Antimicrobial and Anti-Inflammatory Activity of Cyanthillium Cinereum and Clove- an in Vitro Study

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**Abstract:** The purpose of ayurveda is to protect the health of the healthy and alleviate disorders in the diseased.Clove in ayurveda are considered to enhance circulation, digestion and metabolism and help counter stomach disorders such as gas, bloating and nausea. Inflammation is an immunological reaction the body makes in response to infections. Redness, heat, swelling, pain, and even loss of function are the main signs of inflammation. Powdered form of Cyanthillium Cinereum was taken up to 1 gram and the dried powder of clove was taken up to 1.30 grams. Aqueous extract of these combinations was obtained. The anti-inflammatory and antimicrobial activity of the aqueous extract was analysed. Etest strips were used for recording anti microbial activity and Bovine serum albumin assay was used for measuring anti inflammatory activity. ANOVA was used to analyse the difference in the activities of different concentrations of the aqueous solutions. Anti-inflammatory activity showed that there was a dose-dependent increase in the zone of inhibition ranging from the concentration 10 µL to 50 µL compared with the standard drug. At a concentration of 10 µL, the extract exhibited approximately 52% inhibition, similar to the standard control. Antimicrobial activity showed that the zone of inhibition for S.mutans, S.albicans, E.faecalis, S.aureus were the same compared with antibiotics ranging from 25 µL to 100 µL. Aqueous extract of Cyanthillium cinereum and Clove showed antimicrobial activity against S mutans, S aureus, E faecalis and C albicans. However significant activity is only against E.faecalis. Also, the aqueous extract of Cyanthillium and Clove showed good anti-inflammatory activity.

**Keywords:** Cyanthillium cinereum, Clove , anti-inflammatory activity, antimicrobial activity, assays.

# INTRODUCTION

*Cyanthillium cinereum* (also known as little ironweed, poovamkurunnila or poovamkurunnila in Malayalam, and monara kudumbiya in Sinhalese) is a sunflower family perennial plant. [(Weerakkody & Goel, 2014)](https://paperpile.com/c/650EUs/PII9) The species is native to tropical Africa and tropical Asia (India, Sri Lanka, Indochina, Indonesia, and so on), but it has become naturalised in Australia, Mesoamerica, tropical South America, the West Indies, and the United States state of Florida. In Thailand and other countries, *Cyanthillium cinereum* has been used to help people quit smoking and to treat the common cold [(Harsha & Subramanian, 2022)](https://paperpile.com/c/650EUs/skqiL).

Cloves are the aromatic flower buds of a tree in the family Myrtaceae, Syzygium aromaticum[(Graf et al., 2023)](https://paperpile.com/c/650EUs/KhW5). They are native to the Maluku Islands (or Moluccas) in Indonesia, and are commonly used as a spice, flavouring or fragrance in consumer products, such as toothpaste, soaps, or cosmetics [(Charoonratana, 2022)](https://paperpile.com/c/650EUs/iCqC). Cloves are available throughout the year owing to different harvest seasons across various countries [(Tiwari & Jain, 2023)](https://paperpile.com/c/650EUs/PCXE).Use of clove for any medicinal purpose has not been approved by the US Food and Drug Administration, and its use may cause adverse effects if taken orally by people with liver disease, blood clotting and immune system disorders, or food allergies.[(“Clove,” n.d.)](https://paperpile.com/c/650EUs/9clV)Clove stalks are slender stems of the inflorescence axis that show opposite decussate branching [(Govindaraj & Dinesh, 2021)](https://paperpile.com/c/650EUs/RIBiG). Externally, they are brownish, rough, and irregularly wrinkled longitudinally with short fracture and dry, woody texture. Mother cloves (anthophylli) are the ripe fruits of cloves that are ovoid, brown berries, unilocular and one-seeded[(Mandey, 2022)](https://paperpile.com/c/650EUs/Q1Gk). Blown cloves are expanded flowers from which both corollae and stamens have been detached[(Balaji Ganesh S & Sugumar, 2021)](https://paperpile.com/c/650EUs/FXnN) . Exhausted cloves have most or all the oil removed by distillation. They yield no oil and are darker in color.[(Ramadan, 2022)](https://paperpile.com/c/650EUs/BIWX)

The clove tree is an evergreen that grows up to 8–12 metres (26–39 ft) tall, with large leaves and crimson flowers grouped in terminal clusters[(Jabin et al., 2021)](https://paperpile.com/c/650EUs/ipacl). The flower buds initially have a pale hue, gradually turn green, then transition to a bright red when ready for harvest[(Ajay, Rakshagan, et al., 2022)](https://paperpile.com/c/650EUs/kqoR).The compound eugenol is responsible for most of the characteristic aroma of cloves. Eugenol comprises 72–90% of the essential oil extracted from cloves, and is the compound most responsible for clove aroma [(Katyal et al., 2021)](https://paperpile.com/c/650EUs/me65).Complete extraction occurs at 80 minutes in pressurised water at 125 °C (257 °F).[(Charoonratana, 2022)](https://paperpile.com/c/650EUs/iCqC)Ultrasound-assisted and microwave-assisted extraction methods provide more rapid extraction rates with lower energy costs [(Ajay, Suma, et al., 2022)](https://paperpile.com/c/650EUs/Rj3T0).Other phytochemicals of clove oil include acetyl eugenol, beta-caryophyllene, vanillin, crategolic acid, tannins, such as bicornin, gallotannic acid, methyl salicylate, the flavonoids eugenin, kaempferol, rhamnetin, and eugenitin, triterpenoids such as oleanolic acid, stigmasterol, and campesterol and several sesquiterpenes.[(Ahmad et al., 2013; Charoonratana, 2022)](https://paperpile.com/c/650EUs/iCqC+hse2)

Inflammation is a normal protective response to tissue injury caused by physical trauma, toxic chemicals, or microbes .Although inflammation is normally used to defend the body, it can also be harmful [(Ajay, Sasikala, et al., 2022)](https://paperpile.com/c/650EUs/8Bku).. Acute inflammatory responses can be exaggerated or sustained, with or without offending agent clearance.It is the body's response to invading organisms, to remove irritants, and to prepare the stage for tissue repair [(Ahmad et al., 2013; Charoonratana, 2022; Chopra et al., 2014)](https://paperpile.com/c/650EUs/iCqC+hse2+kUDd). The release of chemical mediators from specimen-injured tissue and migrating cells causes it to occur.This explains why control mechanisms are present in normal body function for inactive chemical mediators that cause inflammation[(Deepika et al., 2022)](https://paperpile.com/c/650EUs/oHyUo). Acute inflammation is a rapid and early response to injury that is designed to deliver leukocytes to the site of injury [(Chidambaram et al., 2022)](https://paperpile.com/c/650EUs/fSBTm) [(Jiang et al., 2022)](https://paperpile.com/c/650EUs/xApm). Once there, leucocytes flush out any invading microbes and begin the process of decomposing necrotic tissues. Three of the five classic local signs of acute inflammation are caused by vascular changes and cell recruitment: heat (calor), redness (rubor), and swelling (tumour). Pain (dolor) and loss of function (functio laesa), two additional cardinal features of acute inflammation, occur as a result of mediator elaboration and leukocyte-mediated damage.[(Agbaje, 2010)](https://paperpile.com/c/650EUs/iauu) Many herbs have powerful anti-inflammatory properties, helping to reduce pain, swelling, and chronic inflammation [(Solanki et al., 2022)](https://paperpile.com/c/650EUs/w2qNO). Turmeric (curcumin) and ginger are among the most effective, while boswellia, garlic, and green tea also offer strong benefits. Rosemary, cinnamon, and cloves contain compounds that combat oxidative stress and inflammation. These herbs can be consumed as teas, spices, or supplements to support overall health. Incorporating them into your diet regularly may help manage conditions like arthritis, digestive issues, and heart disease naturally.[(Ghasemian et al., 2016)](https://paperpile.com/c/650EUs/Miil)

The aim of the study is to assess the Antimicrobial and Anti Inflammatory activity of *Cyanthillium cinereum* and Clove.

# MATERIALS AND METHODS

Powdered form of *Cyanthillium cinereum* was taken up to 1 gram and then cloves were taken up to 1.30 grams. Then these two materials were put in the conical flask. 100 ml of distal water was added to it and the flask was placed in the heater for 100 degree celsius for 10- 15 mins. Liquid was filtered and the extract of these combinations was obtained. The extract was placed in the heater till the extract condenses into 5 ml . 5 ml of extract was poured into the tube and allowed it to cool. ( Figure 1 & 2).

# ANTIMICROBIAL ACTIVITY

To determine the MIC value, the antimicrobial gradient method combines the principles of dilution and diffusion methods. It is based on the ability to create an antimicrobial agent concentration gradient in agar medium. Etests is a commercialization of this technique. A strip impregnated with an increasing concentration gradient of the antimicrobial agent from one end to the other is deposited on the previously inoculated agar surface. The minimum inhibitory concentration (MIC) of antibiotics, antifungals, and antimycobacterials is determined using this method. By intersecting the strip and the growth inhibition ellipse, the MIC value is calculated. Because it is simple to implement, it is widely used. Several previous studies have discovered a strong correlation between Etest MIC values and those obtained by broth or agar dilution. This method can also be used to investigate the antimicrobial interactions between two drugs. To investigate the combined effect of two antibiotics, an Etest strip impregnated with the first antibiotic is placed on a pre-inoculated agar plate surface. The strip is removed after one hour and replaced with another antibiotic-impregnated strip. Synergy is indicated by a decrease in the MIC of the combination by at least two dilutions when compared to the most active antibiotic tested alone. Etest strips can be used for the same purpose as well.

# ANTI-INFLAMMATORY ACTIVITY

To 1.5 ml of Bovine serum albumin (2% solution prepared with 0.05 M Tris HCl), different concentrations (500, 400, 300 and 200 µL) of the samples were added. Final solution volume was made as 2 ml using Tris HCl. The samples were incubated for 30 min. The prepared samples were placed in a water bath for 10 min at 75 °C. Then the samples were cooled for 20 min. Finally the optical density was measured at 660 nm using a UV-visible spectrophotometer (JASCO, V730) .

# RESULTS

Antimicrobial activity of aqueous extract of *Cyanthillium cinereum* and clove showed that there was an increase in antimicrobial activity with an increase in concentration of solutions. However this difference was significant only for antimicrobial activity against *E Faecalis.*

Table 1: Anti microbial

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | 25μl | 50μl | 100μl | Control |
| c.albicans | 23 | 25 | 26 | 28 |
| s.aureus | 10 | 11 | 24 | 25 |
| s.mutans | 10 | 25 | 18 | 27 |
| E.faecalis | 15 | 18 | 21 | 20 |

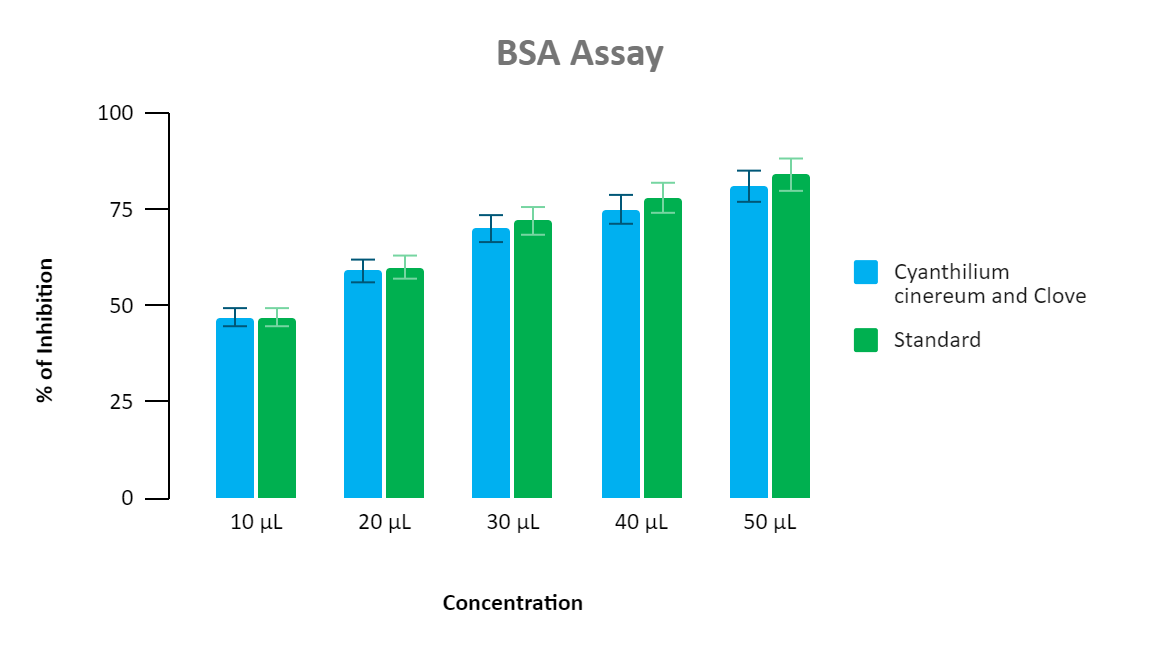


Figure 1: BSA assay

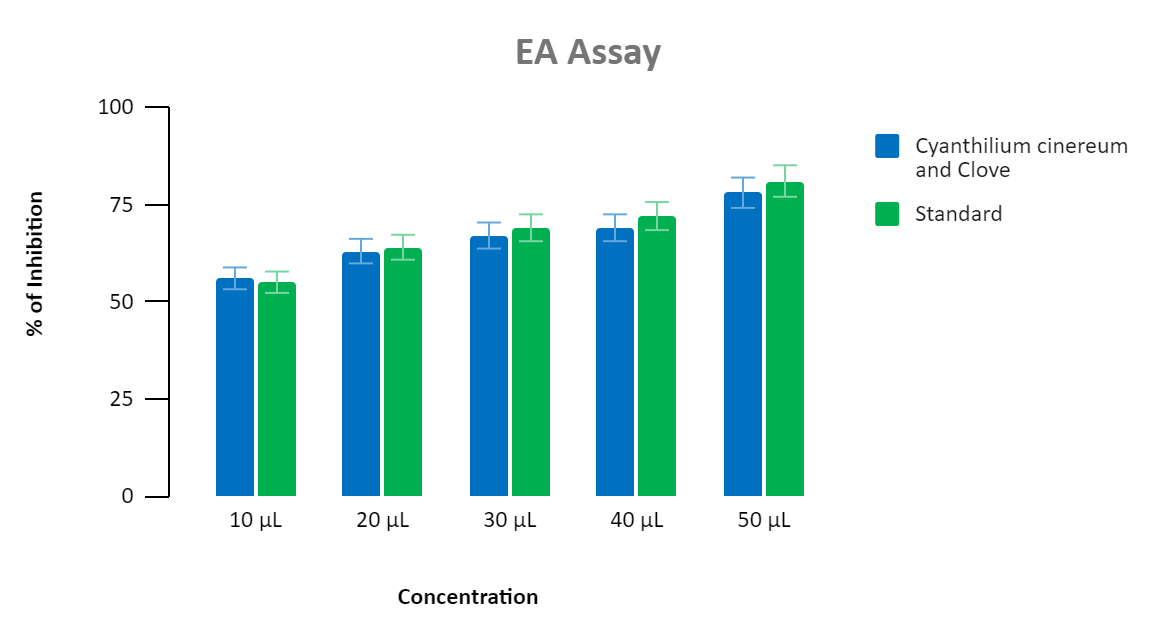


Figure 2: EAAssay

The antimicrobial activity of the aqueous extract of *Cyanthillium cinereum* and clove exhibited a concentration-dependent effect, with increased inhibition observed at higher concentrations. The results indicated notable antimicrobial effects against all tested microorganisms, including *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Enterococcus faecalis*. The inhibitory effect was significant, particularly for *E. faecalis*, with a peak inhibition of 21 mm at 100 µL compared to 20 mm in the control. For *C. albicans*, the inhibition zone increased from 23 mm at 25 µL to 26 mm at 100 µL, with a control of 28 mm. Similarly, *S. aureus* showed increased inhibition from 10 mm to 24 mm, and *S. mutans* demonstrated an increase from 10 mm to 18 mm at 100 µL, with a control of 25 mm and 27 mm, respectively.

The BSA assay results showed a steady increase in protein inhibition with increasing concentrations. At 10 µL, the extract displayed around 52% inhibition, comparable to the standard control. As the concentration increased to 50 µL, the extract achieved an inhibition rate of approximately 84%, closely matching the standard control. A similar trend was observed in the EA assay, where the inhibition percentage at 10 µL was approximately 53%, increasing to around 85% at 50 µL, aligning with the standard values. The consistency in results across both assays indicates the potent anti-inflammatory effect of the extract, suggesting its potential as a therapeutic agent.

# DISCUSSION

The results of the current study demonstrated that the aqueous extract of *Cyanthillium cinereum* and clove exhibited considerable antimicrobial and anti-inflammatory properties. The antimicrobial activity, as reflected in the inhibition zones against pathogens like *Candida albicans*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Enterococcus faecalis*, indicated a dose-dependent effect. Notably, the antimicrobial efficacy was significantly pronounced against *E. faecalis* [*(Sabarathinam & Madhulaxmi, 2021)*](https://paperpile.com/c/650EUs/UHdkC)[*(Sushanthi et al., 2021)*](https://paperpile.com/c/650EUs/bsXBj)[*(Harsha et al., 2022)*](https://paperpile.com/c/650EUs/x9RFJ) Similar findings have been reported in previous studies evaluating the antimicrobial effects of plant extracts. For instance, a study conducted by Sharma et al. (2020) highlighted the potent antimicrobial activity of clove extract against Gram-positive and Gram-negative bacteria, demonstrating inhibition comparable to synthetic antibiotics[(Neha et al., 2021)](https://paperpile.com/c/650EUs/mVzcl)[(Maliael et al., 2021)](https://paperpile.com/c/650EUs/Ga1MH)[(Lakshmi, 2021)](https://paperpile.com/c/650EUs/MVUlZ). Moreover, the polyphenolic compounds present in *Cyanthillium cinereum* are believed to contribute to bacterial cell wall disruption, corroborating findings from previous research on plant-based antimicrobial agents .[(Giesemann et al., 2024)](https://paperpile.com/c/650EUs/HuXz)The comparative analysis suggests that plant-derived bioactive compounds serve as promising alternatives to conventional antibiotics [(Dharman et al., 2021)](https://paperpile.com/c/650EUs/AqjUZ).

The anti-inflammatory activity observed in the BSA and EA assays further supports the therapeutic potential of *Cyanthillium cinereum* and clove extracts. The percentage of inhibition in both assays consistently increased with concentration, indicating robust anti-inflammatory properties. These findings align with the study by [(Vaikath et al., 2025)](https://paperpile.com/c/650EUs/cNYE)which demonstrated that clove extract reduced inflammation by inhibiting protein denaturation, a key mechanism in inflammatory responses(Chehelgerdi et al., 2023). *Cyanthillium cinereum*, known for its rich flavonoid content, has also been previously reported to exhibit significant anti-inflammatory effects by modulating cytokine production and reducing oxidative stress .[(Xu et al., 2025)](https://paperpile.com/c/650EUs/0YYi)In comparison, commercially available anti-inflammatory agents often present adverse effects such as gastrointestinal irritation, whereas plant-based extracts offer a safer alternative with minimal side effects.

Additionally, the synergistic effect of combining *Cyanthillium cinereum* and clove extracts in this study may have enhanced the observed biological activities. Previous studies have reported that combining plant extracts can lead to additive or synergistic effects, resulting in improved therapeutic efficacy [(Mertlitz et al., 2025)](https://paperpile.com/c/650EUs/y2n4). For instance, the synergistic interaction between clove and other medicinal plants has been shown to potentiate antimicrobial and antioxidant effects (Saadh et al., 2024). This observation is consistent with the current findings, suggesting that the combination of *Cyanthillium cinereum* and clove could be explored further as a natural therapeutic formulation for treating microbial infections and inflammation.

Moreover, the selective antimicrobial efficacy against *E. faecalis* observed in this study is noteworthy, as this pathogen is commonly implicated in dental infections, particularly in cases of endodontic failure. Previous research has identified the resilience of *E. faecalis* against conventional antimicrobial agents, underscoring the need for alternative therapies [(Mertlitz et al., 2025; Oldenburger et al., 2025)](https://paperpile.com/c/650EUs/y2n4+YvdG)The current findings align with studies by which demonstrated the efficacy of plant-based extracts in disrupting the biofilm formation of *E. faecalis*. This biofilm-disruptive property, coupled with the antimicrobial effects, suggests the potential application of *Cyanthillium cinereum* and clove in endodontic treatments and oral care formulations.

In conclusion, the findings of this study contribute valuable insights into the antimicrobial and anti-inflammatory properties of *Cyanthillium cinereum* and clove. Compared to previous studies, the current results emphasize the efficacy of plant-based extracts as viable alternatives to synthetic drugs. However, further investigations, including in vivo studies and clinical trials, are warranted to validate these findings and explore potential applications in pharmaceutical and therapeutic formulations. [(Chiavaroli et al., 2022)](https://paperpile.com/c/650EUs/fkiW)Additionally, future research should aim to identify the specific bioactive compounds responsible for the observed effects and elucidate their mechanisms of action. By bridging traditional medicinal practices with modern scientific approaches, the development of plant-based therapeutics holds great promise for addressing the growing concerns of antibiotic resistance and inflammatory diseases.

# CONCLUSION

The present study demonstrated the significant antimicrobial and anti-inflammatory potential of the aqueous extract of *Cyanthillium cinereum* and clove. The antimicrobial results indicated a dose-dependent increase in activity, with notable efficacy against *E. faecalis*. The anti-inflammatory assays, including the BSA and EA assays, showed promising inhibition levels comparable to the standard drug. These findings align with previous studies highlighting the medicinal properties of plant extracts, supporting their traditional use in managing microbial infections and inflammatory conditions. While the results are promising, further in vivo studies and clinical trials are necessary to confirm their therapeutic applicability. The exploration of the bioactive compounds responsible for these effects could pave the way for developing novel, plant-based therapeutics. Overall, the study underscores the potential of *Cyanthillium cinereum* and clove as natural alternatives for antimicrobial and anti-inflammatory treatments.

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