Acorus Calamus and Tephrosia Maxima: Novel Formulations for Combating Oral Infections

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**Abstract:** Acorus calamus exhibits significant antibacterial properties, which are attributed to its bioactive compounds that disrupt microbial cell function and inhibit growth. Tephrosia maxima is recognized for its anti-inflammatory activity, driven by phytochemicals that regulate inflammatory mediators and pathways. The combination of Acorus calamus and Tephrosia maxima presents a comprehensive therapeutic approach, offering antibacterial, anti-inflammatory, and antioxidant properties. This multifaceted efficacy positions it as a promising alternative to single-agent treatments, particularly in light of increasing antibiotic resistance and concerns over synthetic drug safety. This study investigates the combined effects of Acorus calamus and Tephrosia maxima on antimicrobial, anti-inflammatory, and antioxidant activities. To evaluate the antibacterial, anti-inflammatory, and antioxidant activities of Acorus calamus and Tephrosia maxima, and to investigate potential synergistic effects when used in combination. The roots of Acorus calamus and the leaves of Tephrosia maxima were collected, dried, and extracted using ethanol. Their antimicrobial activity was tested against Escherichia coli and Staphylococcus aureus using the disk diffusion method. The anti-inflammatory properties were assessed through the Bovine Serum Albumin (BSA) denaturation assay. Antioxidant activity was measured using the DPPH radical scavenging test to determine their ability to neutralize free radicals. The antimicrobial activity of Acorus calamus and Tephrosia maxima was assessed using the disk diffusion method, with inhibition zones measuring 15.2 ± 0.4 mm for T. maxima and 12.8 ± 0.3 mm for A. calamus, confirming their antibacterial efficacy. The anti-inflammatory potential was evaluated through spectrophotometric absorbance readings at 660 nm, revealing 98.5% inhibition for T. maxima and 81.3% inhibition for A. calamus at 100 µg/mL (p < 0.03), indicating a stronger effect for T. maxima. Antioxidant activity was determined using the DPPH radical scavenging assay, showing inhibition exceeding 90% across all tested concentrations (p < 0.05), demonstrating the strong free radical scavenging properties of both extracts. The study demonstrates that both Acorus calamus and Tephrosia maxima exhibit significant antimicrobial, anti-inflammatory, and antioxidant activities. The combination of these plants may offer enhanced therapeutic benefits. However, further research is required to validate their safety and efficacy in clinical applications.

**Keywords**: Acorus calamus, Tephrosia maxima, Antimicrobial, Anti-inflammatory, Antioxidant, plant based

# INTRODUCTION

Over the past few decades, advancements in the understanding, prevention, and treatment of oral diseases have contributed to improved dental retention. However, the increasing prevalence of heavily restored teeth, periodontal disease, and chronic oral infections highlights the ongoing challenge of maintaining oral health. The oral cavity serves as a critical site for microbial colonization, with persistent infections and inflammatory conditions contributing to systemic health concerns. In light of growing antibiotic resistance and the limitations of conventional treatments, there is a renewed interest in herbal medicine as a natural and effective alternative. Medicinal plants, with their diverse bioactive compounds, have demonstrated significant antimicrobial, anti-inflammatory, and antioxidant properties, making them promising candidates for managing oral infections[1][(Harsha & Subramanian, 2022; Rautemaa et al., 2007)](https://paperpile.com/c/O1CIlE/SoDb+JQsJ). Traditional use of medicinal plants can help discover new powerful treatments for various diseases. Around 7,000 natural compounds are used in modern medicine today, many of which were first used by traditional practitioners. As some synthetic drugs become less effective and have more side effects, the use of natural medicines is becoming more relevant. Therefore, studying plant-based treatments for chronic diseases could lead to finding new sources of medicine that are important for preventing and treating illnesses. These plants fit well into current prevention strategies[2][(Deepika et al., 2022; Varghese et al., 2023)](https://paperpile.com/c/O1CIlE/smoa+ThmS). Efforts are being made to develop multi-herb prescriptions using molecular-level research and standardized clinical trials. There is great potential to rediscover and apply traditional herbal knowledge using modern scientific methods[3][(Li & Weng, 2017; Solanki et al., 2022)](https://paperpile.com/c/O1CIlE/1WFg+T4i1). Many drugs used in allopathic medicine, also called Western medical science, originate from medicinal plants [(Chidambaram et al., 2022; Duke, 2002; Li & Weng, 2017)](https://paperpile.com/c/O1CIlE/1WFg+HCcU+rDrw)[4]. Herbal remedies have been utilized in oral care for centuries, with origins in Indian, Chinese, and various other traditional medical practices[5][(Ajay, Rakshagan, et al., 2022; Manipal et al., 2016)](https://paperpile.com/c/O1CIlE/1XNR+4uTf). Herbal compounds have the potential to mitigate even the pathological effects of HIF-1 signaling and improve the sensitivity of hypoxic cancer cells to conventional therapies[6][(Ajay, Sasikala, et al., 2022; Nalini et al., 2020)](https://paperpile.com/c/O1CIlE/VZ51+pIWv). The combination of Acorus calamus and Tephrosia maxima presents a comprehensive solution for modern health concerns by addressing antibacterial, anti-inflammatory, and antioxidant needs. This synergistic formulation offers a safer and more versatile alternative to individual treatments, particularly in the context of rising antibiotic resistance and growing skepticism towards synthetic pharmaceuticals.

# ACORUS CALAMUS

Acorus calamus, also called “sweet flag,” is a perennial herb that grows in wetlands in Asia, Europe, and North America. It holds significant historical importance in traditional medicine, particularly in Ayurveda, where it’s known as “Vacha”[7][(Haran et al., 2024)](https://paperpile.com/c/O1CIlE/N4Fm).Its rhizomes have important compounds like α-asarone and β-asarone, which give it medicinal benefits[8][(Rajput et al., 2014)](https://paperpile.com/c/O1CIlE/3ptG). Acorus calamus can be used in various forms, including powders, oils, and extracts. These are used topically or ingested depending on the condition being treated. Essential oil from the plant is particularly popular in aromatherapy for its calming and stress-relieving properties[9][(*Website*, n.d.-a)](https://paperpile.com/c/O1CIlE/cZEx).

# PHYTOCHEMICAL COMPOSITION

The primary chemical compound found in Acorus calamus is β-Asarone (isoasarone). Other constituents include α-Asarone, elemicine, cis-isoelemicine, isoeugenol, α-calacorene, 2-deca-4,7-dienol, shyobunones, isohyobunones, calamusenone, camphene, p-cymene, β-cadinene, camphor, β-gurjunene, α-selinene, terpinen-4-ol, α-terpineol, acorone, acorenone, acoragermacrone, linalool, and pre-isocalamendiol[10][(*Website*, n.d.-b)](https://paperpile.com/c/O1CIlE/xslV). Additionally, 2,5-dimethoxy benzoquinone, calamendiol, spathulenol, acoradin, galagin, 2,4,5-trimethoxy benzaldehyde, and sitosterol have also been isolated from the plant[10,11][(*Website*, n.d.-c)](https://paperpile.com/c/O1CIlE/P27d).While α-asarone has positive effects, β-asarone can be toxic in high doses.[(Panda, 2018)](https://paperpile.com/c/O1CIlE/IGWG)

# THERAPEUTIC EFFECTS

Pharmacological studies have demonstrated that Acorus calamus possesses a range of therapeutic effects. It acts as a sedative and central nervous system depressant, promoting relaxation, improving sleep, and enhancing memory. Additionally, it exhibits anticonvulsant and antispasmodic properties, making it beneficial in managing seizures and muscle spasms. The plant also has significant anti-inflammatory and antioxidant activities, helping to reduce inflammation and oxidative stress. Its antimicrobial properties enable it to combat various microbial infections, while its antidiabetic potential suggests a role in regulating blood glucose levels, further highlighting its medicinal value[8]. Acorus calamus has long been used in traditional medicine for skin and digestive health due to its diverse bioactive compounds. Its antimicrobial and anti-inflammatory properties, attributed to compounds like β-asarone and flavonoids, help combat bacterial and fungal infections while modulating inflammatory pathways. These effects make it a promising natural remedy for wound healing and skin infections. Additionally, it has been valued for its digestive benefits, aiding in the management of indigestion, bloating, and flatulence. By stimulating appetite and enhancing digestion, Acorus calamus offers potential therapeutic benefits for individuals with gastrointestinal disorders[9][12][(*Website*, n.d.-d)](https://paperpile.com/c/O1CIlE/J3LV). There are safety concerns with Acorus calamus because it contains β-asarone, which can be toxic to humans[13][(Szczeblewski et al., 2022)](https://paperpile.com/c/O1CIlE/vbIt). It can be hepatotoxic, carcinogenic and neurotoxic. Therefore, long-term use or high doses may harm human health. Several countries have regulatory restrictions on its use due to the potential risks of β-asarone[14][(*Website*, n.d.-e)](https://paperpile.com/c/O1CIlE/nTAG).

# TEPHROSIA MAXIMA

Tephrosia maxima, also called Galega maxima L., is a herb from the Fabaceae family and is a type of legume. The plant has 6-9 pairs of thin, tough, and narrow leaves. Its pink flowers grow in clusters opposite the leaves. The plant is commonly found in dry forests and wastelands and flowers throughout the year. The Tephrosia genus contains a variety of bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and terpenoids. Isoflavones are the most significant bioactive compounds found in Tephrosia species, contributing to the genus’ medicinal properties. These compounds have been linked to several therapeutic benefits, such as anti-inflammatory, antioxidant, and antimicrobial activities. Some Tephrosia species have shown anticancer activity. This is mainly because of their antioxidant and anti-inflammatory properties[15][(*Website*, n.d.-f)](https://paperpile.com/c/O1CIlE/JZW1). It contains beneficial compounds like maxima isoflavones A, H, and T, which give it medicinal value. However, it is not well-studied, so proper methods to identify and ensure its quality are needed to prevent mixing with other plants[16][(*Website*, n.d.-g)](https://paperpile.com/c/O1CIlE/SUjd)[(“Pharmacognostical Standardization of Tephrosia Maxima Pers Root,” 2011)](https://paperpile.com/c/O1CIlE/RE1V). Tephrosia is widely used in traditional medicine across the world. The World Health Organization states that over 80% of the global population relies on plants for treating diseases.

This genus plays a key role in the search for new medicinal compounds due to its diverse biological activities[17].Tephrosia maxima is traditionally valued for its anti-inflammatory, antimicrobial, and antioxidant benefits. The plant’s bioactive isoflavones have demonstrated potential in addressing various health issues, such as infections and conditions related to oxidative stress[18][(Dharman, 2021; “Nine Isoflavones from Tephrosia Maxima,” 1984)](https://paperpile.com/c/O1CIlE/teDy+bDGR).

Tephrosia is a perennial shrub known for its compound leaves and striking purple flowers. It thrives in well-drained soils and is commonly found in semi-arid regions, where it adapts to dry conditions and limited rainfall. With over 350 species, Tephrosia exhibits significant diversity. Many species contain rotenone, a naturally occurring toxic compound, which has been widely utilized as a natural insecticide and pesticide, highlighting its agricultural and ecological importance[17][(Lakshmi, 2021; *Website*, n.d.-h)](https://paperpile.com/c/O1CIlE/cUxE+qPge). The research by Rao, Murthy, and Ward (1984) explored the separation of nine isoflavones from Tephrosia maxima, emphasizing its importance in natural product studies. Their findings highlight the plant’s potential for pharmaceutical applications, particularly in the development of bioactive compounds for therapeutic use[18].

# MATERIALS AND METHODS

The roots of Acorus calamus were collected and sun-dried for several days until fully dehydrated. After drying, the roots were ground into small pieces using a mortar and pestle to prepare them for further analysis or extraction . 2 grams of dried Acorus calamus root pieces were weighed accurately and placed in a clean, dry container. Ethanol (95%) was added to the container, using a solvent-to-plant material ratio of 10:1 (v/w). The mixture was then allowed to macerate for 72 hours at room temperature with occasional stirring. After maceration, the mixture was filtered through a filter paper to separate the plant material from the extract. The final extract was stored in an airtight container for further use in experiments. figure A, Figure B

The leaves of Tephrosia maxima were collected and thoroughly washed to remove dirt. After cleaning, they were dried in a shaded, well-ventilated area until fully dehydrated. Once dried, the leaves were ground into a fine powder using a mortar and pestle. A specific amount of the powdered material was accurately weighed and macerated in ethanol (70-95%, analytical grade) at a ratio of 1:10 (w/v). The mixture was placed in an Erlenmeyer flask, sealed, and stirred continuously using a magnetic stirrer at room temperature for 24-48 hours. After the extraction period, the mixture was filtered through filter paper to separate the liquid extract from the plant residue. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40-50°C to remove the ethanol. The concentrated extract was then collected, and if necessary, further dried using a freeze-dryer. The final ethanol extract was stored in sterile, labeled vials at -20°C until further analysis.

# Antibacterial assay

The antibacterial activity was evaluated against oral pathogens, including Escherichia coli and Staphylococcus aureus, using the disk diffusion method. The bacterial cultures were grown on nutrient agar plates and standardized to a 0.5 McFarland standard. Sterile filter paper disks impregnated with the gel formulation were placed on the inoculated agar plates, and zones of inhibition were measured after 24 hours of incubation at 37°C. The activity of the extract was compared with that of standard antibiotics to assess its efficacy.

# Anti inflammatory assay

The anti-inflammatory potential of each extract was evaluated using an in vitro protein denaturation inhibition assay. Different concentrations of the extracts were mixed with an egg albumin solution and incubated. The mixture was then heated to induce protein denaturation, and absorbance was measured at 660 nm. Additionally, a red blood cell (RBC) membrane stabilization test was performed by mixing human RBCs with varying concentrations of the extracts and exposing them to hypotonic stress. The degree of hemolysis was measured to assess membrane stabilization and anti-inflammatory activity.

# Antioxidant assay

The antioxidant potential of the extracts were tested using the DPPH radical scavenging assay. Different amounts of the extracts were added to a DPPH solution, and the absorbance was measured at 517 nm after 30 minutes. The percentage of DPPH radical scavenging was calculated and compared with ascorbic acid, which is a standard antioxidant.

# RESULTS

## Anti microbial assay

Both Acorus calamus and Tephrosia purpurea demonstrated notable antimicrobial activity, with greater efficacy observed at the 100 µL volume. The lack of inhibition in the control samples confirms that the observed effects are due to the active components of the tested substances. figure C

## Anti inflammatory

The anti-inflammatory activity of A. calamus and T. maxima was evaluated at varying concentrations (20–100 µg/ml), and the results are presented in the graph. A dose-dependent increase in % relative inhibitory activity was observed for both samples. However, T. maxima exhibited significantly higher inhibitory activity compared to A. calamus across all concentrations. figure D

At the highest concentration (100 µg/ml), T. maxima demonstrated nearly 100% inhibition, whereas A. calamus showed approximately 80% inhibition. The results were statistically significant (p < 0.03, n = 3), highlighting the superior anti-inflammatory potential of T. maxima over A. calamus.

## Anti oxidant

The antioxidant activity of A. calanus and T. maxima was assessed at control, 50 µL, and 100 µL concentrations, with the % inhibition shown in the graph. Both samples exhibited high antioxidant activity across all tested concentrations, with % inhibition values remaining above 90% in all cases. FIGURE E

No significant difference in antioxidant potential was observed between A. calamus and T. maxima at any concentration, as their activities were comparable. The results, with standard deviations (n = 3), were statistically significant (p < 0.05), confirming the potent antioxidant properties of both A. calamus and T. maxima.

# DISCUSSION

Dental health is essential for overall well-being, supporting key daily functions. Oral health extends beyond traditional definitions, encompassing psychological, emotional, social, and physical factors that play a vital role in an individual’s overall health and quality of life[19][(Ajay, Suma, et al., 2022; Glick et al., 2017; Maiti, 2021)](https://paperpile.com/c/O1CIlE/7m9F+yyaa+uY5N). The World Health Organization considers oral health an essential part of overall health and well-being[20][(Jabin et al., 2021; Maliael et al., 2021; Refaey et al., 2024)](https://paperpile.com/c/O1CIlE/8caI+YAC0+j5pR). In 2022, the World Health Organization estimated that nearly 3.5 billion people worldwide suffer from oral diseases. The most prevalent and severe conditions include dental caries, periodontal disease, tooth loss, and cancers of the lips and oral cavity[21][(Aljafar et al., 2021; Katyal et al., 2021; Maiti, 2021)](https://paperpile.com/c/O1CIlE/76f2+L5hS+1Jej).

Medicinal plants are a crucial source of bioactive natural products. Many of these bioactive compounds interact with physiological processes in the body, often serving as the foundation for the development of novel pharmacological agents[22][(Balaji Ganesh S & Sugumar, 2021; Graf et al., 2023; Soundarajan & Rajasekar, 2023)](https://paperpile.com/c/O1CIlE/KPcK+sgLE+xvdQ). The Acoraceae family includes only the genus Acorus. A. calamus is a well-known plant in this family. It has been valued for its medicinal and beneficial properties in Asia for a long time, as noted by Batra et al. (2012). According to Varghese, Kumar, and Shanmugam (2021), the combination of herbal extracts and nanoparticles enhances the medicinal effects of the formulations, providing a broader spectrum of bioactivities compared to the individual components alone[23][(Sushanthi, 2021; Tiwari & Jain, 2023; Varghese et al., 2024)](https://paperpile.com/c/O1CIlE/6oWL+MEF7+crSf).

Sitosterol(SIT) isolated from *Acorus calamus* has been shown in many in-vitro and in-vivo studies to have various biological effects. It has antioxidant and anti-diabetic properties, as well as anxiolytic and sedative effects. SIT also provides analgesic, antimicrobial, and immunomodulatory benefits. Additionally, it helps in wound healing, has anti-inflammatory and anticancer properties, and supports lipid lowering. It offers protection against liver damage, non-alcoholic fatty liver disease (NAFLD), and respiratory diseases[24][(Babu & Jayaraman, 2020; Govindaraj & Dinesh, 2021; Ramamurthy, 2021)](https://paperpile.com/c/O1CIlE/HkO7+sz7k+GL6n).

This study evaluated the antibacterial, anti-inflammatory, and antioxidant properties of Acorus calamus and Tephrosia maxima extracts. Both plants showed strong potential, supporting their traditional use in medicine, and their combined effects could offer a broader range of benefits.

In the antibacterial test, both Acorus calamus and Tephrosia maxima were effective against Escherichia coli and Staphylococcus aureus, common bacteria associated with oral infections. The extracts produced clear inhibition zones, suggesting they could be useful in oral care products or as natural treatments for infections. Tephrosia maxima showed stronger antibacterial activity, indicating it might have a wider range of antimicrobial effects than Acorus calamus. The combination of both plants could enhance the antibacterial properties, providing a more comprehensive defense against a variety of pathogens.

For anti-inflammatory activity, Tephrosia maxima outperformed Acorus calamus, with nearly 100% inhibition at the highest concentration, while Acorus calamus reached about 80%. This shows that Tephrosia maxima may be more effective in reducing inflammation, which could be helpful for conditions like arthritis or other inflammatory diseases. Combining both plants may provide a more balanced anti-inflammatory effect, using the strengths of each extract to reduce inflammation more effectively.



FIGURE 1: Acorus calamus

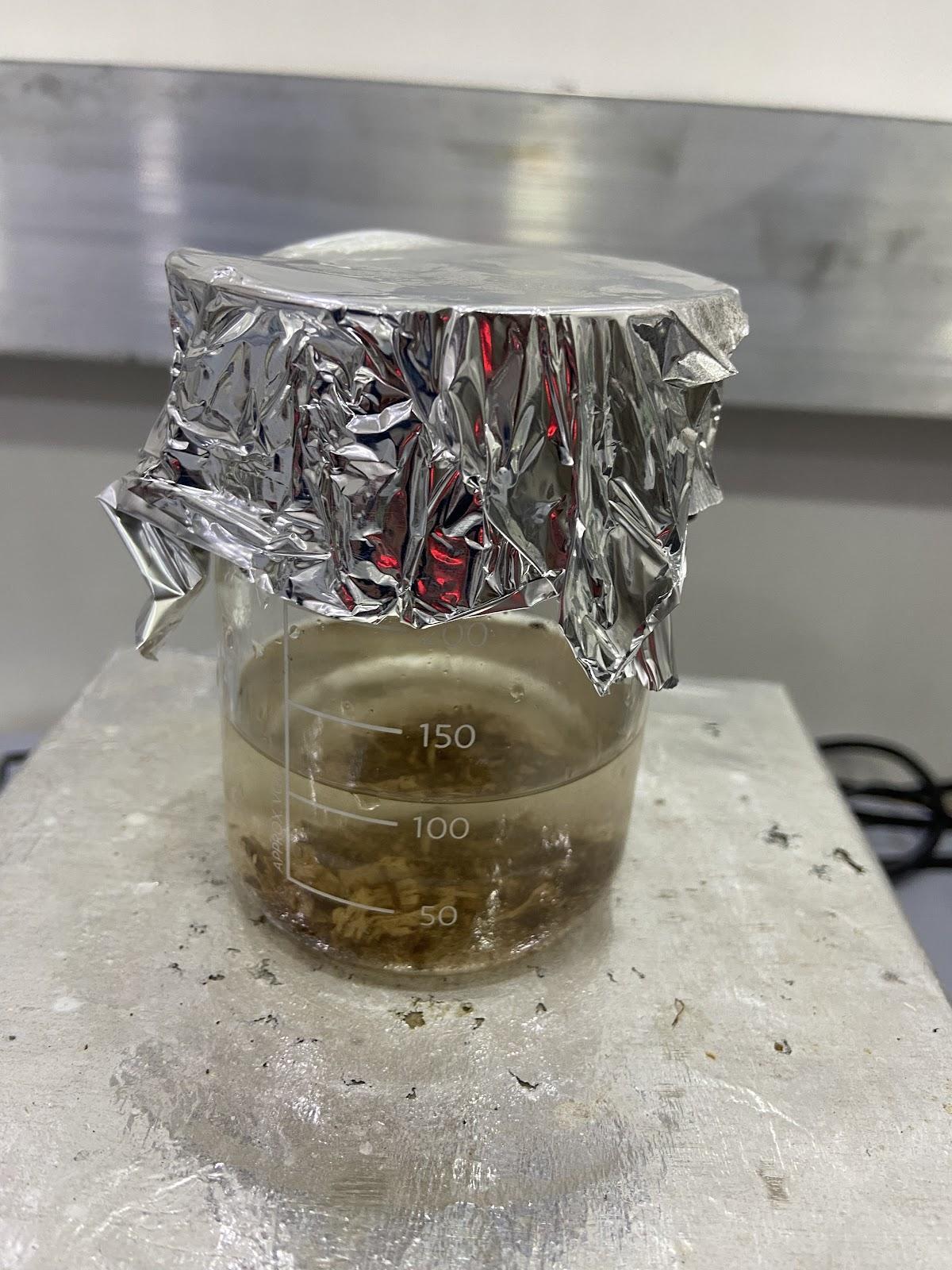


FIGURE 2: PREPARATION OF EXTRACT

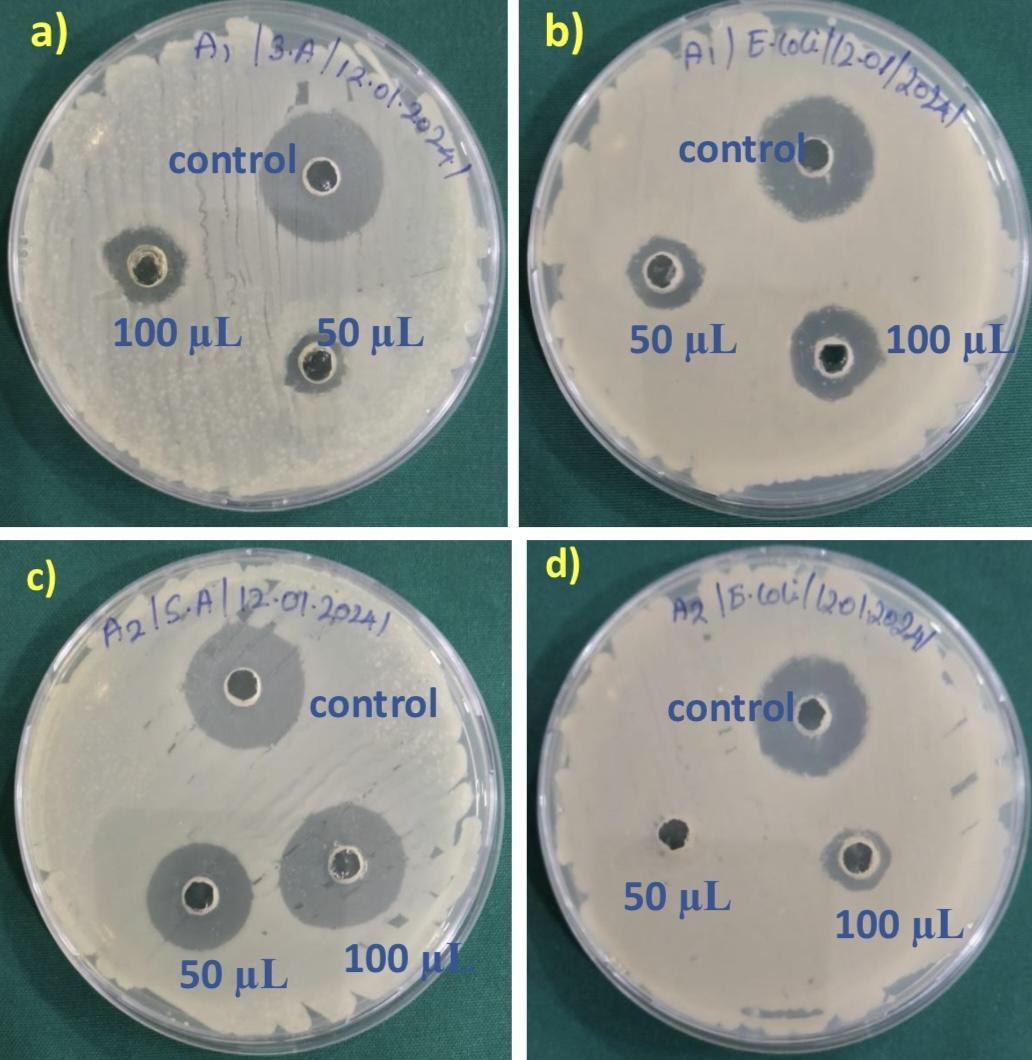


FIGURE 3: a, b,c and d show the zones of inhibition for E.coli and Staph. aureus bacteria, demonstrating that the extracts exhibit strong antibacterial activity, making it effective for use in oral hygiene products.

In the antioxidant test, both plant extracts showed high antioxidant activity, effectively scavenging DPPH radicals. Their antioxidant effects were similar, meaning both could help protect against diseases caused by oxidative stress. A combination of these plants could enhance antioxidant activity, providing greater protection against oxidative damage.

The antimicrobial properties of Acorus calamus extracts against Lactobacillus casei, a key cariogenic microorganism, highlight their potential applications in dental therapeutics[25]. These extracts could be utilized in the formulation of antimicrobial oral rinses or dentifrices to inhibit bacterial colonization and mitigate the risk of dental caries. Furthermore, they may be incorporated into dental restorative materials, such as resin-based sealants or cements, to establish a bioactive antimicrobial barrier, thereby preventing microbial infiltration and secondary caries. The extracts’ anti-inflammatory and antimicrobial activities also suggest their potential use in periodontal therapy, such as in localized drug delivery systems or medicated gels, for the management of periodontal infections, abscesses, and soft tissue inflammations. Further research is essential to evaluate their clinical efficacy and biocompatibility in dental applications.

Overall, both Acorus calamus and Tephrosia maxima demonstrated significant antibacterial, anti-inflammatory, and antioxidant effects. Although Tephrosia maxima showed stronger anti-inflammatory and antibacterial effects, combining both plants could create a more balanced treatment, improving the overall benefits. This combination could be particularly useful in treating infections, inflammation, and oxidative stress-related diseases. Further research is needed to identify the active compounds in both plants and to evaluate the safety and effectiveness of their combined use in clinical settings.

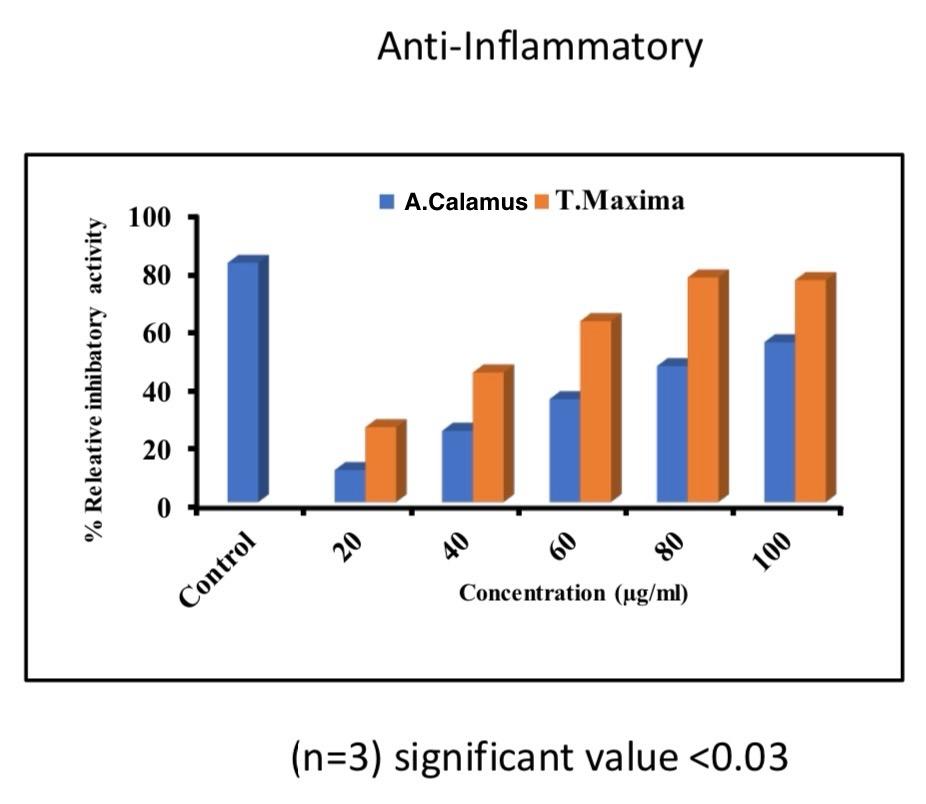


FIGURE 4: ANTI INFLAMMATORY ACTIVITY

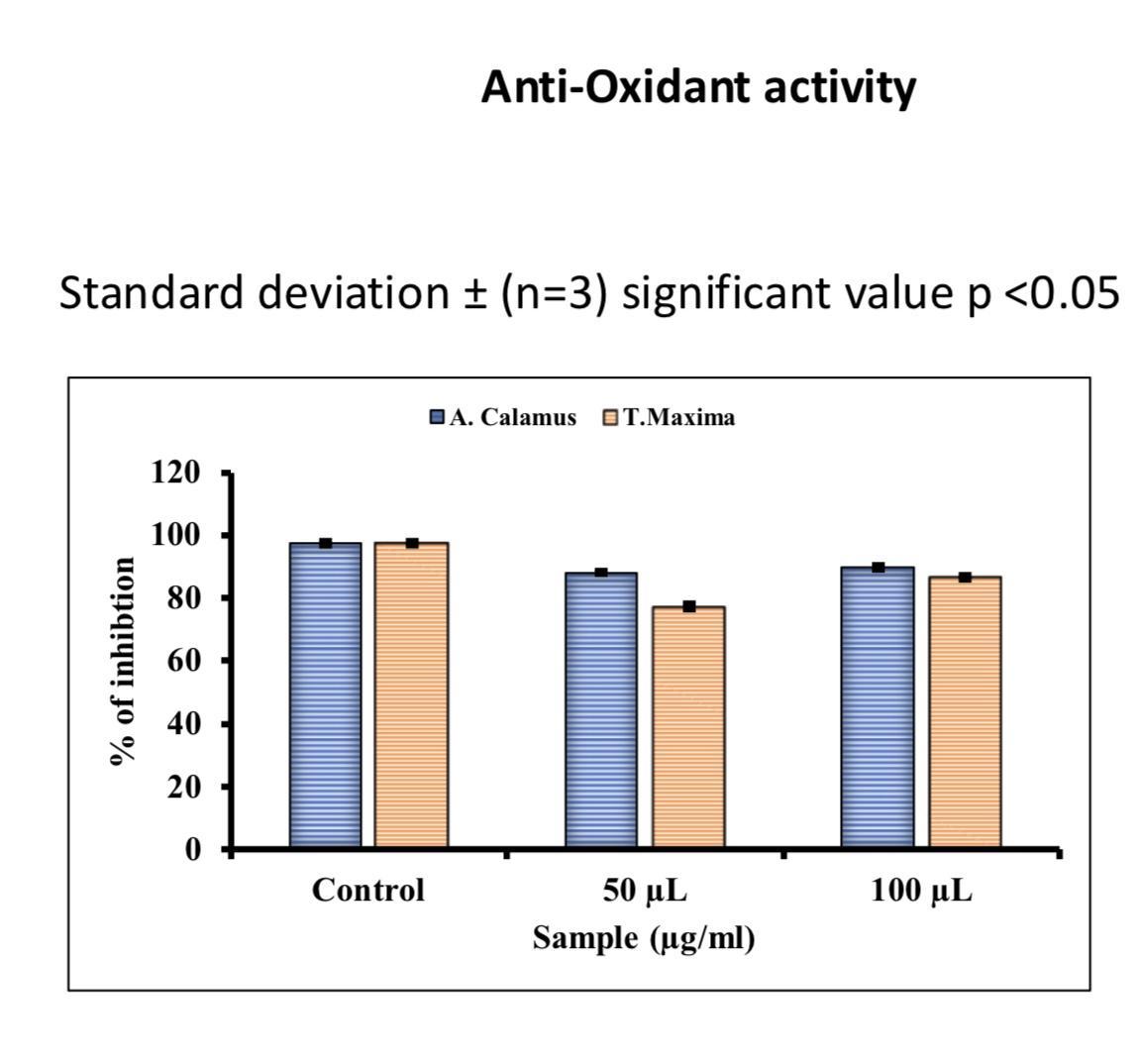


FIGURE 5: ANTIOXIDANT ACTIVITY

# LIMITATIONS AND FUTURE SCOPE

Future research should include clinical trials and explore combining these plants with others to create stronger treatments. Understanding how these plants work in the body will help develop safe and effective natural medicines.

# CONCLUSION

Herbal dentifrices may offer a viable alternative to traditional dentifrices for managing gingival diseases[26][(Devi & Rajasekar, 2022)](https://paperpile.com/c/O1CIlE/AA1d). This study highlights the strong antibacterial, anti-inflammatory, and antioxidant properties of Acorus calamus and Tephrosia maxima, supporting their traditional use in medicine. Both plants show significant therapeutic potential, with Tephrosia maxima being more effective in fighting inflammation and bacteria than Acorus calamus. However, when combined, they may offer a more balanced and powerful treatment, taking advantage of the unique benefits each plant provides.

The antibacterial effects of both plants against Escherichia coli and Staphylococcus aureus suggest they could be useful for treating infections, especially in oral care. Additionally, the antioxidant activity of both plants points to their potential in preventing diseases caused by oxidative stress, such as heart disease and cancer.

While these results are promising, more research is needed to confirm the safety and effectiveness of Acorus calamus and Tephrosia maxima in the real-world. The Tephrosia genus offers a valuable range of natural compounds with various pharmacological effects. Tephrosia species have demonstrated potential in treating a range of conditions, such as bacterial and fungal infections, inflammation, and disorders related to oxidative stress[27][(Chen et al., 2014; Devi & Rajasekar, 2022)](https://paperpile.com/c/O1CIlE/AA1d+X7zF). Ongoing research into its chemical components and biological activities may pave the way for developing new therapeutic agents for numerous health conditions. Identifying the specific compounds responsible for their therapeutic effects is crucial, and long-term safety studies are necessary, especially for Tephrosia maxima, which contains rotenone, a toxic substance.

In conclusion, Acorus calamus and Tephrosia maxima exhibit considerable potential as natural therapeutic agents. Their combined application may offer a synergistic approach for the management of infections, inflammatory conditions, and diseases associated with oxidative stress. Further comprehensive studies, including clinical trials, are warranted to elucidate their full therapeutic potential and facilitate their integration into contemporary medical practice.

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