Antibacterial Activity of Centella Asiatica Against Streptococcus Mutans and Enterococcus Faecalis

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**Abstract:** Throughout history, plants have been integral to medicinal practices, offering remedies for various ailments. Centella asiatica (CA), a perennial herbaceous creeper, holds significant medicinal value. Utilized by diverse ethnic groups for centuries, it has emerged as a preferred antibacterial option due to its wide availability and established medicinal use. Its clonal nature contributes to a rich array of bioactive compounds, making it versatile for addressing health concerns. As antibiotic resistance rises, CA's efficacy against bacteria gains attention. Its holistic healing potential, including antioxidant and anti-inflammatory properties, suggests broader therapeutic applications. With minimal side effects, CA presents a promising natural remedy for modern healthcare challenges. The present study aims to assess the antibacterial efficacy of Centella asiatica. Centella asiatica dried powder was weighed at 3 gms, 200ml of distilled water was added to it. The resulting mixture underwent boiling at 50°C for a duration of 20 minutes until the emergence of water bubbles and vapor. Subsequently, the resultant aqueous solution was subjected to analysis to evaluate its antibacterial effect against pathogenic microorganisms of oral cavity. Streptococcus mutans exhibited the maximum zone of inhibition in the conducted experiment. The findings from this study lead to the conclusion that Centella asiatica demonstrates promising antibacterial activity against both Streptococcus mutans and Enterococcus faecalis. The study findings establish the antibacterial activity of *centella asiatica* and against Str. Mutans and E. Faecalis.

**Keywords:** Medicinal plants, oral pathogen, green synthesis.

**Running title:** Antibacterial activity of *Centella* *asiatica*

# INTRODUCTION

Plants have been revered for millennia for their medicinal properties, serving as a bridge between ancient wisdom and modern scientific insights. Among these botanical treasures, Centella Asiatica, a member of the Umbelliferae family, has emerged as a significant contributor to human health and wellness[(Aparna et al., 2021)](https://paperpile.com/c/Yai7Rz/tVak). Thriving in various regions such as India, Pakistan, Sri Lanka, South Africa, and Eastern Europe, this resilient herbaceous creeper, known by various names including mandukaparni, Indian pennywort, and gotu kola, holds a prominent place in traditional medicine, particularly in Ayurveda (1), (2), (3).

Centella asiatica is esteemed for its multifaceted therapeutic properties, ranging from its reputed blood-purifying attributes to its acclaimed efficacy in addressing hypertension, enhancing cognitive function, and promoting longevity[(*Website*, n.d.)](https://paperpile.com/c/Yai7Rz/uHdS). Revered for centuries, this botanical gem transcends cultural boundaries, finding mention in ancient sacred texts and earning recognition in the Indian pharmacopeia of the nineteenth century (4).

In contemporary contexts, Centella Asiatica continues to attract scientific interest, especially for its role in wound healing and dermatological care[(Laghari et al., 2023)](https://paperpile.com/c/Yai7Rz/EW9V). Its aqueous extracts have emerged as promising agents in tissue repair, cellular proliferation stimulation, and collagen synthesis, thereby facilitating faster recovery and scar mitigation[(Larjava, 2012)](https://paperpile.com/c/Yai7Rz/vWKT). Additionally, studies have highlighted its neuroprotective abilities, particularly in elevating gamma-aminobutyric acid (GABA) levels in the brain, which confer anxiolytic and anticonvulsant effects (5), (6), (7).

Beyond its dermatological and neurological applications, Centella Asiatica boasts a range of pharmacological activities, from gastroprotective effects to antimicrobial properties[(*Anti-Inflammatory Potential Mouthwash Formulated Using Clove Ginger Mediated Zinc Oxide Nanoparticles: Vitro Study*, n.d.)](https://paperpile.com/c/Yai7Rz/FYyF). Its efficacy against bacterial and fungal pathogens underscores its potential as a natural therapeutic agent against infectious diseases. However, despite empirical evidence and historical reverence, gaps persist in understanding its underlying mechanisms and full therapeutic potential (8).

Centella Asiatica extracts have been traditionally used for wound healing, with increasing research supporting these claims[(Chokkattu et al., 2023)](https://paperpile.com/c/Yai7Rz/WRtw). Additionally, it can revitalize the nervous system, improve attention span and concentration, and mitigate aging (9).

Preclinical studies have reported increased cellular proliferation and collagen synthesis at wound sites with the application of aqueous Centella Asiatica extracts. Furthermore, its ability to increase cerebral GABA levels explains its traditional use as an anxiolytic and anticonvulsant. The herb has also demonstrated effectiveness in inhibiting gastric lesions induced by ethanol administration (10), (11), (12).

Centella Asiatica is generally well-tolerated, with minimal side effects such as skin allergy, headache, stomach upset, burning sensations and extreme drowsiness[(Sreevarun et al., 2023)](https://paperpile.com/c/Yai7Rz/0PeG). Despite extensive studies on its biological components and mechanisms of action, there is still room for understanding its complete therapeutic potential (13).

This plant has become a primary choice for researchers seeking antimicrobial substances due to its availability and widespread use in various ethnic treatments. While biological mechanisms underlying its actions have been postulated, additional scientific evidence is necessary to support its growing utilization .

Given its remarkable therapeutic potential, further comprehensive research is pivotal. The objective of the current investigation is to evaluate Centella Asiatica's antibacterial efficacy against Streptococcus mutans and Enterococcus faecalis, contributing to our understanding of its potential in combating bacterial infections .

# MATERIALS AND METHODS

The Centella asiatica extract is prepared from commercially available dry powder form - three grams is weighed (Fig 1). 200ml of distilled water was added to the powder, mixed well and boiled at 50°C for 20 minutes duration until water bubbles and vapor appeared. Subsequently, the mixture underwent centrifugation at 8000 rpm for 10 minutes (Fig 2). Filtering of the resultant extract was done using a Buchner funnel with the aid of Whatman filter paper no.1, a process that required considerable time. The filtered mixtures were transferred to conical flasks covered with aluminum foil and subjected to orbital shaking for 24 hours, following guidance from laboratory technicians. The extracts (Fig 3) were then placed in flasks which are pre- weighted before undergoing drying. This procedure was validated by experts in nanotechnology. Finally, the Centella asiatica plant extract was ready for antibacterial activity assessment.

Test organisms: Two microorganisms are used in this research as test organisms consisting of *Streptococcus mutans* and *Enterococcus faecalis*

## Antibacterial Activity

The evaluation of Centella Asiatica's antibacterial activity against Streptococcus mutans and Enterococcus faecalis was conducted using MHA agar (14). Muller Hinton agar sterilized at 120lbs for 45 minutes, poured into plates, was allowed to solidify prior to wells being cut (15). Plates were swabbed with test organisms sequentially followed by loading with nanoparticles at different concentrations. Incubation of the plates were done at 37°C for 24 hours. After incubation, the zone of inhibition was measured to assess antibacterial activity, and correlation analysis was performed (Fig 4).

# RESULTS AND DISCUSSION

The antibacterial activity against oral pathogens shows a positive correlation with the increase in concentration (mm)depicted on the X-axis correlating with the Y-axis depicting the zone of inhibition in mm

The medicinal plants derive their therapeutic and pharmaceutical significance from the diverse chemical components they contain[(Jain & Verma, 2022)](https://paperpile.com/c/Yai7Rz/vSz3). These plants have been recognized for their myriad therapeutic benefits. India has seen extensive research on plants with medicinal benefits, reflecting the growing utilization of traditional natural remedies worldwide[(Marya et al., 2022)](https://paperpile.com/c/Yai7Rz/fRb7). Researchers worldwide have extensively explored the effects of plant extracts on bacteria, highlighting the widespread utility of medicinal plants in everyday life. (1), Centella Asiatica demonstrates effectiveness in treating wounds, including infectious wounds, burns, and hypertrophic scars. Its key constituents are primarily pentacyclic triterpenes, with prominence of asiaticoside, madecassoside, Asiatic acid, and madecassic acid[(Ramamurthy et al., 2022)](https://paperpile.com/c/Yai7Rz/JD01). Additionally, Centella Asiatica exhibits antioxidant, anti-inflammatory, and antimicrobial properties, and it aids in the healing of gastric ulcers. Research findings suggest that Centella Asiatica exerts bactericidal effects on pathogenic microorganisms. In the present study, the zone of inhibition for Streptococcus mutans at 25μL was 9mm, at 50μL was 10mm, at 100μL was 13mm, and at antibiotic μL was 28mm[(Chokkattu et al., 2022)](https://paperpile.com/c/Yai7Rz/QfaU). The zone of inhibition for Entero faecalis at 25μLwas 9mm, at 50μL was 9mm, at 100μL was 10mm and at antibiotic μL, was 32mm. The correlation analysis graph illustrated a positive relationship between concentration and the zone of inhibition.

Table 1: Antimicrobial activity of aqueous solution of CentellaAsiatica*.* in gram positive bacteria with different concentration(μL) and zone of inhibition(mm)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| NAME OF THE PATHOGEN | ZONE OF INHIBITION [Concentration in mm] | | | |
|  | 25μL | 50μL | 100μL | ANTIBIOTICμL |
| Streptococcus mutans | 9mm | 10mm | 13mm | 28mm |
| Entero faecalis | 9mm | 9mm | 10mm | 32mm |

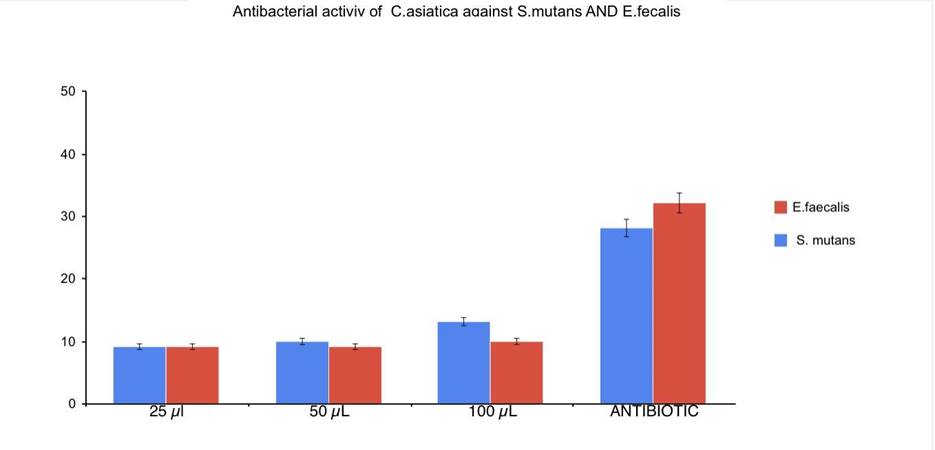


Figure 1: The graph representing the antibacterial activity of *Centella Asiatica*

Streptococcus mutans displayed the highest zone of inhibition among the two oral pathogens tested[(Merchant et al., 2022)](https://paperpile.com/c/Yai7Rz/90cn). The selection of this plant in our study aligns with its traditional use, though further research is warranted to validate its efficacy against disease-causing microorganisms (Chehelgerdi et al., 2023). In a previous study, the antibacterial activity was assessed against both Gram-positive and Gram-negative organisms[(Pandiyan et al., 2022)](https://paperpile.com/c/Yai7Rz/pmEr). The stem bark extract exhibited notable antibacterial activity against Enterobacter aerogenes, Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus, while the leaf extract demonstrated maximum activity against Escherichia coli, Alcaligenes faecalis, and Pseudomonas aeruginosa. P. santalinus showcases remarkable antioxidant, antimicrobial, and anti-inflammatory properties.[(Belcaro et al., 2011)](https://paperpile.com/c/Yai7Rz/ThCsk),[(Wiegand et al., 2008)](https://paperpile.com/c/Yai7Rz/mmg5Y),[(Ouchi et al., 2010)](https://paperpile.com/c/Yai7Rz/zzSOA),[(Thakurdesai et al., 2015)](https://paperpile.com/c/Yai7Rz/2Paw7), Furthermore, our findings corroborate those of previous research, which highlighted the efficacy of Centella asiatica root and leaf extracts in wastewater treatment, owing to their bactericidal effect on E. coli and other pathogens. While recent reports have emphasized the antimicrobial potential of C. asiatica, our study underscores that root extracts exhibit superior effectiveness against bacteria, fungi, and even yeast. (20),(21),(22),(23),(25), (26).

The outcomes of this investigation suggest a promising potential for the development of antimicrobial compounds derived from Centella asiatica, indicating a path towards more effective and promising treatments (24). Promoting the integration of medicinal plants into dental practice represents a significant step forward in holistic healthcare[(Ganapathy 2021)](https://paperpile.com/c/Yai7Rz/Q4if). These botanical wonders offer a rich tapestry of therapeutic effects, ranging from combating inflammation to thwarting bacterial and fungal infections, thereby augmenting traditional dental treatments [(Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/Yai7Rz/MWdD+MiEy). Their escalating adoption worldwide underscores not only their efficacy but also their remarkable safety profile, devoid of adverse effects commonly associated with synthetic compounds (Saadh et al., 2024). Furthermore, their profound antimicrobial properties make them formidable adversaries against a spectrum of harmful microorganisms, potentially revolutionizing oral hygiene practices.

However, beyond their immediate clinical utility, the preservation of traditional knowledge surrounding medicinal plants assumes paramount importance[(Ganapathy 2021)](https://paperpile.com/c/Yai7Rz/nKg8). This reservoir of ancient wisdom not only serves as a treasure trove of natural remedies but also holds the key to unlocking novel therapeutic agents. As such, fostering awareness and documenting historical records of these botanical remedies becomes imperative in safeguarding their legacy and harnessing their full potential for the betterment of human health. Failure to do so risks the loss of invaluable resources, forever depriving future generations of the benefits conferred by these natural wonders. Thus, a concerted effort to preserve and propagate this knowledge is essential, ensuring that the healing power of medicinal plants continues to enrich and inspire generations to come[(Wadhwani et al., 2022)](https://paperpile.com/c/Yai7Rz/6ijR).

The current study focused solely on evaluating the antibacterial activity of Centella Asiatica against two oral pathogens[(Aparna et al., 2021)](https://paperpile.com/c/Yai7Rz/tVak). However, future research endeavors could expand this scope by testing the efficacy of Centella Asiatica against a broader range of oral pathogens. By conducting comprehensive studies encompassing a diverse array of microbial strains, researchers can gain deeper insights into the plant's antibacterial potential and identify specific compounds responsible for its activity.

Furthermore, the aqueous solution of Centella Asiatica utilized in this study holds promise as a source for the development of novel antibacterial drugs. Through rigorous investigation and extraction processes, researchers can isolate and characterize bioactive compounds present in the solution. These compounds can then be further studied and optimized to create potent antibacterial agents for therapeutic use in combating oral infections.

By broadening the scope of research to include a greater variety of oral pathogens and harnessing the therapeutic potential of Centella Asiatica, future studies hold the promise of uncovering new avenues for the development of effective antibacterial treatments, ultimately benefiting oral health and overall well-being.



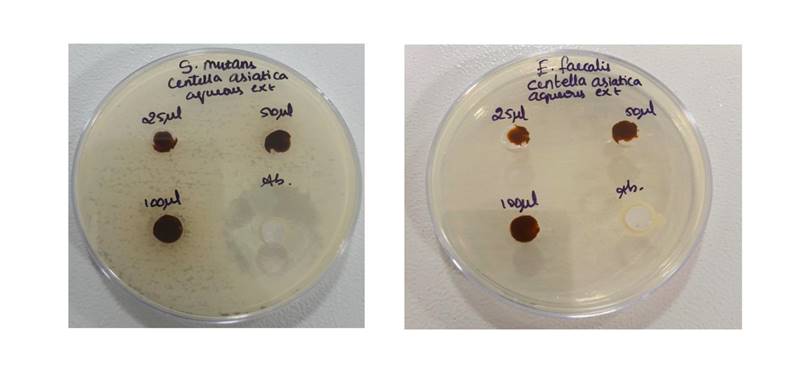
Figure 2: Dry form of *Centella* *asiatica*



Figure 3: Mixture of 200 ml of distilled water and the *Centella* *asiatica* powder



Figure 4: *Centella* *asiatica* extract from the mixture after boiling, filtering and shaking in orbital shaker.



1. (b)

Figure 5: Antibacterial activity of aqueous solution of *Centella Asiatica* against oral pathogens showing zone of inhibition analyzed by well diffusion method.

# CONCLUSION

The current study confirms the antibacterial activity of Centella Asiatica extracts against pathogens such as Streptococcus mutans and Enterococcus faecalis. However, to fully understand how these extracts exert their antimicrobial effects, further investigations are warranted. Elucidating the precise mechanism of action and identifying the biologically active ingredients responsible could pave the way for the development of new drugs, enhancing healthcare services with safe and effective treatments.

The remarkable antimicrobial potential displayed by Centella Asiatica underscores the importance of conducting more extensive and detailed research. By delving deeper into its efficacy and versatility, researchers can unlock valuable insights that may lead to the discovery of novel therapeutic strategies. Consequently, advancing our understanding of Centella Asiatica's antimicrobial properties holds considerable promise for improving healthcare outcomes and addressing the challenges posed by infectious diseases.

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