

Free radical scavenging activity of herbal formulation based mouthwash using Green tea and black tea extract

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ABSTRACT

BACKGROUND: Due to the presence of polyphenols and flavonoids, the tea plant *Camellia sinensis* is well known for its abundant antioxidant capabilities. These bioactive substances are essential for reducing oxidative stress, which is a major contributor to plaque development and gingival inflammation, by neutralizing free radicals. Mouthwashes made of herbs, especially tea-based ones, are becoming more and more popular as safer substitutes for traditional chemicals like chlorhexidine.

AIM: The aim of this study was to evaluate the free radical scavenging activity of a herbal mouthwash formulated using green and black tea extracts and to compare its effectiveness with the standard chlorhexidine.

MATERIALS AND METHODS: Fresh tea leaves were extracted by boiling in distilled water for 15 minutes, followed by filtration to obtain aqueous extracts. The prepared extracts were subjected to antioxidant evaluation using DPPH and hydrogen peroxide (H_2O_2) scavenging assays. ELISA plates were prepared and incubated at $37^\circ C$ for 24 hours. Different concentrations (10–50 μL) of the extracts were tested, and absorbance was measured using a microplate reader. Statistical analysis was performed using SPSS software.

RESULTS AND DISCUSSION: The results demonstrated a concentration-dependent increase in antioxidant activity in both DPPH and H_2O_2 assays. The herbal mouthwash exhibited free radical scavenging activity comparable to chlorhexidine across all tested concentrations. The synergistic effect of polyphenols from green tea and flavonoids from black tea contributed to the enhanced antioxidant potential. Clinically, the formulation showed effectiveness as a plaque inhibitor and was preferred due to its better taste and convenience, suggesting improved patient compliance for long-term use.

CONCLUSION: The herbal tea-based mouthwash is as effective as chlorhexidine in controlling plaque regrowth and gingival inflammation, with the added advantage of reduced side effects such as tooth staining. This highlights its potential as a safe, natural, and effective alternative for routine oral hygiene.

Keywords: Herbal mouthwash, Green tea, Black tea, Antioxidant activity, H₂O₂ scavenging

INTRODUCTION

The tea plant is called *Camellia sinensis* by botanists. These are small, bushy and 3-4 feet high. Tea leaves are picked 3-4 times each year from spring to autumn. The two main types of tea, black and green are made from the same *Camellia sinensis* plant. Black tea is more commonly consumed, accounting for 75% of the world's consumption and about 98% of the international tea trade. Green tea is popular in China and Japan and is gaining popularity in many Western countries due to its recognized health benefits. Green tea is a type of "unfermented" aged tea made by drying and steaming fresh leaves. The catechins contained in green tea are called epigallocatechin-3-gallate (EGCG). (1) Catechin helps prevent cell damage and provides other beneficial effects. Hot drinking tea may have a better cleansing effect compared to cold supplements. Black tea has a beneficial effect on the gastrointestinal tract. By affecting hormonal balance and antioxidant function, it affects motility, absorbability, microflora, etc. Black tea improves bone density. It is also antiviral due to its enzyme inhibitory and receptor blocking properties. Its role in gastrointestinal, liver, and prostate cancers has been confirmed, but its effect on urinary tract cancer is uncertain and requires further research.(2)

There is growing interest around the world in drinking tea which may be associated with the antioxidant effects of polyphenols that fight the harmful effects of environmental free radicals. Green tea has polyphenols, including the four major catechins- epigallocatechin gallate (EGCG), epigallocatechin gallate (ECG), epigallocatechin (EC), epigallocatechin (EGC). Contains four major catechins. (3) Green tea also contains minerals such as carotenoids, tocopherols, ascorbic acid, Cr, Mn, Se and Zn, as well as certain phytochemicals. The various reported therapeutic and biological activities of catechins lead to a reduced incidence of various pathological conditions including cardiovascular disease, stroke, obesity and cancer. These effects are partly due to the antioxidant and free radical scavenging effects of the polyphenol component of green tea.(3,4)

Previous studies have shown that green tea polyphenol catechins, especially (-)-(EGCg) and (-)-(ECg) can inhibit the growth of various gram-positive and gram-negative bacteria with moderate potency. There is evidence that these molecules may help fight common oral infections such as tooth decay and periodontal disease.(5) Previous literature has also shown that about the biological activity of green tea, less research has been cited for its use as an anti-plaque agent, and no data on its comparative effect with chlorhexidine gluconate (gold standard) are available. Numerous studies have demonstrated the effectiveness and usefulness of antiseptic mouthwashes containing the active ingredients chlorhexidine and essential oils for the prevention

and control of the formation of plaque and gingival inflammation when used in addition to mechanical treatment. (6)

Chlorhexidine is still considered the gold standard for its antibacterial activity but due to its side effects such as discoloration, taste changes, oral mucosal desquamation and supragingival calculus formation it cannot be used long-term, so alternative active ingredients based on herbal extracts are especially important. There is evidence of the beneficial effects of plant extracts in case of gingival inflammation and plaque accumulation or subgingival periodontal disease microorganisms. (7) Therefore, more and more people around the world are turning to natural herbal products for both prevention and treatment of various illnesses. Mouthwash, a safe and effective delivery system for antibacterial agents, can play an important role in plaque reduction and various other illnesses. A suitable mouthwash is a mouth disinfectant that reduces exposure to oral pathogens, prevents bad breath, has caries prevention properties, heals gum wounds and ulcers and provides an excellent supply of fluoride(8). Over the last two decades, herbal medicines and the tendency to use natural or organic remedies, especially for long-term and routine use have become more and more popular. The aim of this study is to qualify the effectiveness of the free radical scavenging activity of herbal formulation using green and black tea extracts.

MATERIALS AND METHODS

Fresh leaves of green and black tea (*Camellia sinensis*) were collected and thoroughly washed with distilled water to eliminate dust and surface impurities. The cleaned leaves were then subjected to aqueous extraction by boiling in distilled water for approximately 15 minutes, allowing the release of bioactive constituents such as polyphenols, catechins, and flavonoids. The extracts were allowed to cool to room temperature and subsequently filtered using filter paper to remove particulate matter, yielding a clear solution. The filtrates were stored under appropriate conditions until further analysis.

For the evaluation of antioxidant activity, different concentrations of the prepared herbal extracts (10–50 μL) were prepared. These were added to ELISA plates, ensuring proper pipetting and uniform distribution. The plates were then incubated at 37°C for 24 hours, facilitating sufficient interaction between the phytochemical components of the extracts and the free radicals present in the assay system.

The antioxidant potential was assessed using standard in vitro assays, namely the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the hydrogen peroxide (H_2O_2) scavenging assay. In the DPPH assay, the ability of the extracts to donate hydrogen atoms and neutralize stable free radicals was measured, while the H_2O_2 assay evaluated the capacity of the extracts to scavenge reactive oxygen species. A standard control, chlorhexidine, was included for comparative evaluation. Following incubation, absorbance readings were recorded using a microplate reader at appropriate wavelengths, and the percentage inhibition of free radicals was calculated.

All experiments were performed under standardized conditions, and the obtained data were compiled and analyzed statistically using SPSS software. Appropriate statistical tests were applied to determine the significance and reliability of the results, ensuring the validity of the study outcomes.

RESULTS

The antioxidant potential of the herbal mouthwash formulated using green and black tea extracts was evaluated using DPPH and H_2O_2 scavenging assays, and the findings demonstrated strong free radical scavenging activity.

In the DPPH assay (Figure 1), both the standard (chlorhexidine) and the herbal extract showed a steady increase in percentage inhibition with increasing concentration (10 μ L to 50 μ L). The herbal formulation exhibited inhibition values very close to the standard across all concentrations. At lower concentrations (10–20 μ L), the herbal extract showed slightly lower but comparable activity, while at higher concentrations (40–50 μ L), the difference became minimal, indicating near-equivalent antioxidant efficiency.

Similarly, in the H_2O_2 scavenging assay (Figure 2), a concentration-dependent increase in antioxidant activity was observed. The herbal extract demonstrated inhibition values comparable to the standard throughout the tested range. At certain concentrations (around 20–30 μ L), the herbal extract showed nearly equal or marginally better activity than the standard.

Overall, both assays confirm that the antioxidant activity is dose-dependent, increasing with concentration, and that the herbal mouthwash performs comparably to chlorhexidine, the standard reference.

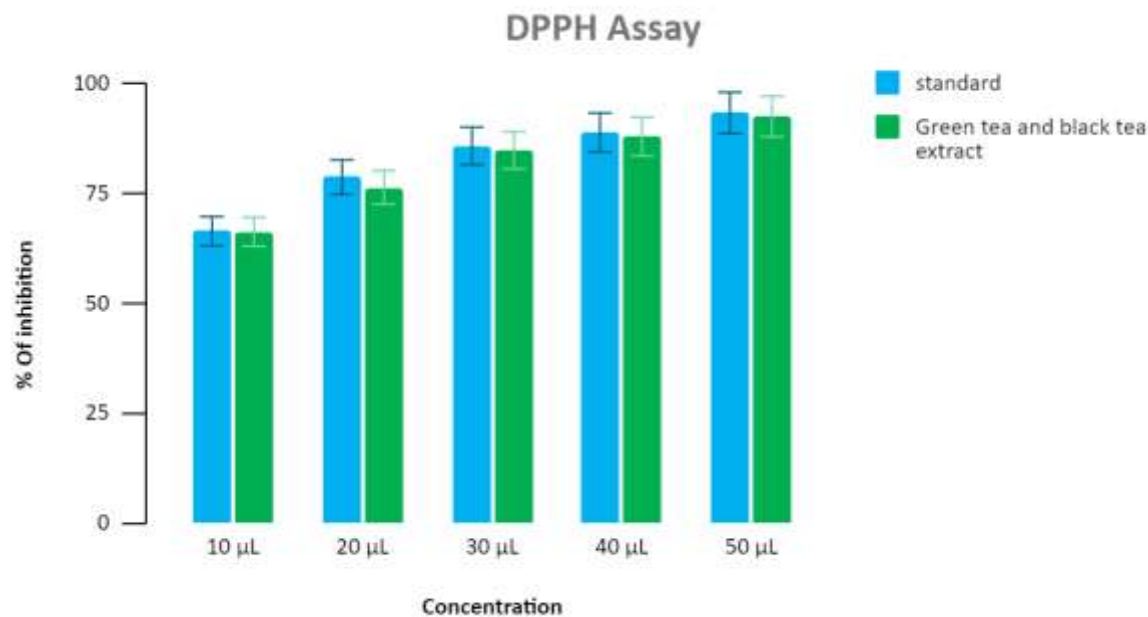


Figure 1: DPPH assay between the standard (chlorhexidine) and the herbal extract

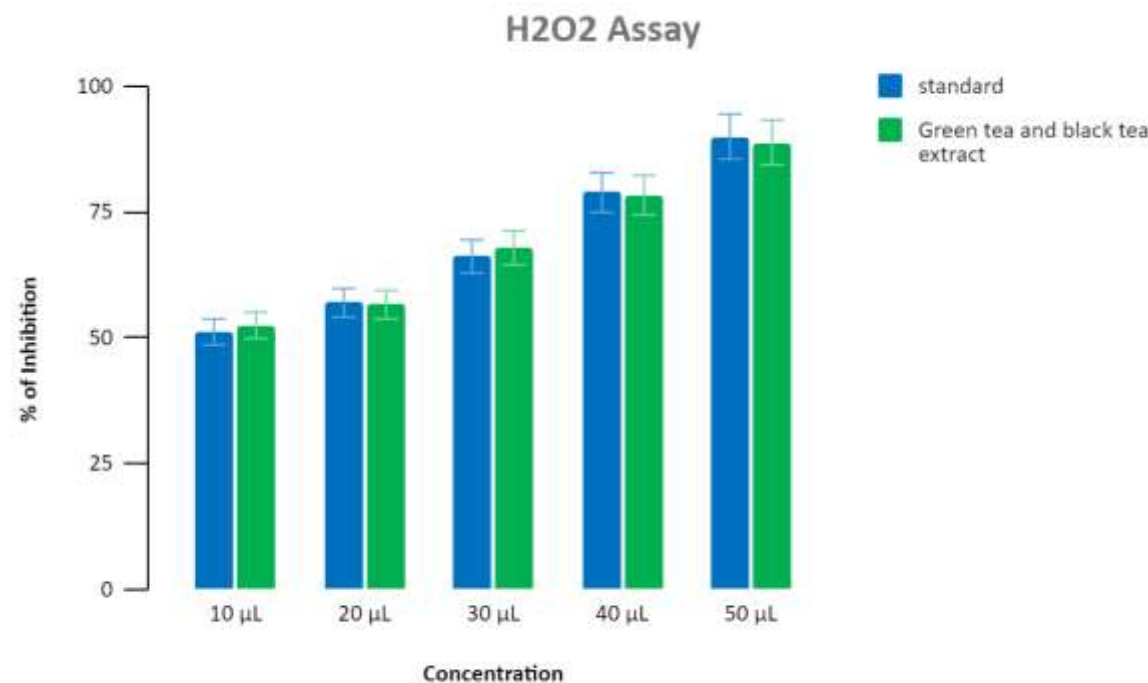


Figure 2: H2O2 assay between the standard (chlorhexidine) and the herbal extract

DISCUSSION

The findings of the present study highlight the significant antioxidant potential of the herbal mouthwash formulated using green and black tea extracts. The observed activity can be primarily attributed to the rich presence of bioactive compounds in tea, particularly polyphenols in green tea and flavonoids in black tea. These compounds are well known for their ability to donate hydrogen atoms or electrons, thereby neutralizing free radicals such as DPPH and reactive oxygen species like hydrogen peroxide. The combination of both extracts appears to produce a synergistic effect, enhancing the overall antioxidant capacity beyond what either component might achieve alone.

The concentration-dependent increase in activity observed in both assays further supports the efficacy of the formulation, indicating that higher concentrations of these phytochemicals lead to greater free radical neutralization. This is particularly important in the oral environment, where oxidative stress plays a crucial role in the initiation and progression of plaque formation and periodontal inflammation. By reducing oxidative stress, the herbal mouthwash may help in preventing cellular damage in gingival tissues and limiting the growth of plaque-forming microorganisms.(8,9)

From a clinical standpoint, the comparable performance of the herbal formulation to chlorhexidine is highly significant. While chlorhexidine is considered the gold standard for plaque control, its long-term use is often limited due to side effects such as tooth staining, altered taste sensation, and mucosal irritation.(10) In contrast, the herbal mouthwash demonstrated similar efficacy without these drawbacks, making it a more favorable option for routine and prolonged use.

Additionally, patient acceptability plays a key role in the success of any oral hygiene product. The preference for the herbal mouthwash in terms of taste and ease of use suggests better compliance, which is essential for achieving consistent therapeutic outcomes. The natural origin of the formulation also aligns with the growing interest in herbal and alternative medicine, further increasing its appeal.

Overall, the study supports the potential of tea-based herbal formulations as effective, safe, and patient-friendly alternatives to conventional chemical mouthwashes. However, further clinical trials and long-term studies are recommended to validate these findings and standardize the formulation for widespread use.

CONCLUSION

The study concludes that the herbal mouthwash formulated using green and black tea extracts is as effective as chlorhexidine in preventing plaque regrowth and gingival inflammation, with the added benefits of reduced tooth staining, better taste, and increased patient compliance, making it appropriate for long-term and routine oral hygiene use. This further highlights the

10.48047/jocaaa.2025.34.12.98

significance of herbal medicines in dentistry, which offer a safe, natural, and effective alternative to synthetic agents, and with further research and standardization, could play a key role in preventive dental care.

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