Research Article

Antimicrobial Effect of Coleus Amboinicus L. Extract Against Oral Pathogens: an in-Vitro Study

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Abstract

Objective: Oral diseases such as periodontitis and dental abscesses are largely driven by microbial infections. The rise in antimicrobial resistance has created an urgent need to explore alternative therapeutic agents, particularly those derived from medicinal plants.

Material and Methods: This study aimed to evaluate the in vitro antimicrobial activity of ethanolic extracts of Coleus amboinicus leaves against oral pathogens isolated from clinical cases of subgingival calculus and periapical abscesses. Leaf samples of C. amboinicus were collected, shade dried, and extracted using the hot percolation method with ethanol. Clinical isolates were obtained from patients attending the Periodontics and Conservative Dentistry departments. The isolated pathogens were cultured and identified, and antimicrobial susceptibility was assessed using the standard disc diffusion technique.

Results and Conclusion: Among the identified organisms, Enterococcus faecalis showed a zone of inhibition of 5 mm, indicating sensitivity to the plant extract. In contrast, Streptococcus mutans and Escherichia coli were resistant under the tested conditions. The findings suggest that C. amboinicus possesses selective antimicrobial activity, particularly against E. faecalis, a common endodontic pathogen. Further studies are recommended to optimize extract concentration, isolate active constituents, and develop potential formulations for oral healthcare applications.

Key Words Coleus amboinicus; oral pathogens; antimicrobial activity; herbal extract; Enterococcus faecalis; in vitro study.

INTRODUCTION

Oral diseases pose a significant public health challenge due to their widespread prevalence and incidence across populations. These diseases can have a profound impact on overall health, quality of life, and economic burden, making their prevention and treatment a critical aspect of healthcare systems worldwide. (1) The human oral cavity harbours a diverse microbiota, consisting of bacteria, fungi, viruses, and protozoa, many of which play a crucial role in maintaining oral homeostasis. However, an imbalance in these microbial communities can lead to the development of various oral diseases. Among the most common oral diseases are periodontitis, dental abscesses, and pulpitis, all of which have a strong microbial etiology. Periodontitis, a chronic inflammatory disease affecting the supporting structures of the teeth, is primarily driven by periodontal pathogens such as Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans. (2) Similarly, dental abscesses result from bacterial infections within the pulp or periodontal tissues, while pulpitis occurs due to bacterial invasion of the dental pulp, often leading to severe pain and potential tooth loss. Given the microbial origins of these conditions, targeted antimicrobial therapies are essential for effective management.

Conventionally, synthetic antimicrobial agents, including antibiotics and antiseptic mouthwashes, have been widely used to control oral infections. However, the increasing emergence of antimicrobial resistance (AMR) among oral pathogens has raised significant concerns. The widespread and often indiscriminate use of antibiotics has contributed to the selection of resistant bacterial strains, diminishing the efficacy of conventional treatments and complicating the management of infectious oral diseases. (3) This growing threat necessitates the exploration of alternative therapeutic strategies that are both effective and sustainable. In this context, natural herbal remedies have gained attention for their potential antimicrobial properties. Various medicinal plants and phytochemicals have demonstrated promising antibacterial, anti-inflammatory, and antioxidant activities against oral pathogens. Unlike synthetic antimicrobial agents, herbal formulations often exhibit a lower risk of adverse effects and minimal potential for resistance development. (4) The incorporation of natural compounds into oral healthcare regimens could serve as an adjunct or alternative to traditional antimicrobial therapies, offering a holistic approach to managing oral infections. (5)

Given the rising concerns over microbial resistance and the limitations of existing treatments, it is imperative to explore novel therapeutic interventions that are both effective and sustainable. This manuscript aims to highlight the significance of oral microbial infections, discuss the challenges posed by antimicrobial resistance, and evaluate the potential of natural herbal remedies as a viable approach to combatting infectious oral diseases.

Coleus amboinicus (KARPURAVALLI), scientifically known as Plectranthus amboinicus, belongs to the family Lamiaceae. It is a semi-succulent, perennial plant characterized by its thick, fleshy leaves and a distinct spicy, oregano-like aroma and flavor. This plant is widely cultivated and utilized for multiple purposes, including culinary, ornamental, and medicinal applications. (6) Due to its rich phytochemical composition, C. amboinicus has been traditionally valued for its various health benefits.

One of the key attributes of Coleus amboinicus is its therapeutic potential, which is primarily linked to the presence of bioactive antioxidant compounds, including flavonoids. These compounds play a crucial role in neutralizing free radicals, reducing oxidative stress, and protecting cells from damage. (7) As a result, this plant has been explored for its potential in promoting overall health and preventing various diseases. In addition to its antioxidant properties, Coleus amboinicus exhibits significant antibacterial activity. Research has demonstrated its effectiveness against various bacterial strains, making it a promising candidate for developing natural antimicrobial agents. Moreover, scientific investigations have explored the efficacy of C. amboinicus in combating halitosis-causing bacteria through in vitro studies. The results have shown promising antibacterial effects, suggesting its potential use in oral healthcare products to help reduce bad breath and improve oral hygiene. (8-10)

Previous studies have primarily tested the antibacterial properties of Coleus amboinicus in its essential oil form. However, in the present study, the plant extract is being evaluated in its ethanolic extract form to further analyse its antimicrobial efficacy of oral pathogens isolated from patients.

Materials and Methods:

A total of 10 subgingival calculus and 5 periapical abscesses were collected from the patients attended outpatient ward of the Periodontics and Conservative & Endodontics department of Rajas Dental College and Hospital within the time period from October 10,2024 to November 15,2024 (Figure: 2). Patients under age group of 20-40 years were included in the study and those with systemic complications like diabetes, cardiovascular anomalies, pregnancy and patient with long term corticosteroid therapy were excluded from the study. The leaf samples Coleus amboinicus were collected from a cultivated garden in Tirunelveli. The plant was identified based on its morphological characteristics such as thick succulent leaves with a distinctive aromatic scent (Figure :1). The leaves were washed thoroughly with water to remove dirt and other contaminants and then shade dried at room temperature for 7 to 10 days. The dried leaves were then ground into a fine powder using a laboratory blender and stored in an airtight container until further use.



Fig: 1 Collection of Coleus amboinicus leave

Plant Extraction method: Hot Percolation method

25g of the dried powder of Coleus amboinicus leaves was loaded in the Soxhlet apparatus and extracted by continuous hot percolation method. Ethanol (250ml) was poured into the round bottom flask which served as the extraction solvent and the extraction was conducted at 60°C for 6 hours, allowing continuous percolation of ethanol through the plant material. The obtained extract was filtered through Whatman No.1 filter paper to remove plant residues. The filtrate was concentrated using a rotary evaporator under reduced pressure at 40°C for 1 hr to remove ethanol and obtain a semisolid crude extract. The extracts were collected in sterile falcon tubes and stored at 4°C for further analysis.

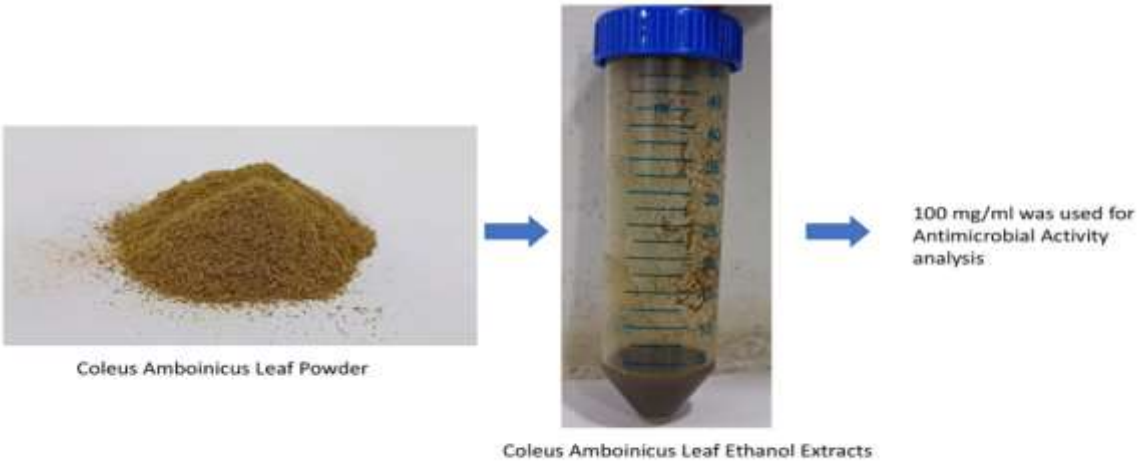

Fig 2: Processing of coleus amboinicus leaf extracts

Processing of Clinical Samples:

The clinical samples were collected on different days and the microbiological analysis was carried out at the central research laboratory of Rajas Dental College and Hospital. The samples were processed under aseptic condition to ensure the accuracy of microbial isolation. The specimen was diluted using sterile sodium chloride solution to maintain isotonicity and facilitate microbial suspension. The diluted samples were then inoculated on nutrient agar plates and incubated at 37°C for 24 hrs.



Fig 3: Collection of clinical samples

Antimicrobial sensitivity testing:

Among the isolated microorganism, E. faecalis, E. coli and S. mutans were predominantly present in the clinical samples and were selected for susceptibility testing against the leaf extract of Coleus amboinicus by standard disc diffusion method. The inoculum of the bacterial strains was prepared and adjusted to 0.5 MacFarland standard. Muller-Hinton agar plates were used and lawn culture was done. The leaf extracts were impregnated in the sterile filter paper disc of 6mm size and are allow to dry. Using sterile forceps, it was then placed in the culture plate. The plates were then incubated at 37°C overnight. After incubation using standard ruler the zone size was measured.

RESULTS:

Isolation of organisms:

Following incubation, the plates were examined for microbial growth and the colonies were isolated and reinoculated in MacConkey agar, Mitis salivarius agar and SDA (Saburaoud's Dextrose Agar) and incubated at 37°C for 24 hrs for the identification of the organisms. The culture plate was examined for the microbial growth (Figure:4). Gram Staining was performed and the organisms was identified. (Table:1).

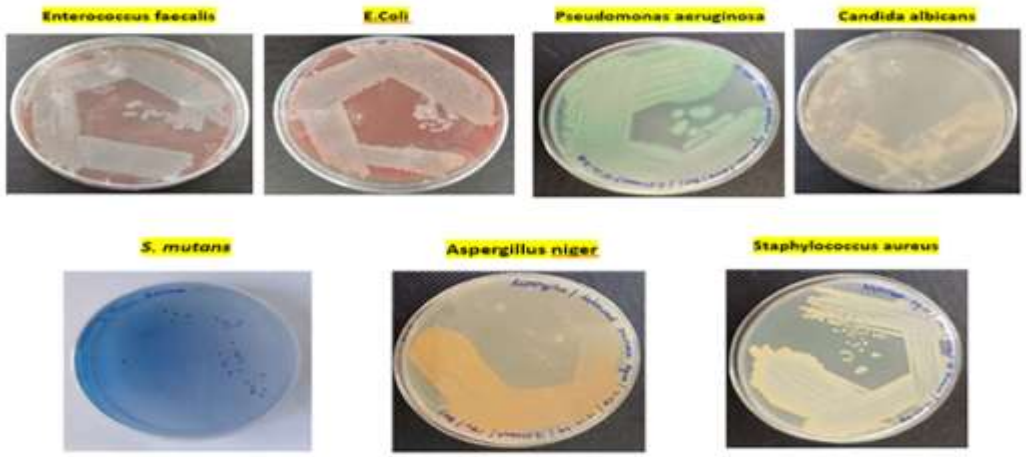

Fig 4: Microbial growth from clinical samples

Table 1: Microorganisms isolated from clinical samples

Gram Positive Bacteria	Gram Negative Bacteria	Fungi
Staphylococcus aureus	Escherichia coli	Aspergillus niger
Streptococcus mutans	Pseudomonas aeruginosa	Candida
Enterococcus faecalis		

The table represents the different microorganisms isolated from the clinical sample

Antimicrobial sensitivity testing:

Among the three strains, Enterococcus faecalis had a zone size of 5mm in the particular concentration of the leaf extract. The other organisms were resistant to the leaf extract.



Fig 5: Antimicrobial Sensitivity testing of the Bacterial Isolates

DISCUSSION:

Oral diseases are primarily caused by microbial imbalances, and antimicrobial therapy plays a critical role in their management. However, the rise of antimicrobial resistance (AMR) has posed a significant challenge to conventional treatment strategies. In recent years, researchers have increasingly explored herbal and plant-based alternatives due to their bioactive potential and lower resistance risk compared to synthetic antimicrobials (11,12).

Coleus amboinicus (L.), commonly known as Indian borage, has shown promise due to its rich phytochemical profile, including flavonoids, phenols, and terpenoids, which contribute to its antimicrobial, anti-inflammatory, and antioxidant effects (13,14). In the current study, ethanolic leaf extracts of C. amboinicus were evaluated for their antimicrobial activity against oral pathogens isolated from patients with subgingival calculus and periapical abscesses. The microbial isolates included Enterococcus faecalis, Streptococcus mutans, and Escherichia coli—all of which are known contributors to periodontal and endodontic infections (15,16).

Among the tested organisms, only E. faecalis showed a measurable zone of inhibition (5 mm), indicating a mild sensitivity to the extract. This is noteworthy, considering that E. faecalis is a resilient organism often implicated in failed root canal treatments and is known for its resistance to standard antimicrobial agents (17). Previous in vitro studies have also reported inhibitory effects of C. amboinicus essential oils and extracts against E. faecalis, confirming its potential as an alternative remedy (11,18).

On the other hand, S. mutans and E. coli were resistant to the extract. The resistance of S. mutans might be attributed to its ability to form robust biofilms, which protect it from many antimicrobials, especially those with weaker penetration properties (9). Similarly, the gram-negative outer membrane of E. coli can act as a barrier to phytochemicals, reducing their antimicrobial efficacy (11).

The extraction method employed in this study, hot percolation with ethanol as solvent, is well-suited for isolating bioactive polar compounds (11). Ethanol has been shown to effectively extract antibacterial constituents from C. amboinicus leaves, although the efficacy can vary depending on the solvent concentration, exposure time, and temperature (12).

While essential oils of C. amboinicus have been extensively studied, there is relatively limited data on ethanolic extracts. However, essential oils have demonstrated strong antibacterial and antibiofilm activity against oral microbes including Streptococcus mutans and Candida albicans, and similar trends are beginning to emerge for ethanolic extracts, though more work is needed to confirm these effects across various concentrations and microbial strains. (11,15)

One limitation of the current study is the use of crude extracts without fractionation or phytochemical quantification. Further studies employing purified fractions or synergistic herbal combinations may improve antimicrobial efficacy. Moreover, testing with higher extract concentrations and larger clinical isolates could offer deeper insight into potential applications of C. amboinicus in dental care products such as mouthwashes, gels, or gum paints (12,14).

CONCLUSION:

This in vitro investigation demonstrates that Coleus amboinicus ethanolic leaf extract exhibits antimicrobial activity against Enterococcus faecalis, a common and persistent endodontic pathogen. The extract did not exhibit significant inhibitory effects against Streptococcus mutans or Escherichia coli, suggesting a selective antimicrobial spectrum. The findings support the potential utility of C. amboinicus as a plant-based adjunct in managing resistant oral infections.

Given its traditional medicinal relevance and observed activity, C. amboinicus merits further exploration through advanced phytochemical analysis, concentration optimization, and broader-spectrum antimicrobial testing. Future studies should also consider synergistic effects with other herbal extracts and explore the formulation of dental products such as mouth rinses or gels. Such developments may offer effective, natural, and sustainable alternatives to conventional antimicrobial agents, particularly in the context of rising antibiotic resistance.

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CONFLICT OF INTEREST:
None of the authors has any conflict of interest.

AUTHORS CONTRIBUTION:

Suja contributed concept, design, extract preparation. microbiological assay, data analysis, manuscript framing. Akash Mithran contributed to the design, concept . All the CRRI in the study contributed to the sample collection, extract preparation.

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