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SHORT COMMUNICATION

# Comparison of the Antibacterial Effectiveness of *Achyranthes aspera* Extract *vs.* Tetracycline - An *In Vitro* Investigation

**Paavai Ilango[1*], G. D. Gomathi[1], P. Sivaranjani[2], Ayswarya V. Vummidi[1], A. J. Ashmi[1] and S. Saisuruthi[1]**

[1]Department of Periodontology, Priyadarshini Dental College and Hospital, Pandur, Tiruvallur - 631203, Tamil Nadu, India; drpaavaiilango@gmail.com

[2]Department of Dentistry, Arunai Medical College and Hospital, Thiruvannamalai - 606603, Tamil Nadu, India

## Abstract

**Background:** Periodontitis is an inflammatory disease of microbial origin, affecting the tooth supporting structures. Various chemical agents have been used to modulate this disease; however, the side effects are found to outweigh the benefits. Of late, various herbal agents have been experimented to assess its efficacy to treat periodontitis. One among them is *Achyranthes aspera*, an herb that possess antimicrobial and anti-inflammatory property. Therefore, we conducted a pilot study to evaluate its antibacterial properties. **Aim:** To assess the antibacterial effectiveness of *A. aspera* against periodontitis causing pathogens (*Prevotella intermedia* and *Porphyromonas gingivalis*) in an *in vitro* setting and to compare its effectiveness with that of tetracycline. **Methods:** Bacterial strains were cultured and inoculated into *A. aspera* extract plate and tetracycline plate. The plates were incubated at 37°C, and the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined for both. **Results:** The inhibition zones produced by *A. aspera* at a concentration of 50 mg/ml measured 2.4±0.02 mm and 2.8±0.22 mm in diameter for *P. gingivalis* and *P. intermedia*, respectively. At 100 mg/ml, the inhibition zones increased to 5.6±0.43 mm and 6.1±1.3 mm for *P. gingivalis* and *P. intermedia*, respectively. Tetracycline at 30 µg, positive control showed inhibition zones of 14.9±1.1 mm and 15.6 ±1.2 mm in diameter for *P. gingivalis* and *P. intermedia*, respectively.

**Major Findings:** *A. aspera* extracts have shown substantial inhibition of periodontal pathogenic bacteria in *in vitro* experiments making it to be considered a promising alternative for anti-infective therapy.

**Keywords:** *A. aspera*, Minimum Inhibitory Concentration, Periodontitis, *P. gingivalis*, *P. intermedia*, Tetracycline

## 1. Introduction

Periodontitis is a complex infectious immune-inflammatory disease originating from microbial origin, characterized by the gradual deterioration of periodontal structures. The development of this disease is influenced by the intricate interplay of oral microorganisms, environmental factors, and the host's immune response. Clinically, periodontitis manifests as clinical attachment loss, evident through the presence of periodontal pockets, gingival recession, horizontal or vertical bone loss, and tooth mobility[1]. Periodontal pockets create an ideal niche for the proliferation of pathogenic bacteria such as *P. intermedia*, *Aggregatibacter actinomycetemcomitans* and *P. gingivalis*. Periodontal health can be efficiently enhanced by eradicating the periodontal microorganisms through mechanical therapy, along with antimicrobial agents[2]. Periodontal treatment aims at targeting specific periodontal microbes with minimal side effects, thereby regulating the destructive host-defense response. Currently available

*Author for correspondence

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chemotherapeutic agents are effective in combatting microbial challenges, but are invariably associated with issues like antimicrobial resistance, tooth discoloration, and altered taste perception. To minimize the side effects of conventional agents in management of periodontitis, newer herbal options are being explored. Herbal therapy, or phytotherapy, is gaining popularity due to its naturally occurring phytochemicals, which have demonstrated comparable clinical efficacy to conventional chemotherapeutic agents[3]. *A. aspera*, also known as Prickly chaff flower, *Apamarga* (in *Ayurveda*), *Chirchira*, *Latjeera*, or Chaff-flower, commonly referred to as devil's horsewhip, is a weed species from the *Amaranthaceae* family. It is widely found in various regions of India and other tropical countries. The roots, shaft, leaves and seeds possess valuable medicinal properties and has been used in traditional medicine in Australia, India, and Kenya. Studies on this herb have revealed its therapeutic potential, showcasing a range of properties such as hypoglycemic, hypolipidemic, anti-inflammatory, antimicrobial, larvicidal, antioxidant, diuretic, cardiostimulant, antihypertensive, analgesic, immunostimulant, antipyretic, antispasmodic, antifertility and hepatoprotective effects[4]. Additionally, extracts of *A. aspera* have been shown to inhibit the growth of pathogenic fungal strains as well in minimal amounts. These extracts are rich in various phytoconstituents, including alkaloids, flavonoids, saponins, steroids, and terpenoids, known for their therapeutic properties[5].

Given these properties, *A. aspera* extract is proposed as a potential therapeutic alternative to chemotherapeutic agents, particularly in microbial diseases like periodontitis. To assess the suitability of using this extract in periodontal diseases, an *in vitro* study was designed. This study aimed to assess the antibacterial efficacy of *A. saspera* against periodontitis-causing pathogens, including *P. gingivalis* and *P. intermedia*, and to compare its effectiveness with tetracycline, a commonly used locally delivered antimicrobial agent.

## 2. Materials and Methods

This *in vitro* microbiological analysis was carried out by the Department of Periodontics at Priyadarshini Dental College and Hospital.

### 2.1 *Achyranthes aspera* Powder Preparation

Fresh *A. aspera* plant weeds (401486) were obtained. The weeds were cleaned and dried under sunlight for 3 consecutive days. The dried weeds were grinded to obtain fine powder. The powder was stored in air tight container until further use[1].

### 2.2 Extract Preparation

100mg of *A. aspera* powder was soaked in chloroform for 24 hours. The extract was then filtered through Whatman No.1 filter paper and subjected to Soxhlet extraction for 4 hours. The filtrate was air-dried using a vacuum desiccator, and the resulting residue was stored at 4°C for future use[6].

### 2.3 Bacterial Strains and Media

Laboratory strains of *P. gingivalis* (ATCC 3277) were cultured in Mueller-Hinton broth at 37°C for 3 hours. The bacterial suspensions were standardized to a density of approximately $1 \times 10^8$ CFU/ml and then diluted with media to achieve a concentration of $10^5$ CFU/ml. *P. gingivalis* was grown in tryptic soy broth supplemented with 5 µg/ml hemin and vitamin K for 72 hours at 37°C in an anaerobic chamber. The bacterial cell suspension was adjusted to $10^6$ CFU/ml for *P. gingivalis*. *P. intermedia* ATCC 25611 strains were revived in 5 ml thioglycollate broth and incubated for eight days at 35°C under anaerobic conditions. Subsequently, they were seeded on blood agar and incubated for 48 hours at 35°C under anaerobic conditions. Gram stains were performed on isolated colonies to verify strain morphology and purity[7].

### 2.4 Antimicrobial Susceptibility Testing

100 µl of each bacterial suspension was spread on two nutrient agar plates[7].

### 2.5 *Porphyromonas gingivalis* Culture Plate

Five uniform wells with a size of 6 mm were prepared on the *P. gingivalis* strain culture plate. A volume of 25 µl of the test solution, at concentrations of 25 mg/ml, 50 mg/ml, and 100 mg/ml, was added to the corresponding wells on the plate[7].

### 2.6 *Prevotella intermedia* Culture Plate

A volume of 25 µl of the experimental test solution at concentrations of 25 mg/ml, 50 mg/ml, and 100 mg/ml was applied to sterilized 6 mm blank discs, which were then placed onto the *P. intermedia* culture plate. All

impregnated discs were ensured to be fully dried in a 45°C incubator for 18 to 24 hours before application on bacteria[8]. Negative control discs loaded with 10% DMSO were used for both culture plates. Positive controls consisted of 30 µg of tetracycline for all strains. Discs impregnated with the extract were applied to the inoculated Mueller Hinton agar once it had completely dried, using sterile forceps. The discs were gently pressed to ensure uniform contact with the agar surface. Within 15 minutes of application, the plates were placed in an anaerobic jar and incubated at 37°C for 48 hours. After incubation, plates were evaluated only if there was confluent or nearly confluent bacterial growth. The diameter of the inhibition zones surrounding the treated and control discs was measured to assess antibacterial activity. If present, the diameters were measured to the nearest whole millimeter using a ruler. Each test was performed in triplicate for both the extract concentrations and the positive control to ensure consistency, and the average of the three replicates was calculated for each concentration.

### 2.7 Statistical Evaluation

Statistical analysis was executed using one-way analysis of variance (ANOVA) followed by the Least Significant Difference (LSD) test for further evaluation. A significance threshold of $P<0.05$ was established, and the data were processed using SPSS (version 11).

## 3. Results

Analysis of the different concentrations (25 mg/ml, 50 mg/ml, 100 mg/ml) of *A. aspera* extract using the disc diffusion method revealed significant microbial inhibition at concentrations of 50 mg/ml and 100 mg/ml. The inhibition zones measured 2.4±0.02 mm and 2.8±0.22 mm in diameter for *P. gingivalis* (Figure 1) and *P. intermedia* (Figure 2), respectively, at 50 mg/ml. At 100 mg/ml, the inhibition zones measured 5.6±0.43 mm and 6.1±1.3 mm in diameter for *P. gingivalis* (Figure 1) and *P. intermedia* (Figure 2), respectively. Tetracycline at 30 µg as a positive control showed inhibition zones of 14.9±1.1 mm and 15.6 ±1.2 mm in diameter for *P. gingivalis* and *P. intermedia*, respectively. No inhibition zone was observed for the negative control. (Table 1) The Minimum Inhibitory Concentration (MIC) of *A. aspera* extract against *P. gingivalis* and *P. intermedia* was ascertained to be 50 mg/ml, while the Minimum Bactericidal Concentration (MBC) was ascertained at 100 mg/ml, indicating the extract's potent antimicrobial properties at these specific concentrations (Table 2).

**Table 1.** Antibacterial activity of *A. aspera* extract on *P. gingivalis* and *P. intermedia* at different concentrations

| Test Compound | Bacterial Zone of Inhibition (mm) | |
|---|---|---|
| | ***P. gingivalis*** | ***P. intermedia*** |
| ARE (25mg/ml) | Nil | Nil |
| ARE (50mg/ml) | 2.4 ± 0.02*# | 2.8 ± 0.22*# |
| ARE (100mg/ml) | 5.6 ± 0.43**# | 6.1 ± 1.3**# |
| Tetracycline | 14.9 ± 1.1*** | 15.6 ± 1.2*** |
| Negative Control | Nil | Nil |

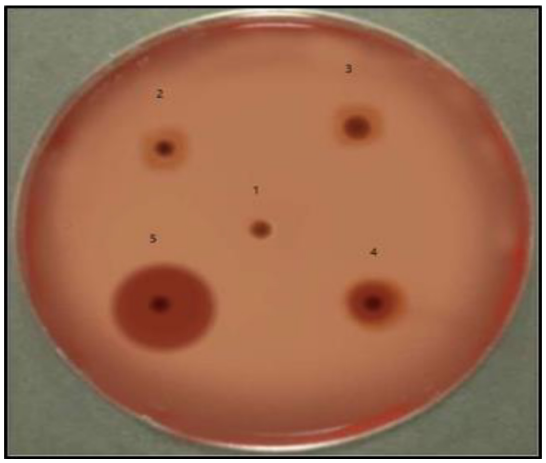
ARE – *A. aspera* extract

Nil - No inhibition zone. Each value is expressed as mean ± SD (n = 3). *p<0.05; **p<0.01; ***p<0.001 statistically significant as compared with negative control. #p<0.001 statistically significant as compared with positive control.

**Table 2.** Minimum inhibitory concentration and minimum bactericidal concentration of *A. aspera* against *P. gingivalis* and *P. intermedia*

| Mean MIC and MBC (mg/ml) | | |
|---|---|---|
| **Bacteria** | **MIC(mg/ml)** | **MBC(mg/ml)** |
| *P. gingivalis* | 50 | 100 |
| *P. intermedia* | 50 | 100 |

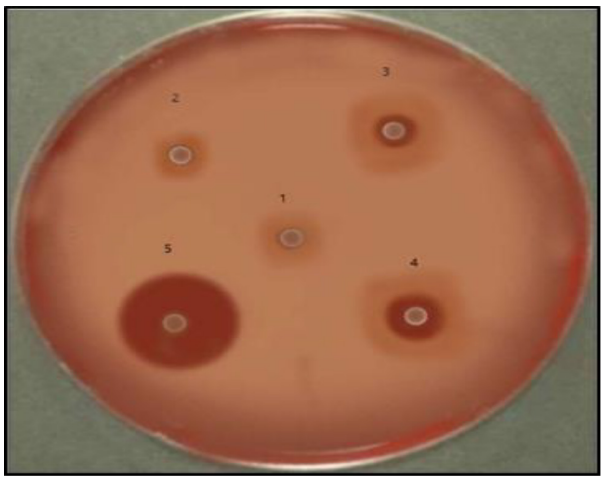
MIC - Minimum Inhibitory Concentration; MBC - Minimum




ARE - *A. aspera extract*

Minimum inhibitory concentration and minimum bactericidal concentration of *P. gingivalis* shows 1. Negative control; 2. ARE (25mg/ml); 3. ARE (50mg/ml); 4. ARE (100mg/ml); 5. Tetracycline.

**Figure 1.** Disc diffusion method showing zone of inhibition *P. gingivalis*.




ARE – *A. aspera*.
Minimum inhibitory concentration and minimum bactericidal concentration of *P. intermedia* shows 1. Negative control; 2. ARE (25mg/ml); 3. ARE (50mg/ml); 4. ARE (100mg/ml); 5. Tetracycline.

**Figure 2.** Disc diffusion method showing zone of inhibition *P. intermedia*.

## 4. Discussion

Periodontitis is a multifactorial oral disease that impacts approximately one-third of the adult population and is characterized by the destruction of connective tissue and the apical pathological migration of the junctional epithelium, resulting in pocket formation, tooth mobility, and ultimately tooth loss. Although the pathogenesis of periodontitis has transitioned from an infectious origin to an inflammatory etiology (Impede model), key pathogens such as *A. actinomycetemcomitans*, *P. gingivalis*, and *P. intermedia* significantly contribute to the disease's progression. *P. gingivalis,* a black-pigmented gram-negative anaerobic *bacillus* is considered as a significant perio-pathogen due to its involvement in periodontal disease progression and tissue destruction. *P. gingivalis* is a well-known organism that produce a plethora of virulence factors such as lipopolysaccharides, fimbriae, which has the ability to penetrate the periodontal structures or the periodontal tissues that leads to direct or indirect tissue destruction by inflammation[9]. *P. intermedia,* an orange complex bacterium, on the other hand, exhibits an increased inflammatory response, releasing proinflammatory cytokines such as IL-8 and IL-6 from stromal and immune cells[10]. It functions as a bridging species between early and late colonizers, thereby augmenting the growth conditions for other bacteria, especially those within the red complex. Thus, *P. intermedia* contribute to the biofilm formation disrupting the host immune defenses leading to the tissue destruction. The main goal in managing periodontitis is to eliminate the inflammatory response by removing the bacterial biofilm. The current challenges in treating periodontal disease lie in the limitations of conventional therapies required to be relooked with newer alternative drugs to target the specific periodontal pathogens with minimal side effects. In case of inaccessible areas such as furcation's, and root concavities, local drug delivery agents play a major role in preventing the recurrence of disease. Thus, the most commonly employed methods include scaling and root planing, adjuncted with local drug delivery, subgingival irrigation, systemic antibiotics, host modulation therapy and Laser[11]. Tetracycline and Doxycycline are the broad-spectrum antibiotics most frequently used in periodontal therapy due to its effectiveness against pathogenic bacteria[12]. However, the emergence of antimicrobial resistance and adverse effects, such as tooth discoloration and changes to tooth structure, are undesirable consequences of tetracycline usage[13]. In an effort to address these side effects associated with chemotherapeutic agents, natural compounds such as plant-derived compounds are increasingly being considered in periodontal therapy to modulate microbial interactions and maintain oral health. Herbal medicines encompass natural phytochemicals that present viable alternatives to antibiotics, demonstrating negligible or absent toxicity to human cells. Herbal therapy offers a different approach in the prevention and treatment of oral and periodontal infections[14]. Amidst various antimicrobial herbal agents, the current study evaluates the efficacy of *A. aspera,* as limited studies have been performed with this agent against periodontal disease despite its adequate clinical efficacy on par with conventional drugs like Doxycycline, Tetracycline and the like[15]. *A. aspera* is a medicinal plant recognized for its wide-ranging therapeutic properties, including the treatment of hypertension, fever, asthma, dysentery, diabetes, rheumatism cough and bronchitis. In dentistry, its characteristics- such as antioxidant, antimicrobial, immunomodulatory, anti-inflammatory and wound-healing effects combined with the absence of systemic toxicity, indicate that it could serve as a promising alternative to antibiotics in periodontal

therapy[16]. In our present *in vitro* susceptibility test study, *A. aspera* extract exhibited inhibition zones in both 50 mg/ml and 100 mg/ml against *P. gingivalis* and *P. intermedia* revealing its strong efficacy in the sub *gingival* microflora which eventually leads to decreased inflammation and tissue destruction. The results also indicates that a concentration of 100 mg/ml displayed a minimum bactericidal concentration, while 50 mg/ml exhibited a minimum inhibitory concentration. However, tetracycline at a minimum bactericidal concentration of 30 µg displayed larger inhibition zones than *A. aspera.* Even though tetracycline showed a larger inhibition zone when compared to *A. aspera,* it simultaneously showed certain side effects as mentioned above. The current study demonstrates *A. aspera* efficacy of antimicrobial activity against the key periodontal pathogens with no discernible side effects proving it to be an effective alternative to tetracycline[17,18]. The findings of the current study were clinically corroborated by research conducted by Niharika *et al.*, which indicated a significant reduction in Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL) in periodontitis patients treated with *A. aspera* gel. Similarly, Ramanarayanan Bhoyapati *et al.,* carried out a study using a 4g dose of *A. aspera* root extract for the treatment of chronic periodontitis[1]. The study found that local application of *A. aspera* root extract, when combined with scaling and root planning, produced positive outcomes in managing periodontitis, with no side effects reported. Furthermore, it exhibited strong anti-inflammatory and antioxidant properties[19,20]. Furthermore, numerous studies have demonstrated the antimicrobial activity of *A. aspera* against various cariogenic bacteria, including *Staphylococci, Streptococci, E. faecalis*, and *E. coli*. This effectiveness is attributed to its antibiofilm and quorum sensing inhibitory properties, as well as its low toxicity and favourable pharmacokinetic characteristics[1,21-23]. A systematic review by Pandit *et al.*, strongly reveals the significant antibacterial properties in various herbal medications, coupled with low toxicity and effective plaque control[14]. To the best of our knowledge, there have been no previous studies on the efficacy of *A. aspera* against *P. gingivalis* and *P. intermedia*, though few literatures have analyzed it in clinical applications. Further clinical evaluation with larger sample size and a wider range of microbial efficacy assessments to evaluate the effectiveness of *A. aspera* in periodontal management can substantiate the equivalent herbal alternative in the future antimicrobial therapy for the management of periodontal diseases.

## 5. Conclusion

Extracts of *A. aspera* have demonstrated significant inhibition of periodontal pathogenic bacteria, along with efficacy against other oral bacteria in *in vitro* method. Based on the results of the current study, *A. aspera* extracts can be considered as a valuable alternative source for sustained drug release system that prevents the recolonization of microorganism suggesting their long-term result in anti-infective therapy.

## 6. Author Contribution

Dr. I. Paavai contributed to concepts, design, clinical study, data analysis, manuscript editing and review. Dr. Gomathi. G. D, Dr. Sivaranjani and Dr. V. Vasanth Ayswarya contributed towards data acquisition, data analysis, and manuscript preparation. Dr. Ashmi. A. J and Dr. Saisuruthi S contributed towards literature review and manuscript editing. All authors have read and agreed to the published version of the manuscript.

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