

Preliminary Evaluation of the Antimicrobial, Anti-Inflammatory, Antioxidant, and Cytotoxic Activities of *Taraxacum officinale* Leaf Extract: An *in vitro* Study

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ABSTRACT

Background: *Taraxacum officinale* (dandelion) is known for its bioactive compounds, including flavonoids and phenolic acids, which exhibit antimicrobial, antioxidant, and anti-inflammatory properties. Oral inflammatory lesions, such as oral lichen planus and aphthous stomatitis, are associated with microbial imbalance, oxidative stress, and inflammation. This study evaluates the potential of *T. officinale* leaf extract as a natural alternative for treating these lesions. **Objectives:** To assess the antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities of *T. officinale* leaf extract, and to explore its potential as a therapeutic agent for oral inflammatory lesions. **Materials and Methods:** Ethanol was used to extract bioactive compounds from dried *T. officinale* leaves. Antibacterial activity was tested against *Staphylococcus aureus* and *Escherichia coli* using agar diffusion. Antioxidant activity was evaluated through the DPPH free radical scavenging assay. Anti-inflammatory effects were assessed by measuring protein denaturation inhibition. Cytotoxicity was tested using zebrafish embryos, with survival rates and developmental changes monitored. **Results:** The leaf extract exhibited significant antimicrobial activity, inhibiting both *S. aureus* and *E. coli*, especially at higher concentrations. Antioxidant activity was notable, with near-complete free radical scavenging at higher doses. Anti-inflammatory effects were concentration-dependent, with maximum inhibition of protein denaturation at 100 µg/mL. Cytotoxicity studies revealed dose-dependent toxicity, with reduced survival in zebrafish embryos at 40 µL/mL and higher concentrations. **Conclusion:** *T. officinale* leaf extract demonstrates strong antimicrobial, antioxidant, and anti-inflammatory activities, suggesting its potential as a treatment for oral inflammatory lesions. However, its dose-dependent toxicity highlights the need for further research to determine safe and effective therapeutic doses.

Keywords: *Taraxacum officinale*, Dandelion, Oral inflammatory lesions, Antioxidant activity, Anti-inflammatory activity, Antimicrobial activity, Cytotoxicity, Natural therapeutics.

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Received: 18-11-2024;

Revised: 20-12-2024;

Accepted: 09-01-2025.

INTRODUCTION

Taraxacum officinale, commonly known as dandelion, is a resilient and widely distributed herbaceous plant that has long been utilized in traditional medicine for its versatile therapeutic properties.^[1] Originally native to Eurasia, *T. officinale* has successfully naturalized across North America and other temperate regions, where it thrives in diverse habitats, from grasslands to urban landscapes.^[2] This accessibility, coupled with its rich phytochemical profile, has made dandelion an enduring component in herbal remedies, used to address a wide range of

health issues including infections, inflammatory disorders, and oxidative stress-related conditions.^[3]

The therapeutic potential of *T. officinale* is largely attributed to its high content of bioactive compounds. Among these, flavonoids and phenolic acids play a critical role as potent antioxidants, helping to neutralize free radicals and reduce oxidative stress within the body—a factor implicated in the development of many chronic diseases and cellular aging.^[4] Additionally, sesquiterpene lactones, prominent constituents in dandelion, have demonstrated significant anti-inflammatory and antimicrobial properties in various studies, making *T. officinale* a promising candidate for addressing inflammation and microbial infections.^[1] Polysaccharides present in dandelion, such as inulin, further add to its therapeutic repertoire by supporting gut health and potentially modulating immune responses.^[5]



DOI: 10.5530/pres.20252021

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Oral inflammatory lesions, including conditions such as oral lichen planus, aphthous stomatitis, and periodontal disease, are prevalent in dental and medical practice and present a significant treatment challenge.^[6] These lesions are often driven by persistent inflammation and microbial imbalance within the oral cavity, leading to chronic discomfort and a diminished quality of life for affected individuals.^[7] The management of these conditions typically involves anti-inflammatory or antimicrobial agents, which, while effective, may sometimes be limited by side effects or resistance in long-term use.^[8] This scenario underscores the need for exploring alternative or adjunctive treatments that offer multi-functional therapeutic benefits with a potentially lower risk profile.^[9]

Given the bioactivities of *T. officinale*, its leaf extract emerges as a potential therapeutic agent for oral inflammatory lesions.^[10] Its antioxidant properties may help in reducing oxidative stress, while its anti-inflammatory effects could alleviate chronic inflammation associated with these lesions.^[11] Additionally, the antimicrobial actions of dandelion could contribute to managing oral microbial populations, potentially reducing the incidence or severity of infections that exacerbate inflammatory conditions.^[11] The cytotoxic effects of *T. officinale* leaf extract are also of interest, as they may provide insights into safe dosage levels and further elucidate its potential application in a clinical setting.^[12]

This study, therefore, aims to conduct a preliminary evaluation of *T. officinale* leaf extract, systematically assessing its antimicrobial, antioxidant, anti-inflammatory, and cytotoxic activities. Through this assessment, we aim to provide foundational data that could support the potential integration of dandelion leaf extract as a natural adjunctive treatment for managing oral inflammatory lesions. This research contributes to the broader exploration of plant-based therapeutics, addressing the growing interest in safer, cost-effective, and multi-targeted treatment options in oral health care.

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-2301/24/354.

Plant Extract Preparation

Taraxacum officinale leaves were purchased from Kodaikanal, India. The leaves were dried and extracted with an ethanolic solution. A 100 mL ethanol solution was mixed with 10 g of dried *Taraxacum officinale* leaves and heated at 60°C for 24 hr. After heating, the solution was filtered using Whatman filter paper, and the resulting extract was used for subsequent characterization studies.

Antibacterial Activity

The antibacterial activity of the *Taraxacum officinale* leaf extract was assessed using two bacterial strains: Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922). Bacterial strains were cultured on TrypCase Soy Agar (TSA) plates after being thawed from the frozen stock culture and incubated at 37°C for 18 to 24 hr. The bacteria were transferred to sterile Tryptic Soy Broth (TSB) medium and incubated at 37°C with shaking at 80 rpm for 18 to 24 hr. After subculturing in fresh TSB at a 1:50 ratio, the bacterial suspension was incubated for 2 hr at 37°C with shaking at 80 rpm before being used for inoculation in antibacterial assays.

Antioxidant Activity

The antioxidant potential of *Taraxacum officinale* leaf extract was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Varying concentrations of the leaf extract were mixed with DPPH reagent and incubated in the dark. The absorbance of the reaction mixture was measured at 517 nm using a UV-vis spectrophotometer with an ELISA reader on a 96-well plate. Methanol and DPPH were used as the negative control, and ascorbic acid served as the reference standard. The percentage of free radical scavenging activity was calculated using the formula:

$$\text{Percentage of scavenging effect} = (1 - \alpha/\beta) \times 100$$

where α is the absorbance of the test extract and β is the absorbance of the negative control.^[13]

Anti-inflammatory Activity

Anti-inflammatory activity was evaluated by the inhibition of protein denaturation using the method described by Mizushima et al.^[14] The reaction mixture contained 1% Bovine Serum Albumin (BSA) in aqueous solution and varying concentrations of the *Taraxacum officinale* leaf extract. The pH of the mixture was adjusted with 1 N HCl, followed by heating for 20 min at 37°C and an additional 20 min at 57°C. The samples were then allowed to cool, and the turbidity of the mixture was measured at 660 nm. The percent inhibition of protein denaturation was calculated using the formula:

$$\text{Percentage of Inhibition} = [(A_C - A_S) \times 100] / A_C$$

where A_C is the absorbance of the control and A_S is the absorbance of the test sample.

Toxicity Assay using Zebrafish Embryos

For the toxicity assay, zebrafish embryos (0-5 hr post-fertilization, hpf) were used to assess the potential toxicity of the *Taraxacum officinale* leaf extract. Embryos were collected, rinsed with E3 medium to remove any debris, and dechorionated if necessary. The leaf extract was prepared in a range of concentrations using E3 medium, with a control group consisting of only E3 medium. The

embryos were then transferred to 24-well or 96-well plates, with 10-20 embryos per well, and the test solutions were added to ensure the embryos were fully submerged. The plates were incubated at 28.5°C under standard light-dark cycle conditions (14 hr light/10 hr dark). Observations were made at regular intervals, typically every 24 hr, using a stereomicroscope to monitor endpoints such as mortality, hatching rate, developmental abnormalities, and behavioral changes. In cases where more detailed observations were required, embryos were anesthetized with tricaine. The data were analyzed to determine the concentration of the extract that caused 50% mortality (LC_{50}) or other significant developmental endpoints (EC_{50}), and statistical analyses were performed to compare the treated groups with controls. The results were reported with detailed concentrations, observed effects, and statistical interpretations.^[15]

RESULTS

Antibacterial Activity

The antibacterial activity of *Taraxacum officinale* extract was tested against Gram-positive *Staphylococcus aureus* and Gram-negative *Escherichia coli*. For *Staphylococcus aureus*, the control and 100 μ L treatments displayed clear zones of inhibition, with the 100 μ L sample showing activity comparable to the control, indicating strong antimicrobial effects. Similarly, for *Escherichia coli*, both the control and 100 μ L treatments exhibited prominent inhibition zones, while the 50 μ L sample showed moderate activity (Figure 1). These results suggest that *Taraxacum officinale* possesses strong antimicrobial activity, particularly at higher concentrations, against both Gram-positive and Gram-negative bacteria.

Antioxidant Activity

The antioxidant potential of *Taraxacum officinale* was assessed using the DPPH assay, and the results are presented in a bar graph showing the percentage of inhibition (Figure 2). The control group exhibited a high inhibition rate, close to 100%, indicating strong antioxidant activity. The 50 μ L and 100 μ L concentrations of the extract both demonstrated high antioxidant activity, with

the 100 μ L sample achieving levels nearly equivalent to the control. Statistical analysis ($p < 0.05$) confirms these differences are significant, suggesting that *Taraxacum officinale* maintains high antioxidant activity, particularly at higher concentrations.

Anti-Inflammatory Activity

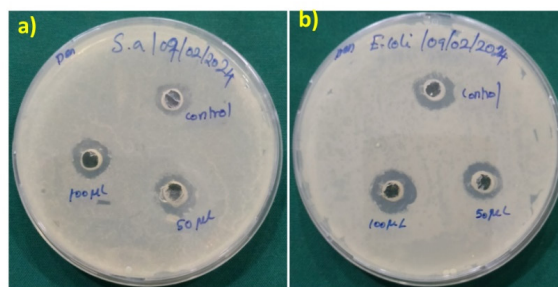
The anti-inflammatory effect of *Taraxacum officinale* was evaluated by measuring the inhibition of protein denaturation at various concentrations (20, 40, 60, 80, and 100 μ g/mL). The bar graph shows a progressive increase in relative inhibitory activity with increasing concentrations (Figure 3). The 20 μ g/mL sample displayed minimal anti-inflammatory effect, whereas the 100 μ g/mL concentration demonstrated the highest inhibitory activity, comparable to or exceeding that of the control. Statistical significance was established ($p < 0.03$), supporting the observation that *Taraxacum officinale* exhibits concentration-dependent anti-inflammatory activity, with peak efficacy at 100 μ g/mL.

Toxicity Assay using Zebrafish Embryos

The toxicity of *Taraxacum officinale* (dandelion) extract on zebrafish embryos was evaluated by monitoring the survival rate at various time intervals (24 hr, 48 hr, 72 hr, 96 hr, and 120 hr). The bar graph illustrates the percentage of live embryos across different concentrations: Control (0 μ L/mL), 20 μ L/mL, and 40 μ L/mL of *Taraxacum officinale* extract. At 24 hr, all groups demonstrated close to 100% live embryos, suggesting no immediate toxic effects. However, as time progressed, the survival rate in treated groups decreased, with the most pronounced reduction observed at 40 μ L/mL after 96 to 120 hr, indicating dose-dependent toxicity (Figure 4). These results demonstrate a concentration and time-dependent toxic effect of *Taraxacum officinale* extract on zebrafish embryos.

DISCUSSION

Oral inflammatory lesions, such as oral lichen planus, aphthous stomatitis, and periodontitis, are complex conditions driven by persistent bacterial infections, oxidative stress, and chronic inflammation.^[16,17] While conventional treatments typically

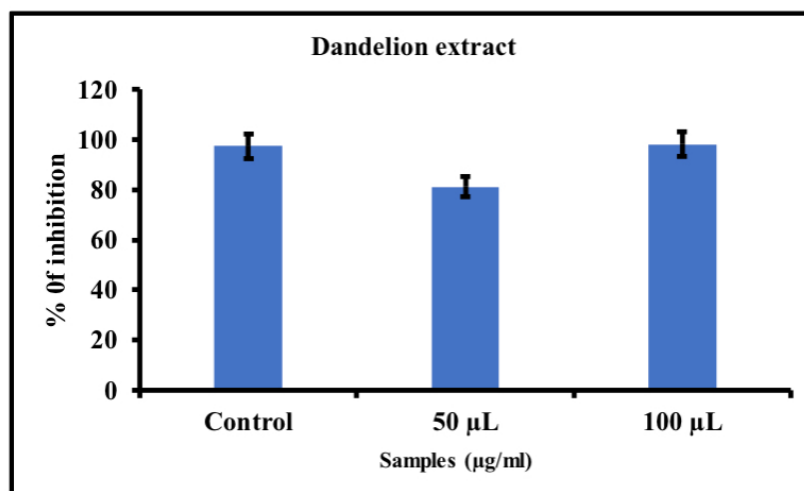


Anti-Microbial activity

Gram-positive *Staphylococcus aureus* (ATCC 25923) and Gram-negative *Escherichia coli* (ATCC 25922).

Figure 1: *Taraxacum Officinale* shows antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*.

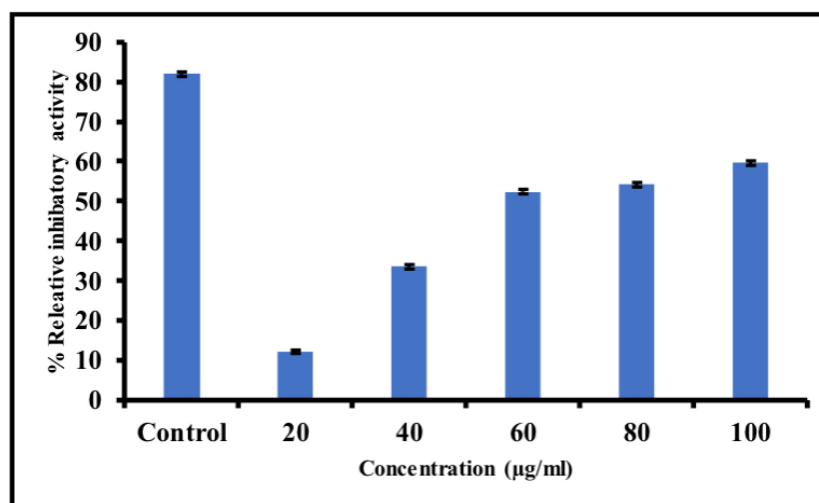
Anti-oxidant activity



Significant value $p < 0.05$

Figure 2: A bar graph showing *Taraxacum officinale* leaf extract's high antioxidant activity at both 50 µL and 100 µL concentrations.

Anti-inflammatory activity



Significant value $p < 0.03$

Figure 3: The Graph represents increasing anti-inflammatory activity with higher concentrations.

include antibiotics, anti-inflammatory drugs, and antioxidants, these approaches are often associated with side effects, including antimicrobial resistance and gastrointestinal issues, especially with long-term use. Consequently, there is a pressing need for safer alternatives, and herbal medicines are increasingly recognized as potential candidates due to their multi-functional bioactive compounds.^[18,19] Extracts like *Taraxacum officinale* (dandelion) offer a promising, natural approach that may address infection, inflammation, and oxidative stress in an integrated manner.^[20]

Our study findings reveal that *T. officinale* leaf extract exhibits notable antimicrobial effects, particularly against *Staphylococcus*

aureus, with moderate effects on *Escherichia coli*. These results align with the findings of Diaz *et al.* (2018), who observed similar antibacterial activities of *T. officinale* against both *S. aureus* and other pathogenic bacteria.^[21] Such natural antimicrobial agents, including plant-derived extracts, have gained attention for their ability to combat bacterial infections while minimizing resistance risks.^[22] These antimicrobial properties highlight the potential role of *T. officinale* in reducing bacterial loads associated with oral lesions, potentially offering an alternative to synthetic antibiotics, which often carry side effects and contribute to resistance issues.^[23]

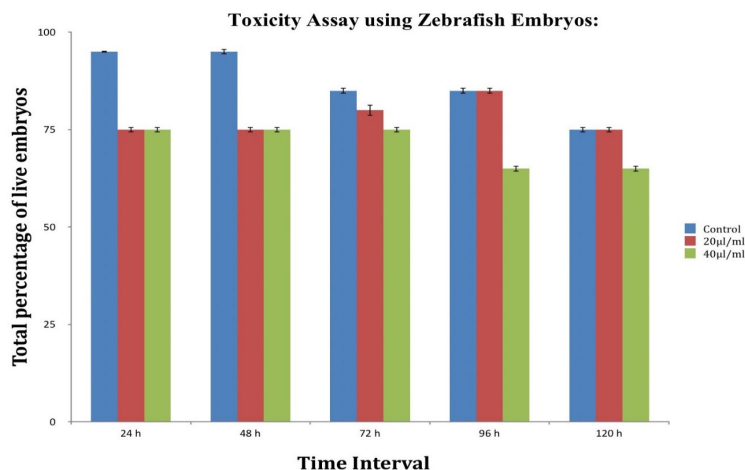


Figure 4: A Bar graph representing *Taraxacum officinale* leaf extract's dose-dependent toxicity on embryos, with higher concentrations (40 µL/mL) causing a greater reduction in live embryos over time.

The study also demonstrated strong antioxidant activity in *T. officinale*, particularly at higher extract concentrations. This outcome is consistent with studies showing the high antioxidant potential of *T. officinale* due to its polyphenolic compounds, such as chlorogenic acid and luteolin.^[24] The DPPH assay, used in this study, is a widely accepted method for quantifying antioxidant activity, as employed in evaluations of other herbal formulations, such as *Suaeda monoica*, *Cocos nucifera* and *Triticum aestivum*.^[25,26] Since oxidative stress contributes significantly to oral lesion progression, these antioxidant properties suggest a role for *T. officinale* in mitigating oxidative damage within the oral cavity, potentially aiding in the prevention and management of such lesions.^[27,28]

Furthermore, the extract exhibited concentration-dependent anti-inflammatory effects, supporting findings by Lis *et al.* (2020) that identified *T. officinale* as an effective natural anti-inflammatory.^[29] This activity may benefit patients with oral lesions by reducing chronic inflammation, a factor that often exacerbates oral lesion severity and delays healing.^[30] The anti-inflammatory properties of *T. officinale* thus suggest that it could serve as a safer alternative to synthetic anti-inflammatory drugs, particularly for patients requiring long-term treatment.^[31]

The observed dose-dependent cytotoxicity in zebrafish embryos indicates that while *T. officinale* has beneficial effects, attention to dosage is essential. Higher concentrations showed increased toxicity over time, in line with another study reporting cytotoxic effects on human hepatic cells.^[32] This emphasizes the importance of identifying therapeutic dosage ranges to ensure safety, especially in clinical applications involving oral administration.^[33]

The broader implications of this study suggest that *T. officinale* could provide a safer, multi-functional approach for managing oral inflammatory lesions, addressing infection, inflammation, and oxidative stress simultaneously.^[34] For individuals with

these lesions, a natural remedy such as *T. officinale* might reduce reliance on synthetic drugs and their associated side effects. However, it is essential to acknowledge the limitations of this study, particularly its *in vitro* design, which may not fully represent the *in vivo* efficacy of *T. officinale*.^[35] Further research through *in vivo* studies is needed to validate these findings and determine the extract's safety and efficacy in clinical settings.^[36]

In summary, the multi-functional bioactivities of *T. officinale*-including antimicrobial, antioxidant, and anti-inflammatory effects-position it as a potential adjunctive treatment for oral inflammatory lesions. This approach may provide a natural complement to conventional treatments, potentially reducing adverse effects associated with synthetic drugs.^[37] However, the study's limitations, including the *in vitro* nature of some assays, suggest a need for further *in vivo* studies to confirm these results in a clinical context. Future research should focus on evaluating safe dosage levels, delivery methods, and long-term effects to support the therapeutic integration of *T. officinale* in oral health care.

CONCLUSION

Taraxacum officinale leaf extract shows significant antimicrobial, antioxidant, and anti-inflammatory activities, making it a promising natural adjunct for managing oral inflammatory lesions. However, its dose-dependent toxicity emphasizes the need for determining safe dosage levels in future *in vivo* studies.

ACKNOWLEDGEMENT

The authors acknowledge the Saveetha Dental College and Hospitals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

This study aimed to investigate the therapeutic potential of dandelion leaf extract. The research focuses on its bioactivities, including antimicrobial effects on *Staphylococcus aureus* and *Escherichia coli*, antioxidant capacity via the DPPH assay, anti-inflammatory properties through protein denaturation inhibition, and its toxicity assessed using zebrafish embryos. Results demonstrate that the extract exhibits strong antimicrobial, antioxidant, and anti-inflammatory activities, particularly at higher concentrations, with dose-dependent toxicity in zebrafish embryos. These findings support the potential of *T. officinale* as a natural therapeutic agent for oral inflammatory lesions, though further *in vivo* research is required to confirm its clinical applicability.

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Cite this article: Kandrikar NF, Ramasubramanian A, Ramani P. Preliminary Evaluation of the Antimicrobial, Anti-Inflammatory, Antioxidant, and Cytotoxic Activities of *Taraxacum officinale* Leaf Extract: An *in vitro* Study. *Pharmacog Res*. 2025;17(1):277-82.