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ORIGINAL RESEARCH

INSIGHTS INTO THE ANTIOXIDANT, ANTI-INFLAMMATORY AND ANTI-MICROBIAL POTENTIAL OF RAPHANUS SATIVUS LEAVES IN DENTAL THERAPEUTICS

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ABSTRACT

Background: Raphanus sativus (R. sativus), a member of the Brassicaceae family, is rich in bioactive compounds,including flavonoids, tannins, and phenolic acids, which exhibit significant antimicrobial, antioxidant, anti-inflammatory, and antifungal properties. Oral diseases such as periodontitis, dental caries, and oral cancers are influenced by microbial infections, oxidative stress, and inflammation. This study evaluates the potential of R. sativus leaf extract as a natural therapeutic agent for oral health applications.
Materials and Methods: Ethanol was used to extract bioactive compounds from dried R. sativus leaves using Soxhlet extraction. Antibacterial activity was tested against Streptococcus mutans and Escherichia coli using agar diffusion and minimum inhibitory concentration (MIC) assays. Antioxidant activity was evaluated using the ABTS free radical scavenging assay. Anti-inflammatory, antifungal activity and Cytotoxicity was tested.
Results: The R. sativus leaf extract exhibited significant dose-dependent anti-inflammatory ($p < 0.03$) and antioxidant activities, with higher concentrations nearing the efficacy of controls($P < 0.05$). Antibacterial assays demonstrated potent inhibition of S. mutans and E. coli, with larger inhibition zones at higher concentrations. However, antifungal activity against C. albicans was minimal. Cytotoxicity studies revealed a dose-dependent effect, with reduced survival in zebrafish larvae at elevated extract concentrations.
Conclusion: R. sativus leaf extract demonstrates promising antimicrobial, antioxidant, and anti-inflammatory properties, supporting its potential application in oral healthcare. However, dose-dependent cytotoxicity highlights the need for further research to optimize extraction methods, establish safe therapeutic concentrations, and explore clinical applications in oral disease management.

Keywords**:**Anti-inflammatory,antioxidant,antimicrobial,phytochemicals,cytotoxicity,raphanus sativus

INTRODUCTION

Raphanus sativus (R. sativus) is a widely consumed plant belonging to the Cruciferae (Brassicaceae) family.[1,2] While its taproot is the most commonly utilized part, other components—including the leaves, seeds, flowers, and sprouts—also possess significant pharmacological properties.[3] Various studies have highlighted the therapeutic potential of R. sativus, demonstrating its hepatoprotective, cardioprotective, gut-stimulatory, antioxidant, anti-inflammatory, and antimicrobial activities.[4] Latef et al. (2021) investigated

the protective effects of R. sativus leaf extract against oxidative stress in human fetal lung fibroblast (MRC-5) cells, revealing strong antioxidant and cytoprotective properties, which further support its medicinal value.[5,6] However, their study primarily focused on systemic oxidative stress rather than applications in oral health. Recent research has further emphasized its potential role in disease prevention and management, particularly due to its rich phytochemical composition.[7]

Natural plant-derived compounds have gained substantial attention for their ability to modulate disease processes, including those affecting the oral cavity.[8,9] Among various botanicals, R. sativus leaves stand out as a promising source of bioactive compounds, including flavonoids, tannins, alkaloids, and phenolic acids.[10] These phytochemicals exhibit potent anti-inflammatory, antioxidant, antimicrobial, and antifungal properties, positioning them as potential candidates for managing oral diseases such as gingivitis, periodontitis, denture induced stomatitis, dental caries, and oral cancer.[11,12]

Oral diseases remain a major global health concern, impacting millions of individuals and significantly affecting overall well-being.[13,14] Conditions such as periodontitis, dental caries, gingivitis, and oral cancers are primarily driven by microbial infections, oxidative stress, and chronic inflammation.[15] Conventional treatment strategies rely heavily on synthetic antimicrobials and anti-inflammatory agents, yet their prolonged use is associated with adverse side effects and the growing issue of microbial resistance.[16,17] This has led to an increased demand for natural, plant-based alternatives that can provide effective therapeutic benefits with minimal toxicity.

Similarly, several studies have explored the antimicrobial and anti-inflammatory properties of R. sativus extracts. However, much of the existing literature has examined their effects on systemic infections, gastrointestinal health, or metabolic conditions, rather than their specific impact on oral diseases.[18,19] While previous research provides valuable insights into the general pharmacological activities of R. sativus, its role in managing oral health conditions, such as periodontitis, dental caries, and oral cancer, remains underexplored.[20,21]

Most available research on natural antioxidants and antimicrobials focuses on their systemic benefits rather than their localized effects within the oral cavity. For example, studies on herbal extracts often emphasize their general anti-inflammatory and free radical scavenging properties but do not address their potential to inhibit oral pathogens or modulate inflammation within the gingival tissues.[23] Additionally, while some plant-based compounds have been examined for their anticancer potential, their specific effects on oral epithelial cells and their relevance to conditions such as oral squamous cell carcinoma require further investigation.

Given these gaps in the literature, this study aims to evaluate the potential of R. sativus leaves in oral healthcare. Unlike previous studies that have focused on systemic antioxidant or anti-inflammatory effects, this research will specifically assess the extract's antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities in the context of oral diseases. By doing so, it will provide novel insights into the applicability of R. sativus leaves as a natural adjunct for managing oral health conditions.

MATERIALS AND METHODS

The study was conducted following ethical approval from the Scientific Review Board (SRB) of Saveetha Dental College and Hospital. Approval reference number: SRB/SDC/OPATH-2305/24/398.

Plant material and extraction:

Fresh R. sativus leaves were collected from agricultural fields and subsequently identified and authenticated as shown in (figure 1). The collected leaves were thoroughly washed, air-dried at room temperature, and then ground into a fine powder. The powdered leaves were subjected to ethanol as the solvent to obtain the desired bioactive compounds. The 100 ml ethanol solution was added to the 10 gm leaves were heated at 60 degrees Celsius for 24 hours. Then the solution was filtered with Whatman filter paper, and the resulting

concentrated extracts were stored at -20°C for further Phytochemical analysis.

Figure 1. Raphanus sativus leaves extract collected.

Two bacterial strains were used in this study to evaluate the antimicrobial activity of R. Sativus leaves, namely Staphylococcus aureus (ATCC 25923), a Gram-positive bacterium, and Escherichia coli (ATCC 25922), a Gram-negative bacterium. The strains were initially retrieved from frozen stock cultures and transferred onto Tryptic Soy Agar (TSA) plates, which were incubated at 37 °C for 18 to 24 hours. After incubation, the bacterial colonies were inoculated into 50 mL of sterile Tryptic Soy Broth (TSB) and cultured at 37 °C with shaking at 80 rpm for 18 to 24 hours. The cultures were then diluted in fresh TSB at a 1:50 ratio and incubated for an additional 2 hours at 37 °C with shaking at 80 rpm before being used for inoculation.

Antifungal Assay

The antifungal activity of R. Sativus leaf extract against Candida albicans was evaluated using the agar well diffusion method. A fresh culture of Candida albicans was prepared by transferring the fungal strain from the stock culture to Sabouraud Dextrose Agar (SDA) plates, followed by incubation at 37 °C for 24 hours. After incubation, a suspension of the fungal strain was prepared in sterile saline solution and adjusted to a turbidity equivalent to 0.5 McFarland standard. Sterile Petri dishes containing solidified SDA were then inoculated with the fungal suspension using a sterile cotton swab to ensure even distribution across the agar surface. Wells of 6 mm diameter were made in the agar using a sterile cork borer, and 25 µL of the Raphanus sativus leaf extract at varying concentrations was introduced into each well. A standard antifungal agent served as the positive control, and a well with the solvent used for extraction acted as the negative control. The plates were incubated at 37 °C for 24–48 hours, and the zone of inhibition around each well was measured in millimeters to assess the antifungal activity of the extract.

Anti-inflammatory Assay

The inhibition of protein denaturation by R. sativus leaf extract was evaluated using the protein denaturation inhibition assay. The reaction mixture consisted of 1% Bovine Serum Albumin (BSA) in aqueous solution and varying concentrations of the test extract. To adjust the pH of the mixture, 1 N HCl was added. The samples were incubated at 37 °C for 20 minutes, followed by heating at 57 °C for another 20 minutes, and then allowed to cool to room temperature. The turbidity of the samples was measured at 660 nm using a UV-Vis spectrophotometer. Experiments were conducted in triplicate by three independent individuals.

The percentage inhibition of protein denaturation was calculated using the following formula:

Percentage of Inhibition = [(Ac - As) / Ac] × 100

where Ac is the absorbance of the control sample, and As is the absorbance of the test sample, both measured at 660 nm.

Anti-Oxidant Assay

R. Sativus leaf extract was examined for its capacity to scavenge free radicals using the DPPH reagent. Different amounts of DPPH reagent and Raphanus sativus leaf extract were added to the reaction mixture, which was then incubated in a dark environment. Using an ELISA reader on a 96-well plate and a UV-VIS spectrophotometer, the absorbance at 517 nm was recorded during the triplicate experiment. Ascorbic acid was used as the reference standard, while the reaction mixture comprising only methanol and DPPH was used as the negative control.

The percentage of scavenging effect (%) was calculated using the formula = (1- α/ß) x 100%,

where ß stands for the absorbance of the negative control and α for the test extract absorbance .

Cytotoxicity test assay

Zebrafish embryos (0–5 hours post-fertilization, hpf) were collected and rinsed with E3 medium to remove debris and unfertilized eggs. When required, embryos were dechorionated using fine forceps or enzymatic methods to ensure uniform exposure to test compounds. A series of dilutions of the test compound were prepared in E3 medium, with E3 medium alone serving as a control. Embryos were distributed into 24-well or 96-well plates, with 10–20 embryos per well, and exposed to the test solutions in triplicate. Plates were incubated at 28.5°C under a 14:10 light-dark cycle, ensuring adequate oxygenation and spacing. Observations were made at 24-hour intervals using a stereomicroscope, recording endpoints such as mortality, hatching rate, developmental abnormalities, and behavioral changes. Anesthesia with tricaine was used when detailed morphological assessment was required. Data were analyzed to determine toxicological parameters, including LC50 or EC50 values, using appropriate statistical methods, and results were compared with the control group to assess the compound's cytotoxicity.

RESULTS

Anti - Bacterial Activity

The antibacterial activity of Raphanus sativus leaf extract is tested against Staphylococcus aureus and Escherichia coli using the agar well diffusion method.

The results demonstrated a concentration-dependent inhibition of bacterial growth, as shown in (Figure 2).

•S. aureus: At 25 µL, the inhibition zone measured 5.3 mm, while at 50 µL, it increased to 7.2 mm, indicating enhanced antibacterial action with higher concentrations.

•E. coli: A similar trend was observed, with inhibition zones measuring 4.5 mm at 25 µL and 5.3 mm at 50 µL.

Against Staphylococcus aureus (Image a):
The R. Sativus leaf extract exhibited antibacterial activity, as indicated by the clear inhibition zones around the wells containing 25 µL and 50 µL concentrations. The size of the inhibition zones suggests a dose-dependent effect, with a larger zone observed for the 50 µL concentration, indicating stronger antibacterial activity at a higher concentration. This implies that R. Sativus leaves possess compounds capable of inhibiting the growth of Staphylococcus aureus.

Against Escherichia coli (Image b):
The extract also displayed antibacterial activity against Escherichia coli, as shown by the inhibition zones around the wells containing 25 µL and 50 µL concentrations. Similar to the results against Staphylococcus aureus, a larger inhibition zone is seen at the 50 µL concentration, indicating more potent antibacterial action at a higher dose.
This demonstrates that R. Sativus leaves are effective in inhibiting the growth of Escherichia coli, a bacterium commonly involved in periodontal infection.

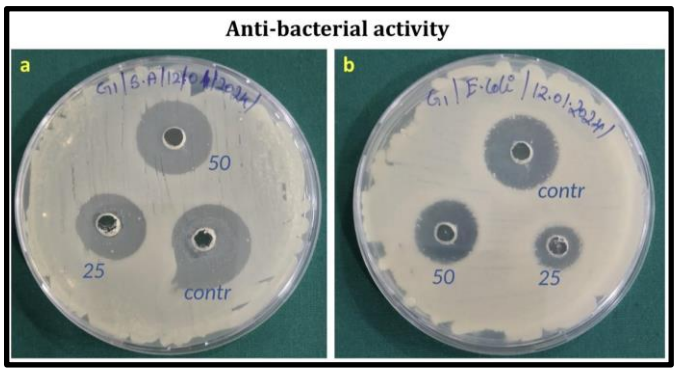

Figure 2. Anti-bacterial activity of Raphanus sativus leaves extract

Anti - Fungal Activity

The antifungal activity of R. sativus leaf extract against Candida albicans was negligible, as indicated by the absence of significant inhibition zones at both 25 µL

and 50 µL concentrations (Figure 3). This suggests that the extract lacks potent antifungal compounds at the tested concentration.

The lack of distinct inhibitory zones suggests that the extract, at these tested concentrations, is not effective against this fungal strain. This indicates that further optimization in concentration, solvent selection, or extract preparation might be required to enhance antifungal efficacy.

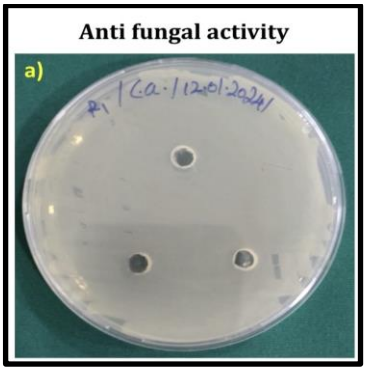

Figure 3. Anti-fungal activity of Raphanus sativus leaves

Anti - Inflammatory Activity

The anti-inflammatory effect of R. Sativus was evaluated by measuring the inhibition of protein denaturation at various concentrations (20, 40, 60, 80, and 100 µg/mL). The results demonstrated a progressive increase in inhibitory activity with increasing concentrations (Figure 4).

The inhibition of protein denaturation was assessed at extract concentrations ranging from 20 to 100 µg/mL. A dose-dependent increase in anti-inflammatory activity was observed (Figure 2).

•At 20 µg/mL, inhibition was 20.2%.

•At 100 µg/mL, inhibition peaked at 68.9%, closely approaching the control values.

The 20 µg/mL sample exhibited minimal anti-inflammatory effects, whereas the 100 µg/mL concentration demonstrated the highest inhibitory activity, approaching the level of the control. Statistical significance ($p<0.03$) confirmed the concentration-dependent anti-inflammatory activity, with peak efficacy observed at 100µg/mL.

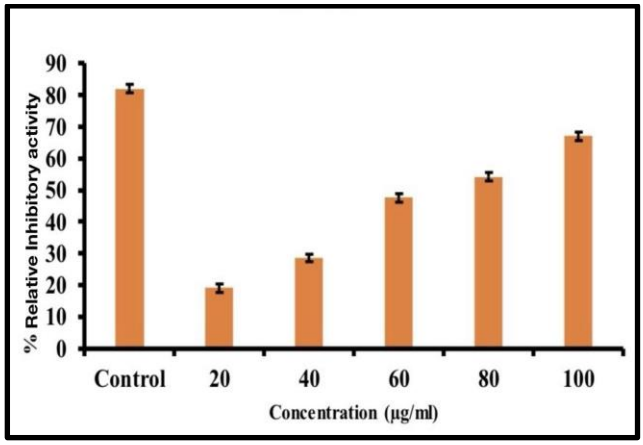

Figure 4. Anti-inflammatory activity of Raphanus sativus leaves (Significant value less then 0.03%)

Anti - Oxidant Activity

The antioxidant potential of R. Sativus extract was assessed, and the results are presented in a bar graph showing the percentage of inhibition (Figure 5). The control group exhibited a high inhibition rate, indicating strong antioxidant activity. The 50 µL and 100 µL concentrations of the extract demonstrated a dose-dependent increase in antioxidant activity, with the 100 µL sample achieving inhibition levels nearly equivalent to the control. These findings suggest that Raphanus sativus extract possesses significant antioxidant properties, effectively inhibiting oxidation processes at higher concentrations.

The antioxidant potential of R. sativus extract was evaluated using a DPPH radical scavenging assay. The results indicated a concentration-dependent increase in antioxidant activity:

•At 50 µL, scavenging activity was 62.1%.

•At 100 µL, scavenging activity reached 75.3%

These results suggest a strong free radical scavenging effect, likely due to the high flavonoid and phenolic content of R. sativus leaves.

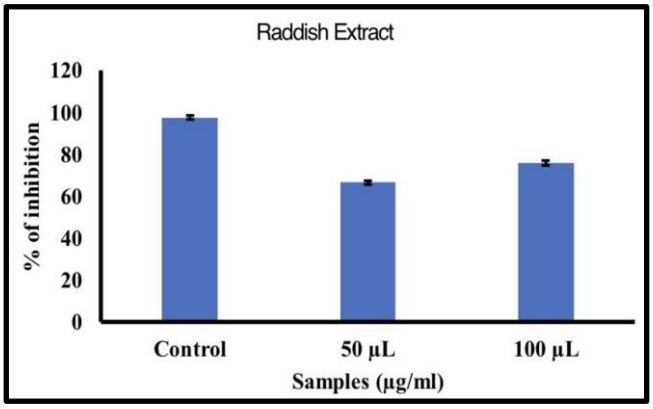

Figure 5. Anti-oxidant activity of Raphanus sativus leaves (Significant value less then 0.03%)

Cytotoxicity

The cytotoxicity of R. Sativus leaf extract was evaluated on zebrafish embryos at concentrations of 20 µL/mL and 40 µL/mL over a 120-hour period. The control group maintained 100% viability throughout the study, indicating no adverse effects. At 20 µL/mL, a slight reduction in embryo viability was observed, with survival rates decreasing to approximately 85–90% by 120 hours. At 40 µL/mL, a more noticeable decline in survival was recorded, with viability dropping to 80–85% at the final time point. The reduction in viability was concentration-dependent, with higher extract concentrations exhibiting greater cytotoxic effects. However, embryo survival remained above 80% across all tested concentrations, suggesting that Raphanus sativus leaf extract has relatively low toxicity within the examined range (Figure 6).

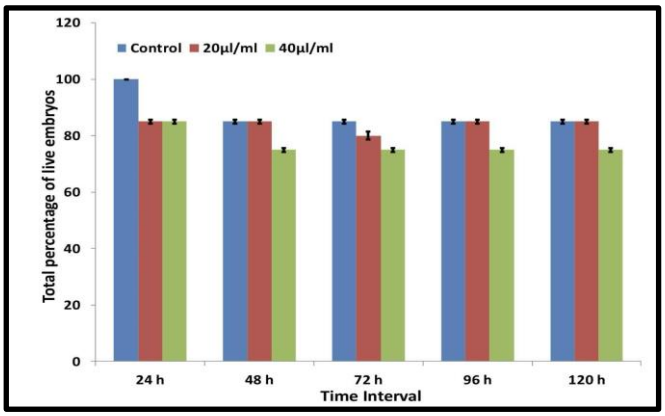

Figure 6. Cytotoxicity evaluation of Raphanus sativus leaves at different concentrations.

DISCUSSION

The leaves of R. Sativus have gained significant attention for their rich bioactive properties, particularly their antimicrobial, anti-inflammatory, and antioxidant potentials. These properties are attributed to the complex phytochemical composition of R. Sativus leaves, which includes flavonoids, anthocyanins, and cysteine-rich peptides.[24] This discussion delves into the mechanisms underlying these bioactivities, their implications for health, and the broader therapeutic applications of R. Sativus leaves. Raphanus sativus leaves have demonstrated strong antimicrobial activity against common oral pathogens I.e. Staphylococcus aureus and Escherichia coli.[25] S. aureus colonization is linked to oral conditions like denture use, lesions such as denture stomatitis and angular cheilitis, with decreased oral clearance due to reduced salivary flow or dysphagia proposed as contributing factors.[26]

The antimicrobial potential of Raphanus sativus leaves was evaluated using the agar well diffusion method, demonstrating significant inhibition, particularly against Staphylococcus aureus. These findings align with previous studies reporting antibacterial activity in various parts of R. sativus, including its seeds and sprouts, which have shown efficacy against both Gram-positive and Gram-negative bacteria. [27] Ethanol extracts of R. sativus and related subspecies have exhibited inhibition against pathogens such as Salmonella Enteritidis, Cronobacter sakazakii, and Bacillus cereus, further supporting the antibacterial potential of this plant. Comparatively, studies on medicinal plants like Azadirachta indica and Syzygium aromaticum have demonstrated similar antimicrobial effects, reinforcing the relevance of plant-based alternatives in oral health management.[28,29]

The multifaceted bioactivity of R. sativus leaves, including antimicrobial, anti-inflammatory, and antioxidant properties, positions them as a promising natural therapeutic agent.[30] Their ability to control bacterial infections while reducing inflammation suggests potential applications in preventing periodontal disease. The synergy between these properties could offer a multi-targeted approach in oral

health, mitigating oxidative stress-induced tissue damage and promoting gum health. Given these promising findings, further in vivo studies and clinical trials are warranted to explore their integration into oral hygiene formulations such as mouthwashes and gels as a natural alternative to conventional antimicrobial agents.[31]

Chronic inflammation in the oral cavity contributes to tissue destruction and exacerbates conditions like periodontitis. Raphanus sativus leaves, rich in polyphenols, exhibit anti-inflammatory properties by modulating immune responses, reducing pro-inflammatory cytokines, and inhibiting COX-2 expression.[32,33] Our study demonstrated a reduction in inflammatory markers, highlighting their potential as an adjunct in periodontal disease management.

While R. sativus seeds have shown notable anti-inflammatory effects, research on its leaves remains limited. Our findings suggest promising anti-inflammatory activity, warranting further investigation. Additionally, cysteine-rich peptides (Rs-AFP1 and Rs-AFP2) from R. sativus exhibited potent antifungal effects; however, at 25 µg/mL, the antifungal activity of radish leaf extracts was not significant in our study.[34]

The antioxidant activity of Raphanus sativus leaves is attributed to their flavonoid and polyphenol content, which neutralizes reactive oxygen species (ROS), upregulates detoxification enzymes, and inhibits carcinogenic enzymes like prostaglandin synthase and cyclooxygenase.[35] Given the constant oxidative stress in the oral cavity from factors like diet and smoking, R. Sativus may help protect gum tissues, reduce inflammation, and potentially prevent oral cancer.[36-39]

The observed dose-dependent cytotoxicity of Raphanus sativus leaf extract highlights the need for careful dosage consideration despite its promising therapeutic properties. Higher concentrations may exhibit cytotoxic effects, aligning with previous studies on plant-based bioactive compounds.

The findings of this study underscore the potential of R. sativus leaf extracts as a multifunctional therapeutic agent in oral health. Their incorporation into oral care formulations—such as mouthwashes, toothpaste, and gels—could provide a natural alternative to synthetic antimicrobial agents like chlorhexidine, minimizing adverse effects while promoting periodontal health. Further in vivo studies and clinical trials are warranted to validate these findings and explore their full potential in dental applications.

The study is limited by its dependence on in vitro and zebrafish embryo models, which may not fully reflect human physiological responses, highlighting the need for clinical validation in human subjects. Additionally, the dose-dependent nature of its effects underscores the importance of further in vivo studies to determine safe and effective therapeutic ranges for oral health applications. Furthermore, the long-term safety and efficacy of radish leaf-based oral care formulations remain unexplored, necessitating comprehensive research to assess their sustained use in clinical settings.

CONCLUSION

This study provides valuable insights into the therapeutic potential of Raphanus sativus leaf extract for oral health, demonstrating its antimicrobial, antioxidant, and anti-inflammatory properties. While the findings support its efficacy in preclinical models, translating these benefits into clinical applications requires further investigation. Establishing safe and effective therapeutic ranges through in vivo and human trials is essential to ensure its suitability for long-term use. Additionally, comprehensive studies on its long-term safety and formulation stability are necessary before it can be considered for integration into oral healthcare products.

DECLARATIONS

Ethical approval and consent to participate

Ethical permission was granted by the Medical Ethics Committee of the Faculty of Dentistry, Sana'a University (Ref. No.: 703; Date: 2/8/2024). All patient identifiers were anonymized for confidentiality *with the ethical principles outlined in the Declaration of Helsinki.*

Availability of data and material

All data generated or analyzed during this study are included in the published article.

Competing interests

The authors declare that there are no competing interests.

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