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Original Article

Antimicrobial Potential of a Raspberry-Sesame Root Herbal Formulation: An *in vitro* Study Targeting Oral Mucositis

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ABSTRACT

Background: Cancer patients frequently experience oral mucositis and oral candidiasis, which are frequently associated with oral pathogens like *Candida albicans* and *Streptococcus mutans*. The increasing prevalence of antimicrobial resistance to conventional antibiotics necessitates the exploration of effective natural alternatives. Sesame (*Sesamum indicum*) and raspberries (*Rubus idaeus*) are rich in bioactive compounds with antioxidant and antimicrobial qualities. The purpose of this study was to assess the antimicrobial efficacy of a herbal formulation made from extracts of sesame and raspberry roots against *Streptococcus mutans* and *Candida albicans*, which may be useful in the treatment of oral candidiasis and mucositis. **Materials and Methods:** Sesame root and raspberry powder were used to create an aqueous herbal formulation. The agar well diffusion method was used to measure antimicrobial activity at concentrations of 25, 50, and 100 µg. Amoxicillin (Amoxyrite) was utilized as the standard control, and *Candida albicans* and *Streptococcus mutans* were employed as test organisms. Zones of inhibition were measured, and Dunnett's multiple comparisons test and ANOVA were used for statistical analysis. **Results:** The antimicrobial effect of the herbal formulation against both pathogens was dose-dependent. At a concentration of 100 µg, the maximum zone of inhibition (16 mm) was seen, indicating activity that was either equal to or greater than that of the conventional antibiotic. Highly significant differences between the herbal formulation and the standard were found by statistical analysis ($p<0.0001$). **Conclusion:** The herbal formulation containing sesame root and raspberries showed strong antimicrobial activity against common oral pathogens. These results point to its potential as a natural substitute antimicrobial agent for oral infections, which calls for additional *in vivo* and clinical testing.

Keywords: Antimicrobial efficacy, Oral pathogens, Raspberry, Sesame extract.

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INTRODUCTION

Microorganisms play a key role in oral diseases such as dental caries, periodontal disease, and other infections through complex biofilm interactions within the oral cavity (Belibasakis, 2020). Antimicrobial therapy remains essential in dental practice, particularly for managing periodontal infections associated with gingival inflammation, bleeding, pain, and tooth mobility (Salehi *et al.*, 2020). However, widespread antibiotic use has contributed to antimicrobial resistance, posing challenges to effective oral healthcare (Purani *et al.*, 2018). Oral mucositis, a common complication of chemotherapy or radiotherapy, involves mucosal inflammation, ulceration, pain, and increased susceptibility to secondary infections, emphasizing the need for safe antimicrobial strategies for compromised oral tissues.

Antibiotics may disrupt the oral microbiome, a dynamic ecosystem essential for maintaining oral health. Continuous exposure to external microorganisms through diet and lifestyle can alter microbial balance, leading to dysbiosis and opportunistic infections (Lee and Hong, 2023). This underscores the need for alternative antimicrobial strategies that target oral pathogens while preserving microbiota equilibrium. Herbal medicine has gained attention as a complementary approach in dentistry due to its biocompatibility, fewer adverse effects, and lower risk of resistance. Many medicinal plants exhibit antimicrobial, anti-inflammatory, antioxidant, and antiviral properties, supporting their potential role in oral disease prevention and management (Budala *et al.*, 2023; Cock *et al.*, 2023; Demkovych *et al.*, 2023; Hakeem *et al.*, 2019).

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Raspberry (*Rubus idaeus*) is recognized as a rich source of bioactive phytochemicals, including phenolic compounds, flavonoids, anthocyanins, ellagic acid derivatives, and ellagitannins, which demonstrate significant antioxidant, anti-inflammatory, and antimicrobial activities (Gomathi *et al*., 2024).

Similarly, sesame (*Sesamum indicum*) contains biologically active compounds such as tannins, saponins, phenolic compounds, flavonoids, lignans, phytosterols, and tocopherols, which contribute to its antimicrobial and health-promoting properties (Du *et al*., 2023). Previous studies have reported the antimicrobial efficacy of sesame extracts against microorganisms associated with dental caries and periodontal disease (Dehiash and Mohammed, 2022; Gomathi *et al*., 2022). Building upon these findings, the present study aims to develop a herbal formulation combining raspberry and sesame root extracts and to evaluate its antimicrobial efficacy using the agar well diffusion method, exploring its potential as a natural and microbiome-friendly alternative for managing oral microbial infections.

MATERIALS AND METHODS

Ethical approval was secured from the Institutional Ethics Committee (Approval Nos. IHEC/SDC/PhD/OMED-1628/21/237 and IEC-FAC/MAR/2025/270). The Declaration of Helsinki guidelines were fully adhered to in this study.

Study Design

The present *in vitro* experimental study was designed to evaluate the antimicrobial efficacy of a herbal formulation containing sesame root extract and raspberry extract against selected oral pathogens. The antimicrobial activity of the herbal formulation was compared with a standard antimicrobial agent across multiple concentrations. The study followed a controlled laboratory-based design, with all experiments conducted under standardized conditions to ensure reproducibility and reliability of results.

Sample Size Justification

Sample size estimation was based on the observed differences in inhibition zones between the standard antimicrobial agent and the Sesame+Raspberry herbal formulation. Using mean differences ranging from 2.0 to 6.0 mm and a pooled standard deviation of approximately 1.0 mm derived from triplicate measurements, the calculated effect size (Cohen's d) ranged from 2.0 to 6.0, indicating a very large effect. An a priori power analysis was performed using G*Power software version 3.1.9.4 with a significance level of 0.05 and a power of 90%, which indicated that two to three samples per group were sufficient to detect statistically significant differences. To enhance accuracy and reproducibility, each concentration was tested in triplicate, resulting in nine observations per group. Evaluation of three concentrations produced a total of 18 measurements per assay, ensuring robust statistical power.

Preparation of *raspberry* and *sesame* root-based herbal formulation

To formulate the herbal formulation of *raspberry* and *sesame* root, precisely 1 g of *sesame* root powder and 1 g of *raspberry* powder were weighed and combined with 100 mL of distilled water in a conical flask. The flask was then carefully placed on a heating mantle, and the mixture was boiled at a temperature of 60°C for 15-20 min. This controlled boiling process was important in extracting the active components from both *raspberry* and *sesame* root powders into the water. Following the boiling step, the concoction underwent filtration using Whatman No. 1 filter paper to eliminate any impurities or solid particles present in the mixture. This filtration process resulted in the acquisition of a clear and filtered herbal formulation containing the beneficial components of both *raspberry* and *sesame* root.

Antimicrobial activity

Agar well diffusion technique

The agar well diffusion method, a widely utilized technique, was used to assess the antimicrobial activity of green-synthesized zinc oxide nanoparticles against two oral pathogens: *Streptococcus mutans* and *Candida albicans*. The procedure began with the preparation of Mueller-Hinton Agar (MHA) plates as the growth medium. Target bacteria were cultured in Mueller-Hinton Broth (MHB) and incubated for 24 hr, after which bacterial concentrations were standardized using McFarland standards. The standardized bacterial cultures were then uniformly swabbed onto the MHA plates. Wells were subsequently created and filled with the *raspberry* and *sesame* root herbal formulation at concentrations of 25, 50, and 100 µg. A comparative analysis with standard antibiotics demonstrated the relative effectiveness of the herbal formulation.

Statistical Analysis

The collected data were expressed as mean±standard deviation. A Two-Way Analysis of Variance (ANOVA) was performed to evaluate the effects of concentration, oral pathogens, and their interaction on antimicrobial activity. *Post hoc* comparisons between the standard and test concentrations were conducted using Dunnett's multiple comparisons test. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using appropriate statistical software.

RESULTS

Antimicrobial Activity

The antimicrobial activity of the raspberry-sesame root herbal formulation was evaluated against *Candida albicans* and

Streptococcus mutans using the agar well diffusion method, demonstrating a clear concentration-dependent response.

Against *C. albicans*, zones of inhibition increased from 12 mm at 25 µg to 14 mm at 50 µg and 16 mm at 100 µg, approaching the standard antibiotic, Amoxyrite (18 mm) (Figure 1A, Figure 2). Similarly, for *S. mutans*, inhibition zones increased from 12 mm to 15 mm and 16 mm across the respective concentrations, exceeding the standard drug (12 mm) at higher doses (Figure 1B, Figure 2).

Against *Candida albicans*, the herbal formulation demonstrated progressive antifungal activity

The antimicrobial activity of the raspberry and sesame root herbal formulation was evaluated (Tables 1 and 2). The results demonstrated a significant effect of formulation concentration, oral pathogen type, and their interaction on antimicrobial efficacy. Concentration was the primary determinant of antimicrobial activity, accounting for 40.42% of the total variation ($p<0.0001$). Pathogen type also contributed significantly (7.27%, $p<0.0001$), indicating differential susceptibility between *Streptococcus mutans* and *Candida albicans*. A significant interaction effect (35.77%, $p<0.0001$) further indicated that the response to increasing concentrations differed between the two pathogens.

Significant differences were observed between the standard drug and the herbal formulation at higher concentrations. For *S. mutans*, both 50 µg and 100 µg concentrations exhibited significantly greater antimicrobial activity than the standard, with mean differences of 3.0 mm and 4.0 mm, respectively ($p<0.0001$). In contrast, for *C. albicans*, all tested concentrations showed significant differences compared to the standard ($p<0.0001$), with the magnitude of difference decreasing at higher concentrations, suggesting improved antifungal efficacy approaching that of the standard drug.

Overall, the findings confirm a significant, concentration-dependent antimicrobial effect of the raspberry and sesame root herbal formulation, with pathogen-specific variations in efficacy.

DISCUSSION

The present study investigated the antimicrobial activity of a herbal formulation derived from raspberry and sesame root against two common oral pathogens, *Candida albicans* and *Streptococcus mutans*. Using the agar well diffusion method, inhibitory effects were assessed at concentrations of 25 µg, 50 µg, and 100 µg, with a standard antibiotic used for comparison. The results demonstrated a concentration-dependent antimicrobial response, with increasing extract concentration producing wider zones of inhibition against both microorganisms. The highest concentration exhibited inhibitory effects comparable to the standard, particularly against *C. albicans*, indicating promising antifungal potential.

These findings are consistent with previous research highlighting the antimicrobial properties of raspberry-derived compounds. For instance, studies examining raspberry extracts have demonstrated bactericidal activity against pathogenic bacteria, supporting the broader understanding that raspberries possess antimicrobial potential (Casalini *et al*., 2023). Similarly, Krzepiłko *et al*. (2021) reported antibacterial activity of raspberry leaf buds against *Staphylococcus aureus* and *Enterococcus faecalis*, indicating that different plant parts contribute diverse bioactive properties. Together, these studies reinforce the relevance of raspberry components as antimicrobial agents. The present findings extend this knowledge by demonstrating the effectiveness of a combined raspberry-sesame formulation against oral pathogens.

Evidence supporting the antimicrobial potential of sesame has also been well documented. Sesame seed extracts have shown antifungal activity against dermatophytic species and other fungal pathogens (Ahmed *et al*., 2009). Additionally, extracts of sesame leaves and seeds have demonstrated inhibitory effects against organisms including *Candida albicans, Streptococcus pneumoniae*, and *Staphylococcus aureus*, highlighting broad-spectrum activity (Bankole *et al*., 2007; Nigam *et al*., 2015). Further investigations have reported concentration-dependent antibacterial efficacy of sesame-derived compounds, confirming their therapeutic relevance (Ahmed *et al*., 2009; Nigam *et al*., 2015). These reports align with the present study, where sesame root contributed to significant antimicrobial activity when combined with raspberry extract.

Statistical evaluation reinforced the reliability of the observed outcomes, with significant effects of concentration, pathogen type, and their interaction on antimicrobial activity ($p<0.05$). *Post hoc* comparisons demonstrated meaningful differences between the herbal formulation and the standard antibiotic at higher concentrations, supporting the formulation's efficacy. The inhibitory activity observed suggests potential application as a natural antimicrobial agent targeting oral pathogens, aligning with broader research exploring plant-based therapeutics in dentistry (Tharani *et al*., 2023).

Despite these encouraging findings, certain limitations must be acknowledged. The *in vitro* design restricts direct clinical translation, and further investigations are required to evaluate formulation safety, stability, and *in vivo* efficacy before clinical application can be considered. Nevertheless, the results highlight the potential of combined herbal approaches as sustainable alternatives for managing oral infections.

Figure 1: Antimicrobial activity of raspberry and sesame root herbal formulation using agar well diffusion technique against oral pathogens A) *C. albicans* B) *S. mutans*.

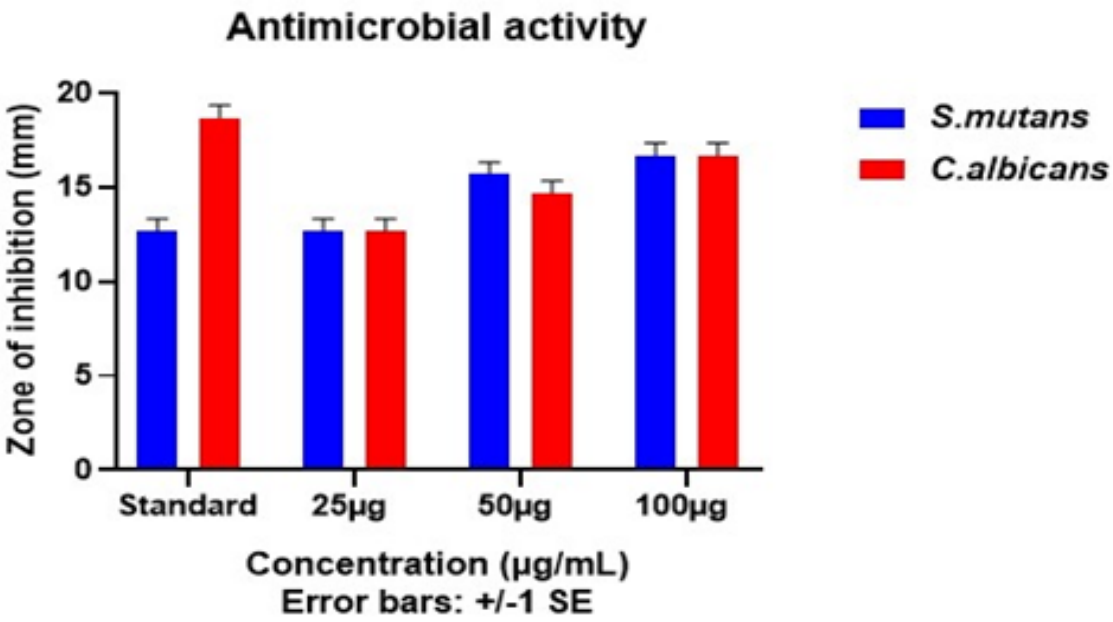

Figure 2: Zone of inhibition produced by *raspberry* and *sesame* root herbal formulation against *S. mutans* and *C. albicans*.

Table 1: ANOVA results for antimicrobial activity of *raspberry* and *sesame* root herbal formulation.

Source of variation	% of total variation	*p* value
Concentration	40.42	<0.0001
Oral pathogens	7.270	<0.0001
Interaction	35.77	<0.0001

Table 2: Dunnett's method for comparing multiple groups.

Dunnett's multiple comparisons test	Mean difference	*p* value
S. mutans		
Standard vs. 50 µg	3.000	<0.0001
Standard vs. 100 µg	4.000	<0.0001
C. albicans		
Standard vs. 25 µg	6.000	<0.0001
Standard vs. 50 µg	4.000	<0.0001
Standard vs. 100 µg	2.000	<0.0001

LIMITATIONS OF THE STUDY

Despite its strengths, the study has certain limitations that must be acknowledged. The *in vitro* experimental design may not fully replicate the complex biological and ecological conditions of the oral cavity. The microbial spectrum was limited to two oral pathogens, and inclusion of additional bacteria associated with periodontal and endodontic infections would provide a broader antimicrobial profile. The absence of phytochemical characterization limits the identification of the specific bioactive compounds responsible for the observed effects. Furthermore, cytotoxicity, safety, and biocompatibility assessments were not conducted, and the formulation's stability and shelf-life were not evaluated, all of which are crucial for clinical application.

CONCLUSION

The present study confirms that the raspberry and sesame root herbal formulation exhibits significant *in vitro* antimicrobial activity against *Candida albicans* and *Streptococcus mutans*. A clear dose-dependent increase in inhibition was observed, with higher concentrations showing greater antimicrobial effectiveness. Statistical analysis further validated the influence of concentration and pathogen type on the observed outcomes. These findings provide preliminary evidence supporting the potential of the herbal formulation as an alternative antimicrobial agent for oral pathogens. However, further *in vivo* and clinical studies are necessary to establish its safety, efficacy, and clinical applicability.

ABBREVIATIONS

ANOVA: Analysis of Variance; **C. albicans:** *Candida albicans*; **g:** Gram; **MHA:** Mueller-Hinton Agar; **MHB:** Mueller-Hinton Broth; **mL:** Milliliter; **mm:** Millimeter; **µg:** Microgram; **p:** Probability value; **PhD:** Doctor of Philosophy; **SD:** Standard Deviation; **S. mutans:** *Streptococcus mutans*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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