights the extract's capacity to mitigate oxidative stress, which is a key factor in protecting cells from damage associated with various chronic conditions. In the H_2O_2 scavenging assay, the polyherbal extract exhibited a significant dose-dependent reduction in hydrogen peroxide levels (Figure 2B), further confirming its antioxidant potential. The results show that 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml of the polyherbal extract has higher percentage of hydroxyl radical scavenging activity than Vitamin E (standard).

The FRAP assay highlights the extract's substantial reducing power, indicating its role in neutralizing oxidative agents and maintaining redox equilibrium within biological systems. The results of the FRAP assay further supported the strong antioxidant activity of the polyherbal extract with a concentration-dependent increase in reducing power (Figure 2C). The findings show that 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml of the polyherbal extract has higher ability to donate electrons and reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) than 0.1 - 1.5 mM $FeSO_4.7H_2O$ in methanol (standard).

Discussion:

The present study aimed to explore the antioxidant potential of a polyherbal extract containing Ocimum sanctum (holy basil) and Cinnamomum zeylanicum (cinnamon) through a series of well-established in vitro assays. The results demonstrated that the polyherbal extract exhibited significant antioxidant activity, as evidenced by its performance in the DPPH, H_2O_2, and FRAP assays. These findings support the hypothesis that the combination of these two herbs has a synergistic effect on antioxidant efficacy, providing a natural alternative for managing oxidative stress in endodontic therapy.

During endodontic infections, excessive production of reactive oxygen species (ROS) exacerbates inflammation, leading to periapical tissue damage, bone resorption, and impaired tissue regeneration.[14,26] ROS, which include free radicals like superoxide anion and hydroxyl radical, as well as non-radical species like hydrogen peroxide, can damage cellular components, leading to apoptosis or necrosis. [27–29] To counteract ROS, the body deploys antioxidant defense mechanisms, including enzymatic systems (e.g., peroxidases) and non-enzymatic antioxidants (e.g., vitamins and uric acid). However, when ROS production exceeds the body's antioxidant capacity, a state known as oxidative stress occurs, leading to further tissue damage. [8,30–32] Oxidative stress plays a critical role in the pathogenesis of endodontic diseases, [7] contributing to tissue destruction and delaying the healing process further complicating the resolution of endodontic infections.[14] This oxidative damage contributes to the breakdown of periapical tissues, particularly bone, during the host defense process. [11,33–35] The ability to neutralize ROS through antioxidant mechanisms is therefore essential for enhancing the success of endodontic treatment, promoting periapical healing, and minimizing complications such as persistent infection and post-treatment inflammation.[6]

Antioxidants have the potential to neutralize these ROS, thereby reducing oxidative stress and promoting tissue healing.[16,36] The inclusion of antioxidants into endodontic procedures, whether as irrigants or intracanal medicaments, could offer additional therapeutic benefits by helping in modulating inflammation, protecting tissues from oxidative damage, and supporting the regeneration of damaged periapical tissues. [26] Recent studies have also underscored the importance of antioxidants in reducing ROS-induced damage to both hard and soft tissues, enhancing the host's immune response, and promoting healing.[37] Given the increasing interest in biocompatible and natural adjuncts in dental care, the antioxidant properties of herbal extracts like those from Ocimum sanctum and Cinnamomum zeylanicum are particularly relevant.[20] These natural compounds could serve as effective alternatives or complements to

conventional chemical agents used in endodontic therapy, offering additional benefits related to ROS neutralization and tissue repair with potentially fewer adverse effects. [38]

Ocimum sanctum, also known as holy basil, is known for its rich content of several phenolic compounds, flavonoids, and tannins that have been shown to exhibit strong free radical scavenging activity.[22,39,40] These compounds can donate electrons to neutralize free radicals, thus interrupting chain reactions that lead to cellular damage.[24] Cinnamomum zeylanicum, on the other hand, contains cinnamaldehyde, eugenol, and other polyphenols which are potent antioxidants known to neutralize free radicals and prevent oxidative damage.[23,41] Cinnamaldehyde, in particular, has been shown to inhibit lipid peroxidation and scavenge ROS, which are critical in preventing oxidative stress-related tissue damage.[23]

While these individual herbal extracts have been studied extensively for their medicinal properties including antioxidant potential, this study employs a novel approach to assess the synergistic antioxidant potential of a polyherbal combination for use in endodontic protocols. A growing body of research supports the concept of polyherbal synergy.[42,43] For instance, several studies have reported that combining multiple herbs with known antioxidant activities resulted in a formulation with significantly improved free radical scavenging abilities compared to the individual components. [37,44] The rationale behind polyherbal formulations lies in their ability to harness the combined effects of various bioactive compounds present in each plant extract that can interact in ways that enhance their overall therapeutic efficacy by potentially targeting multiple pathways simultaneously compared to individual components.[45,46] Such synergism supports the use of these herbal combinations in clinical settings, where complex biological environments may benefit from multifaceted therapeutic interventions [47]. This is crucial in endodontic therapy, where the multifactorial nature of delayed healing, tissue inflammation and infection requires a comprehensive approach to treatment.[2,48]

In the present study, the combination of Ocimum sanctum and Cinnamomum zeylanicum demonstrated a synergistic antioxidant effect, suggesting that these herbal extracts can interact in ways that potentiate each other's effects, resulting in greater neutralization of reactive oxygen species (ROS) and providing a more potent defense against oxidative stress than either would alone. [46] The results from the DPPH assay in the present study showed that the polyherbal extract had a higher scavenging capacity. This indicates that the combination of these two herbs amplifies their antioxidant capacity, possibly through complementary mechanisms of action. In the H_2O_2 assay, the polyherbal extract demonstrated significant hydrogen peroxide scavenging ability, further confirming its potential to mitigate oxidative stress. Finally, the FRAP assay indicated that the polyherbal extract had a strong ability to reduce ferric ions, which is indicative of its capacity to donate electrons and counteract oxidative damage. These findings align with previous research suggesting that combining different herbs with distinct bioactive compounds can lead to a more pronounced therapeutic effect, as the compounds may work together to enhance each other's efficacy.[43,44] This is likely due to the presence of these multiple bioactive compounds that can target different pathways of oxidative stress, leading to a more comprehensive antioxidant effect.[18,46]

The synergistic effects observed in this study provide a scientific basis for the use of polyherbal formulations in endodontics and other fields of dentistry, where oxidative stress plays a pivotal role in disease progression and tissue damage. Additionally, the antimicrobial activity of these herbal extracts could further enhance their role in endodontic therapy. Both Ocimum sanctum and Cinnamomum zeylanicum have been reported to possess significant antimicrobial properties against a wide range of oral pathogens. [49,50] This dual action of reducing oxidative stress while combating infection makes the polyherbal extract a promising candidate for use in root canal irrigation and as an intracanal medicament.[51,52]

Thus, the use of natural antioxidants such as Ocimum sanctum and Cinnamomum zeylanicum in a polyherbal formulation presents a biocompatible alternative to conventional agents such as sodium hypochlorite and calcium hydroxide, potentially reducing the risk of adverse reactions while offering additional benefits related to ROS neutralization and tissue repair. Future research should explore the integration of these herbal extracts into endodontic protocols, evaluating their efficacy in combination with conventional treatments or as standalone agents.

While the findings of this study are promising, several limitations should be acknowledged. The in vitro nature of the assays provides valuable insights into the antioxidant properties of the polyherbal extract but does not fully capture its potential effects in a clinical setting. Further studies are needed to assess the biocompatibility and efficacy of the polyherbal extract in vivo, particularly in terms of its impact on periapical healing and microbial eradication. Moreover, while this study focused on the antioxidant potential of the polyherbal extract, future research should explore its effects on other aspects of endodontic therapy, such as biofilm disruption, dentin conditioning, and long-term outcomes following root canal therapy. Clinical trials are essential to determine the safety and efficacy of these herbal extracts in endodontic practice.

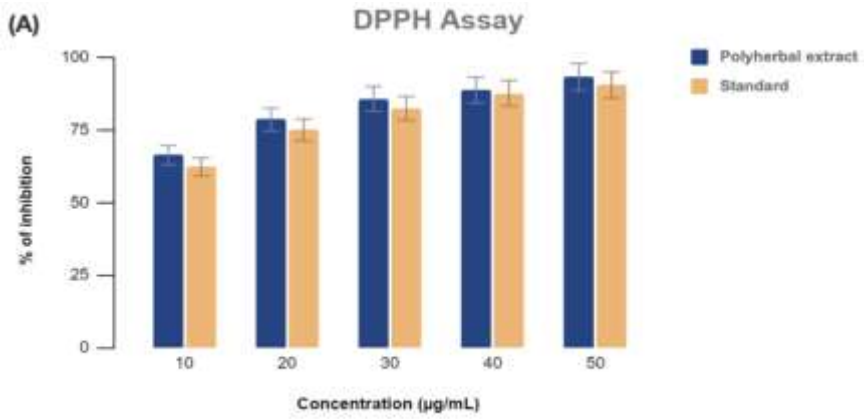
Conclusion:

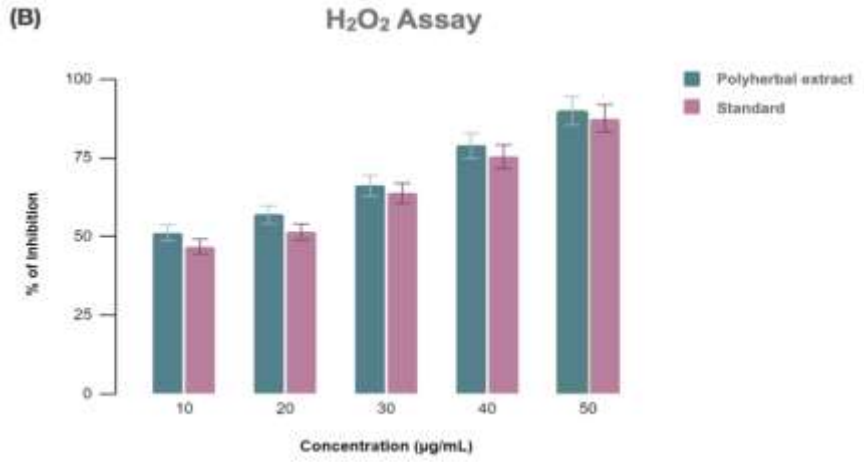
This study highlights the significant antioxidant potential of a polyherbal extract containing Ocimum sanctum and Cinnamomum zeylanicum, demonstrating its ability to neutralize free radicals and mitigate oxidative stress through various in vitro assays. . The synergistic effects of these two herbs offer a promising natural alternative or adjunct to conventional endodontic irrigants and medicaments, potentially improving treatment outcomes by reducing ROS-induced damage and promoting tissue healing. The integration of herbal antioxidants into endodontic therapy could provide a biocompatible, effective, and patient-friendly approach to managing oxidative stress and inflammation, paving the way for future clinical applications.

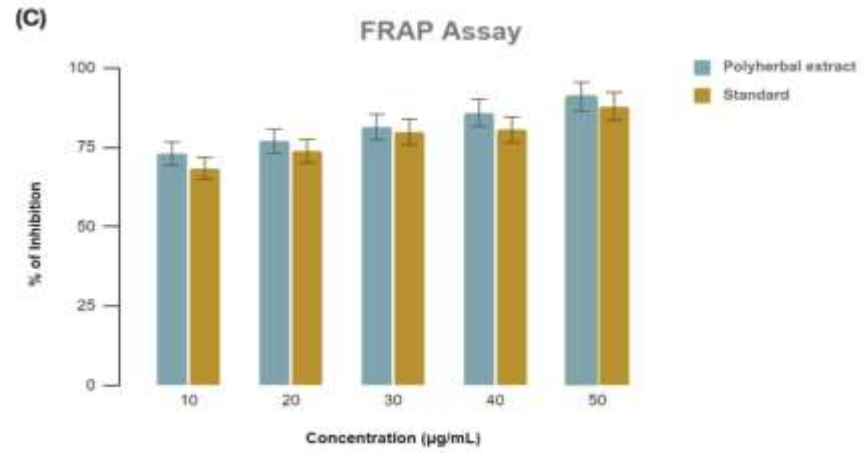
FIGURE LEGENDS:

Figure 1: (A) Five grams of commercially available Ocimum sanctum leaf powder; **(B)** Five grams of commercially available Cinnamomum zeylanicum bark powder; **(C)** Concentration of filtered extract in a laboratory heating mantle to a volume of 5 ml.

Figure 2: Results of antioxidant properties of the polyherbal extract in the conducted assays compared to standards: **(A)** DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging assay; **(B)** Hydrogen Peroxide (H_2O_2) scavenging assay; **(C)** Ferric Reducing Antioxidant Power (FRAP) assay.







Conflict of interest

Authors declare no conflict of interest

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Nil

References:

1. Wong J, Manoil D, Näsman P, et al. Microbiological Aspects of Root Canal Infections and Disinfection Strategies: An Update Review on the Current Knowledge and Challenges. Front Oral Health 2021; 2: 672887.
2. Karamifar K, Tondari A, Saghiri MA. Endodontic Periapical Lesion: An Overview on the Etiology, Diagnosis and Current Treatment Modalities. Eur Endod J 2020; 5: 54–67.
3. Siqueira JF Jr. Microbial causes of endodontic flare-ups. Int Endod J 2003; 36: 453–463.
4. Estrela C, Holland R, Estrela CR de A, et al. Characterization of successful root canal treatment. Braz Dent J 2014; 25: 3–11.
5. Zou X, Zheng X, Liang Y, et al. Expert consensus on irrigation and intracanal medication in root canal therapy. Int J Oral Sci 2024; 16: 23.
6. Patel S, Hans MK, Chander S, et al. Antioxidants in Endodontics: A Strategic Review. J Clin Diagn Res 2015; 9: ZE12–5.
7. Vengerfeldt V, Mändar R, Saag M, et al. Oxidative stress in patients with endodontic pathologies. J Pain Res 2017; 10: 2031–2040.
8. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007; 39: 44–84.
9. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000; 408: 239–247.
10. Lamster IB, Novak MJ. Host mediators in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. Crit Rev Oral Biol Med 1992; 3: 31–60.
11. Chapple IL. Reactive oxygen species and antioxidants in inflammatory diseases. J Clin Periodontol 1997; 24: 287–296.
12. Brock GR, Butterworth CJ, Matthews JB, et al. Local and systemic total antioxidant capacity in periodontitis and health. J Clin Periodontol 2004; 31: 515–521.
13. Mittal M, Siddiqui MR, Tran K, et al. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal 2014; 20: 1126–1167.
14. Dezerega A, Madrid S, Mundi V, et al. Pro-oxidant status and matrix metalloproteinases in apical lesions and gingival crevicular fluid as potential biomarkers for asymptomatic apical periodontitis and endodontic treatment response. J Inflamm 2012; 9: 8.
15. Akalin FA, Baltacioğlu E, Alver A, et al. Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis. J Clin Periodontol 2007; 34: 558–565.
16. Yeung SY, Huang CS, Chan CP, et al. Antioxidant and pro-oxidant properties of chlorhexidine and its interaction with calcium hydroxide solutions. Int Endod J 2007; 40: 837–844.
17. Ordinola-Zapata R, Noblett WC, Perez-Ron A, et al. Present status and future directions of intracanal medicaments. Int Endod J 2022; 55 Suppl 3: 613–636.
18. Veeraswamy SD, Raju I, Mohan S. Phytochemical Evaluation and Antioxidant Potential of Polyherbal Extract Mixture-an In Vitro and In Silico Study. Appl Biochem Biotechnol 2023; 195: 672–692.
19. Sies H. Oxidative stress: oxidants and antioxidants. Exp Physiol 1997; 82: 291–295.
20. Rodríguez-Yoldi MJ. Anti-Inflammatory and Antioxidant Properties of Plant Extracts.

Antioxidants (Basel); 10. Epub ahead of print 7 June 2021. DOI: 10.3390/antiox10060921.
21. Chamseddine R, Abiad RS. Uses of herbal extracts in endodontology: A literature review. BAU Journal - Science and Technology; 5. Epub ahead of print 30 June 2024. DOI: 10.54729/2959-331x.1124.
22. Chaudhary A, Sharma S, Mittal A, et al. Phytochemical and antioxidant profiling of. J Food Sci Technol 2020; 57: 3852–3863.
23. Alizadeh Behbahani B, Falah F, Lavi Arab F, et al. Chemical Composition and Antioxidant, Antimicrobial, and Antiproliferative Activities of Bark Essential Oil. Evid Based Complement Alternat Med 2020; 2020: 5190603.
24. Ghasemzadeh A, Ghasemzadeh N. Flavonoids and phenolic acids: Role and biochemical activity in plants and human. Journal of Medicinal Plants Research; 5. Epub ahead of print 23 December 2011. DOI: 10.5897/JMPR11.1404.
25. Wagner H, Ulrich-Merzenich G. Synergy research: approaching a new generation of phytopharmaceuticals. Phytomedicine 2009; 16: 97–110.
26. Arias Z, Nizami MZI, Chen X, et al. Recent Advances in Apical Periodontitis Treatment: A Narrative Review. Bioengineering (Basel); 10. Epub ahead of print 19 April 2023. DOI: 10.3390/bioengineering10040488.
27. Trivedi S, Lal N. Antioxidant enzymes in periodontitis. J Oral Biol Craniofac Res 2017; 7: 54–57.
28. Reis J, Ramos A. In Sickness and in Health: The Oxygen Reactive Species and the Bone. Front Bioeng Biotechnol 2021; 9: 745911.
29. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem 2015; 30: 11–26.
30. Liskmann S, Vihalemm T, Salum O, et al. Characterization of the antioxidant profile of human saliva in peri-implant health and disease. Clin Oral Implants Res 2007; 18: 27–33.
31. Inchingolo F, Marrelli M, Annibali S, et al. Influence of endodontic treatment on systemic oxidative stress. Int J Med Sci 2014; 11: 1–6.
32. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 2004; 142: 231–255.
33. Mody N, Parhami F, Sarafian TA, et al. Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. Free Radic Biol Med 2001; 31: 509–519.
34. Blagov AV, Summerhill VI, Sukhorukov VN, et al. Potential use of antioxidants for the treatment of chronic inflammatory diseases. Front Pharmacol 2024; 15: 1378335.
35. Subramanian AK, Narayan V, Navaneethan R. Oxidative Stress and Oral Diseases. In: Role of Oxidative Stress in Pathophysiology of Diseases. Singapore: Springer Singapore, 2020, pp. 1–12.
36. Azat Aziz M, Shehab Diab A, Abdulrazak Mohammed A. Antioxidant categories and mode of action. In: Antioxidants. IntechOpen, 2019.
37. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov 2021; 20: 689–709.
38. Karobari MI, Adil AH, Assiry AA, et al. Herbal Medications in Endodontics and Its Application-A Review of Literature. Materials ; 15. Epub ahead of print 25 April 2022. DOI: 10.3390/ma15093111.
39. Joshua A, Gayathri R, Vishnu Priya V. Preliminary phytochemical analysis and total phenolic content of the ethanolic seed extract of Ocimum sanctum. Drug Invention Today; 13
40. Ramamurthy J, Jayakumar ND. Ocimum sanctum and its effect on oral health A comprehensive review. Drug Invention Today 2019; 11: 819–821.
41. Jagadish Rajkumaar R, Rajasekar A, Rajeshkumar S. ANTIMICROBIAL AND ANTIOXIDANT ACTIVITY OF CLOVE AND CINNAMON HERBAL FORMULATION: AN in vitro STUDY
42. Spinella M. The importance of pharmacological synergy in psychoactive herbal medicines. Altern Med Rev 2002; 7: 130–137.

43. Karole S, Shrivastava S, Thomas S, et al. Polyherbal Formulation Concept for Synergic Action: A Review. J Drug Deliv Ther 2019; 9: 453–466.
44. Mapeka TM, Sandasi M, Viljoen AM, et al. Optimization of Antioxidant Synergy in a Polyherbal Combination by Experimental Design. Molecules; 27. Epub ahead of print 29 June 2022. DOI: 10.3390/molecules27134196.
45. Williamson EM. Synergy and other interactions in phytomedicines. Phytomedicine 2001; 8: 401–409.
46. Che C-T, Wang ZJ, Chow MSS, et al. Herb-herb combination for therapeutic enhancement and advancement: theory, practice and future perspectives. Molecules 2013; 18: 5125–5141.
47. Aslam MS, Ahmad MS, Mamat AS, et al. An Update Review on Polyherbal Formulation: A Global Perspective. Syst Rev Pharm 2016; 7: 35–41.
48. Holland R, Gomes JE Filho, Cintra LTA, et al. Factors affecting the periapical healing process of endodontically treated teeth. J Appl Oral Sci 2017; 25: 465–476.
49. Sisodiya M, Ahmed S, Sengupta R, et al. A comparative assessment of pomegranate extract, sodium hypochlorite, chlorhexidine, Myrrh (Commiphora molmol), tulsi extract against , and. J Oral Maxillofac Pathol 2021; 25: 369.
50. Panchal V, Gurunathan D, Thangavelu L. Comparison of antibacterial efficacy of cinnamon extract and calcium hydroxide as intracanal medicament against E. fecalis: An in vitro study. Pharmacogn J 2018; 10: 1165–1168.
51. Kale PP, Raut AW. A proposed classification system for herbal endodontic irrigants. J Conserv Dent 2021; 24: 293–295.
52. Kishan KV, Shah NC, Das DT, et al. Herbal medicaments in endodontics - Current guidelines for studies in India. J Conserv Dent 2019; 22: 411–414.