ANTIMICROBIAL EFFICACY OF *HEMIGRAPHIS COLORATA* ETHANOLIC EXTRACT AGAINST CARIOGENIC BACTERIA ASSOCIATED WITH PRIMARY TEETH: AN IN VITRO STUDY.

Abstract:

Background:
Dental caries is one of the most prevalent oral diseases affecting children worldwide, with Streptococcus mutans and other cariogenic microorganisms playing a major role in its initiation and progression. Increasing interest has been directed towards natural plant-based products due to their antimicrobial potential and biocompatibility. Hemigraphis colorata, a medicinal plant traditionally used for its therapeutic properties, contains various phytoconstituents that may exhibit antibacterial activity.

Aim:
To evaluate the antimicrobial efficacy of Hemigraphis colorata ethanolic extract against Streptococcus mutans and Lactobacillus acidophilus isolated from primary teeth.

Materials and Methods:
An ethanolic extract of Hemigraphis colorata was prepared and its antimicrobial activity was evaluated against Streptococcus mutans and Lactobacillus acidophilus. The antibacterial efficacy of the extract was assessed using appropriate microbiological methods, and the zone of inhibition. The obtained results were compared to evaluate the antibacterial potential of the plant extract.

Results:
The ethanolic extract of Hemigraphis colorata demonstrated antimicrobial activity against Streptococcus mutans and Lactobacillus acidophilus, showing measurable inhibition of bacterial growth. The antibacterial effect varied depending on the concentration of the extract and the bacterial strain tested.

Conclusion:
The findings of this in vitro study suggest that Hemigraphis colorata ethanolic extract possesses promising antimicrobial activity against cariogenic bacteria associated with primary teeth. Further investigations involving phytochemical characterisation, cytotoxicity assessment, and clinical studies are required to establish its potential application as a natural antimicrobial agent in pediatric dentistry.

Keywords: Hemigraphis colorata; ethanolic extract; antimicrobial activity; Streptococcus mutans; Lactobacillus acidophilus; primary teeth; dental caries; pediatric dentistry.

INTRODUCTION

Dental caries is one of the most prevalent chronic diseases affecting children worldwide and continues to be a major public health concern. It is a multifactorial disease resulting from the interaction of cariogenic microorganisms, dietary fermentable carbohydrates, susceptible tooth surfaces, and host factors over time. If left untreated, dental caries can lead to pain, infection, premature loss of primary teeth, impaired mastication, and reduced quality of life. Among the microorganisms associated with dental caries, *Streptococcus mutans* plays a pivotal role in the initiation of the disease through its ability to adhere to tooth surfaces, form biofilms, and produce organic acids from dietary sugars. *Lactobacillus acidophilus* contributes predominantly to the progression of established lesions because of its acidogenic and aciduric characteristics [16].

Various chemical antimicrobial agents have been employed to reduce the cariogenic bacterial load and prevent dental plaque formation. Among these, chlorhexidine has long been regarded as the gold standard because of its broad-spectrum antimicrobial activity and substantivity. Despite its proven effectiveness, prolonged use of chlorhexidine is associated with several undesirable effects, including tooth and tongue staining, altered taste sensation, mucosal irritation, and increased calculus formation. These limitations have encouraged researchers to investigate safer, economical, and naturally derived antimicrobial agents for use in preventive dentistry [10–15].

In recent years, there has been growing interest in herbal medicines because of their wide availability, affordability, biocompatibility, and lower incidence of adverse effects. Medicinal plants have been used for centuries in traditional systems of medicine and continue to serve as an important source of therapeutic agents. The World Health Organization (WHO) has emphasized the importance of strengthening the evidence base for traditional medicine through scientific research to ensure its safe, effective, and appropriate use in healthcare [17]. Numerous medicinal plants have demonstrated antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties, making them attractive candidates for oral healthcare applications [3–9].

Hemigraphis colorata (Blume) H.G. Hallier, commonly known as Murikootti or the "wound healer," is a perennial medicinal herb belonging to the family Acanthaceae and is widely distributed in the Western Ghats of South India, particularly in Kerala. The plant has been used extensively in traditional Ayurvedic and folk medicine for the management of fresh wounds, cuts, bleeding, inflammation, anaemia, and skin disorders. In Kerala, *H. colorata* has gained considerable public recognition because of its traditional medicinal value, and its use has been passed down through generations as a household remedy for wound healing [2,6,7].

Phytochemical investigations have revealed that *H. colorata* contains several biologically active constituents, including flavonoids, phenolic compounds, tannins, alkaloids, terpenoids, and saponins. These phytochemicals are known to exhibit antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities. Experimental studies have demonstrated that topical application of *H. colorata* accelerates wound contraction, collagen deposition, and epithelialization. Furthermore, extracts of the plant have shown antibacterial activity against several pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and other bacterial species [1,2,6,7].

Although the pharmacological properties of *Hemigraphis colorata* have been extensively investigated, there is a paucity of literature regarding its antimicrobial efficacy against oral cariogenic microorganisms. To the best of our knowledge, no published study has specifically evaluated the antibacterial activity of *Hemigraphis colorata* ethanolic extract against the principal cariogenic bacteria, *Streptococcus mutans* and *Lactobacillus acidophilus*, isolated from primary teeth. Considering the increasing demand for herbal alternatives in pediatric dentistry and the traditional medicinal importance of this plant, further scientific evaluation is warranted [1,6,7].

Therefore, the present in vitro study was undertaken to evaluate the antimicrobial efficacy of *Hemigraphis colorata* ethanolic extract against *Streptococcus mutans* and *Lactobacillus acidophilus*, with the aim of exploring its potential as a natural antimicrobial agent for future applications in pediatric preventive dentistry.

MATERIALS AND METHODS

Study Design

The present in vitro experimental study was conducted to evaluate the antimicrobial efficacy of the ethanolic extract of *Hemigraphis colorata* against cariogenic bacteria associated with primary teeth.

Study Setting

The study was carried out at the central Research Laboratory, PMS College of Dental Science and Research, Thiruvananthapuram, Kerala, India, where all microbiological procedures and antimicrobial susceptibility testing were performed under aseptic laboratory conditions.

Plant Collection

Fresh leaves of *Hemigraphis colorata* were collected from the medicinal plant garden of the Government Ayurveda Hospital, Karatte, Thiruvananthapuram, Kerala, India. The collected leaves were thoroughly washed with running tap water followed by distilled water to remove adhering dust and impurities. The cleaned leaves were shade-dried at room temperature until completely dry and then pulverized into a fine powder using a mechanical grinder.

Preparation of Ethanolic Extract

The powdered leaves of *Hemigraphis colorata* were subjected to Soxhlet extraction using 95% ethanol as the extraction solvent. The extraction was continued until the solvent in the siphon tube became colourless, indicating complete extraction of the phytoconstituents. The extract was filtered through Whatman No. 1 filter paper, and the solvent was evaporated to obtain the concentrated ethanolic extract. The extract was stored in sterile amber-coloured containers at 4°C until further use. Before antimicrobial susceptibility testing, the concentrated extract was reconstituted in a sterile solvent to prepare working concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL.

Test Microorganisms

Clinical isolates of *Streptococcus mutans* and *Lactobacillus acidophilus* were obtained from the Central Research Laboratory, PMS College of Dental Science and Research, Thiruvananthapuram, Kerala, India. The isolates had been previously identified using standard microbiological techniques and maintained under appropriate laboratory conditions. Fresh cultures were subcultured before antimicrobial susceptibility testing.

Antibacterial Susceptibility Testing

The antimicrobial activity of the ethanolic extract of *Hemigraphis colorata* was evaluated using the agar well diffusion method.

Four to five well-isolated colonies from fresh cultures of *Streptococcus mutans* and *Lactobacillus acidophilus* were aseptically inoculated into Brain Heart Infusion (BHI) broth and de Man–Rogosa–Sharpe (MRS) broth, respectively. The inoculated broths were incubated overnight at 37°C until the turbidity matched the 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL.

Brain Heart Infusion agar for *Streptococcus mutans* and de Man–Rogosa–Sharpe agar for *Lactobacillus acidophilus* were prepared according to the manufacturer's instructions using distilled water. The media were sterilized by autoclaving at 121°C for 15 minutes, cooled to 45–50°C, and poured aseptically into sterile Petri dishes to a depth of approximately 4 mm, where they were allowed to solidify.

The standardized bacterial suspensions were uniformly spread over the agar surface using sterile cotton swabs to obtain a confluent lawn culture. Wells measuring 6 mm in diameter were prepared in the agar using a sterile cork borer, and the agar plugs were carefully removed. The concentrated ethanolic extract was reconstituted in a sterile solvent to prepare working concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL. Fifty microlitres (50 µL) of each concentration were dispensed into the respective wells. A 30 µg tetracycline

antibiotic disc was placed on each agar plate as the positive control, while a well containing only the sterile solvent served as the negative control.

The inoculated plates containing *Streptococcus mutans* were incubated at 37°C in a 5% CO_2 atmosphere (candle jar) for 24 hours, whereas the plates containing *Lactobacillus acidophilus* were incubated at 37°C under microaerophilic conditions for 24–48 hours.

Following incubation, the diameters of the zones of inhibition surrounding each well and the positive control (tetracycline disc) were measured in millimetres (mm). All experiments were performed in triplicate, and the mean zone of inhibition for each concentration was calculated and recorded.

Outcome Measure

The primary outcome measure was the zone of inhibition (mm) produced by the ethanolic extract of *Hemigraphis colorata* at concentrations of 25 mg/mL, 50 mg/mL, and 100 mg/mL against clinical isolates of *Streptococcus mutans* and *Lactobacillus acidophilus*.

RESULTS

The antimicrobial activity of the ethanolic extract of *Hemigraphis colorata* against *Streptococcus mutans* and *Lactobacillus acidophilus* was evaluated using the agar well diffusion method. The extract exhibited antibacterial activity against both test microorganisms at all three concentrations tested (25 mg/mL, 50 mg/mL, and 100 mg/mL). A concentration-dependent increase in the zone of inhibition was observed for both organisms.

Against *Streptococcus mutans*, the ethanolic extract produced zones of inhibition of **10 mm**, **11 mm**, and **18 mm** at concentrations of **25 mg/mL**, **50 mg/mL**, and **100 mg/mL**, respectively. The positive control, tetracycline (30 µg), produced the highest zone of inhibition (**33 mm**), whereas the sterile solvent (negative control) showed no antibacterial activity.

Similarly, against *Lactobacillus acidophilus*, the ethanolic extract produced zones of inhibition of **11 mm**, **12.5 mm**, and **18 mm** at concentrations of **25 mg/mL**, **50 mg/mL**, and **100 mg/mL**, respectively. The positive control, tetracycline (30 µg), produced a zone of inhibition of **25 mm**, while the sterile solvent did not exhibit any inhibitory effect.

Among the concentrations tested, **100 mg/mL** demonstrated the greatest antibacterial activity against both *Streptococcus mutans* and *Lactobacillus acidophilus*. However, the antibacterial activity of the ethanolic extract was lower than that of the positive control (tetracycline).

178 Table 1. Antimicrobial activity of the ethanolic extract of *Hemigraphis colorata* against
179 *Streptococcus mutans*

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Test Material	Concentration	Zone of Inhibition (mm)
Tetracycline (Positive control)	30 µg	33
H. colorata ethanolic extract	25 mg/mL	10
H. colorata ethanolic extract	50 mg/mL	11
H. colorata ethanolic extract	100 mg/mL	18
Sterile solvent (Negative control)	—	0

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182 Table 2. Antimicrobial activity of the ethanolic extract of *Hemigraphis colorata* against
183 *Lactobacillus acidophilus*

Test Material	Concentration	Zone of Inhibition (mm)
Tetracycline (Positive control)	30 µg	25
H. colorata ethanolic extract	25 mg/mL	11
H. colorata ethanolic extract	50 mg/mL	12.5
H. colorata ethanolic extract	100 mg/mL	18
Sterile solvent (Negative control)	—	0

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185 FIGURE 1: Zone of inhibition of Streptococcus mutans

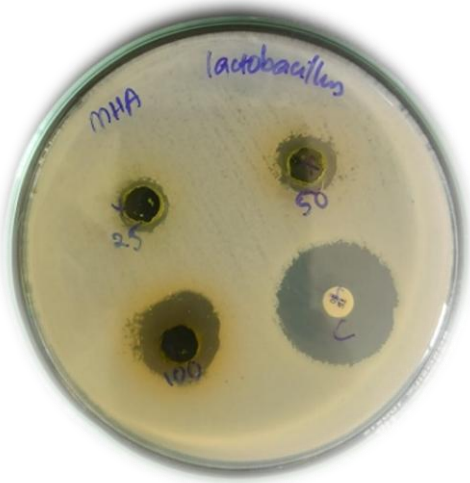

FIGURE 1: Zone of inhibition of *Lactobacillus acidophilus*

DISCUSSION

Dental caries remains one of the most prevalent chronic diseases affecting children worldwide. *Streptococcus mutans* plays a key role in the initiation of dental caries through its ability to adhere to the tooth surface, synthesise extracellular polysaccharides, and produce organic acids that demineralise enamel. *Lactobacillus acidophilus* is predominantly associated with the progression of dentinal caries because of its highly acidogenic and aciduric properties. Therefore, inhibition of these microorganisms is an important strategy in caries prevention and management [16].

Conventional antimicrobial agents such as chlorhexidine, sodium hypochlorite, cetylpyridinium chloride and amine fluoride are widely used in dentistry because of their proven antimicrobial efficacy. However, prolonged use of these agents has been associated with undesirable effects, including tooth staining, altered taste sensation, increased calculus formation, mucosal irritation and cytotoxicity to oral tissues. Consequently, there has been growing interest in identifying plant-derived antimicrobial agents that are effective, economical and associated with fewer adverse effects [4,5,10–15].

Medicinal plants have been used for centuries in traditional systems of medicine, including Ayurveda, because of their diverse therapeutic properties. *Hemigraphis colorata*, popularly known as Murikooti or Muriyanpacha, is an indigenous medicinal herb widely distributed in the Western Ghats of Kerala. The plant is traditionally recognised as a natural first-aid plant, with fresh leaves crushed into a paste and applied directly over cuts and wounds to arrest bleeding and promote rapid wound healing. Owing to these remarkable medicinal properties, *H. colorata* has gained widespread recognition among the people of Kerala and has been highlighted in regional newspapers and public awareness programmes promoting indigenous medicinal plants. Scientific investigations have subsequently validated many of these traditional claims, particularly its wound-healing, anti-inflammatory and antimicrobial activities [2,6,7]. Furthermore, ethanolic extracts generally produce higher extractive yields

than aqueous extracts because ethanol efficiently dissolves a broader spectrum of biologically active compounds. This may explain the appreciable antibacterial activity observed with the ethanolic extract in the present study [1,6,7].

The present study demonstrated that the ethanolic extract of *Hemigraphis colorata* exhibited concentration-dependent antibacterial activity against *Streptococcus mutans* and *Lactobacillus acidophilus*, two of the principal cariogenic microorganisms implicated in the initiation and progression of dental caries. The highest antibacterial activity was observed at a concentration of 100 mg/mL, producing a zone of inhibition of 18 mm against both microorganisms. Although tetracycline demonstrated greater antibacterial activity, the findings indicate that the ethanolic extract of *H. colorata* possesses appreciable antimicrobial potential against cariogenic bacteria.

The antibacterial activity observed in the present study is consistent with the findings of Jeeva et al. [1], who evaluated the antibacterial efficacy of *Hemigraphis colorata* against several pathogenic microorganisms. They reported that the ethanolic extract exhibited superior antibacterial activity compared with aqueous and other solvent extracts and demonstrated inhibitory activity against *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Bacillus cereus* and several other bacterial species. The authors also observed that antibacterial activity increased with increasing extract concentration, which is in agreement with the concentration-dependent antibacterial activity demonstrated in the present study.

Similarly, Saravanan et al. [2] demonstrated that topical application of *Hemigraphis colorata* significantly enhanced wound contraction and epithelialization in experimental animals. The authors attributed these effects to increased collagen deposition and stabilization of collagen fibres by the bioactive constituents present in the plant. These findings indicate that *H. colorata* possesses multiple pharmacological properties and further support its traditional use in wound management.

The pharmacological potential of *H. colorata* has been comprehensively reviewed by Shana et al. [6] and Devi Priya [7], who reported that the plant contains numerous bioactive phytoconstituents including flavonoids, tannins, alkaloids, phenolic compounds, saponins, terpenoids, steroids, coumarins and sterols. These phytochemicals are known to possess antioxidant, antimicrobial, anti-inflammatory, wound-healing and antidiabetic properties. Furthermore, ethanolic extracts have been reported to produce higher extractive yields than aqueous extracts because ethanol efficiently dissolves a broader spectrum of biologically active compounds [1,6,7]. This may explain the appreciable antibacterial activity observed with the ethanolic extract in the present study.

The concentration-dependent increase in the zone of inhibition observed in the present investigation suggests that increasing concentrations of the extract provide greater quantities of bioactive phytochemicals capable of diffusing through the agar medium and inhibiting bacterial growth. Flavonoids and phenolic compounds are known to disrupt bacterial cell membranes, denature proteins and inhibit essential bacterial enzymes. Tannins interfere with

bacterial adhesion and precipitate microbial proteins, while alkaloids, terpenoids and saponins alter membrane permeability and interfere with bacterial metabolism. The synergistic action of these phytoconstituents may therefore account for the antibacterial activity demonstrated against *Streptococcus mutans* and *Lactobacillus acidophilus* [6,7].

The findings of the present study are also supported by investigations on other medicinal plants used in dentistry. Abbaszadegan et al. [3] demonstrated significant antibacterial activity of *Aloe vera* and *Zataria multiflora* essential oils against *Enterococcus faecalis*, suggesting that herbal products can serve as potential alternatives to conventional intracanal medicaments. Vangipuram et al. [4] reported that *Aloe vera* mouthwash significantly improved periodontal health and demonstrated antimicrobial efficacy comparable to chlorhexidine. Likewise, Sharma et al. [5] observed significant improvements in gingival health following the use of herbal mouthwashes, while Ahmadi et al. [9] demonstrated a significant reduction in salivary *Streptococcus mutans* and *Lactobacillus* counts following the use of green tea mouthwash. Collectively, these findings reinforce the growing evidence supporting the use of herbal products as potential adjuncts in preventive and therapeutic dentistry.

From a clinical perspective, the antibacterial activity demonstrated by *Hemigraphis colorata* suggests that this medicinal plant may have potential applications in preventive dentistry. The extract could be explored as a natural ingredient in herbal mouthwashes, cavity disinfectants, varnishes or other oral healthcare products aimed at reducing the cariogenic bacterial load [1,4–7]. However, these applications remain investigational and require further scientific validation before clinical use can be recommended.

The present study has certain limitations. Only two cariogenic bacterial species were evaluated, and the antimicrobial activity was assessed using the agar well diffusion method. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), antibiofilm activity, cytotoxicity and in vivo efficacy were not evaluated. Therefore, the findings should be interpreted with caution and cannot be directly extrapolated to clinical practice.

Future studies should focus on isolating and characterizing the active phytoconstituents responsible for the antibacterial activity of *Hemigraphis colorata*, determining MIC and MBC values, evaluating antibiofilm efficacy, assessing cytotoxicity on oral tissues and conducting well-designed animal and clinical studies. Such investigations may facilitate the development of safe, effective and economical herbal oral healthcare products for the prevention and management of dental caries.

In conclusion, the findings of the present study demonstrate that the ethanolic extract of *Hemigraphis colorata* possesses concentration-dependent antibacterial activity against *Streptococcus mutans* and *Lactobacillus acidophilus*. Although its antibacterial efficacy was lower than that of tetracycline, the extract exhibited appreciable inhibitory activity against both cariogenic microorganisms. These findings provide scientific support for the traditional

medicinal use of *H. colorata* and suggest that it may serve as a promising natural adjunct in the prevention and management of dental caries. Nevertheless, further pharmacological, toxicological and clinical studies are required before its routine application in dentistry can be recommended.

Limitations of the study

The present study was conducted under in vitro conditions, which may not completely replicate the complex environment of the oral cavity, including salivary flow, biofilm interactions, and host-related factors. Although *Streptococcus mutans* is an important cariogenic microorganism, dental caries is a polymicrobial disease; therefore, the antibacterial effects observed against a single bacterial species may not represent the complete clinical scenario.

The study evaluated the antimicrobial activity of plant extracts using laboratory-based methods; however, the bioavailability, stability, toxicity, and effective concentration of these extracts in the oral environment require further investigation. The active phytochemical constituents responsible for the antibacterial effects were not individually isolated and quantified. Additionally, long-term effects, potential side effects, and clinical effectiveness of these extracts were not assessed.

Further in vivo studies and clinical trials are required to validate these findings and determine the potential application of these plant extracts as preventive or therapeutic agents against dental caries.

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