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Original Article

Dentistry Section

Comparative Clinical and Antimicrobial Evaluation of *Abutilon indicum* Mouthwash versus Chlorhexidine in Gingivitis Management: A Prospective Clinical Study on Herbal Approach to Periodontal Therapy

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ABSTRACT

Introduction: Gingivitis, a precursor to periodontitis, is primarily driven by plaque biofilms and the host's inflammatory response. While chlorhexidine is the gold standard mouthwash, its side-effects necessitate safer alternatives. *Abutilon indicum* (*Atibala*) exhibits antimicrobial, anti-inflammatory and antioxidant properties, making it a promising candidate for adjunctive periodontal therapy.

Aim: To evaluate the clinical and antimicrobial efficacy of *Atibala*-based mouthwash in patients with gingivitis and compare its effectiveness with 0.12% chlorhexidine.

Materials and Methods: The present prospective clinical study was conducted at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India, from June to September 2024. Forty systemically healthy participants aged 25-35 years with clinically diagnosed gingivitis were enrolled and assigned into two groups: Group A received 0.12% chlorhexidine mouthwash (control), and Group B received 0.6% *Atibala*-based mouthwash (test). All participants underwent ultrasonic scaling at baseline. They were instructed to use 10 mL of the assigned mouthwash diluted 1:1 with water twice daily for one month. Clinical parameters, including Gingival Index (GI) and Plaque Index (PI), and total microbial count were assessed at baseline and after one month. Supragingival plaque samples were collected to assess microbial load expressed as CFU/mL. Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) Version 23.0 where the intergroup comparison was performed using independent t-test and intragroup comparison was made using paired t-test, with $p<0.05$ considered statistically significant.

Results: Both groups showed a significant reduction in GI, PI, and microbial load from baseline to one month ($p<0.05$). At the one-month follow-up, there was no statistically significant difference between the two groups for GI ($p=0.66$), PI ($p=0.74$), or microbial count ($p=0.77$), indicating comparable efficacy.

Conclusion: *Atibala*-based mouthwash demonstrated effectiveness equivalent to chlorhexidine in managing gingivitis, suggesting its potential as a natural adjunct in periodontal care. Further research is warranted to explore its long-term effects and molecular mechanisms.

Keywords: Chlorhexidine gluconate, Gingival disease, Herbal formulation, Periodontal index, Phytotherapy

INTRODUCTION

Oral health diseases, primarily caused by dental plaque, remain a significant global health burden, affecting individuals across all age groups. Dental plaque is a complex biofilm that forms on the tooth surface due to the interaction between specific bacterial species and dietary components [1]. Over time, if not adequately removed through proper oral hygiene measures, this biofilm matures and serves as a reservoir for pathogenic microorganisms, leading to the development of periodontal diseases. Gingivitis and periodontitis are the most prevalent periodontal diseases, posing major concerns in dentistry. Gingivitis is characterised by inflammation of the gingival tissues without any destruction of the supporting structures of the teeth. If left untreated, gingivitis can progress to periodontitis, a more severe condition that affects both the soft and hard-tissues of the periodontium. Periodontitis is marked by progressive attachment loss, deepening of periodontal pockets, gingival recession, alveolar bone resorption, increased tooth mobility, and, in advanced cases, pathological migration of teeth, ultimately leading to tooth loss [2].

The pathogenesis of periodontal disease is multifactorial, involving both microbial invasion and the host's immune-inflammatory response. While the presence of periodontopathogenic bacteria initiates the disease process, it is the host's exaggerated inflammatory response, mediated by the release of pro-inflammatory cytokines and enzymes that exacerbates tissue destruction [3-5]. The standard treatment for periodontitis involves mechanical debridement through Scaling and Root Planing (SRP), which aims to remove subgingival plaque and calculus deposits. However, SRP has certain inherent limitations, including difficulty in accessing deep periodontal pockets, furcation areas, and anatomical complexities that hinder complete bacterial elimination [6]. Moreover, some pathogenic bacteria possess the ability to invade gingival tissues, making them less susceptible to mechanical therapy alone [7]. Consequently, a variety of adjunctive therapies have been explored to enhance periodontal treatment outcomes. These include chemical plaque control agents, systemic and local antibiotics, host modulation therapies, vitamin supplements, and herbal-based interventions [8-10]. Among these, chlorhexidine mouthwash is widely regarded as the gold standard due to its broad-spectrum antimicrobial activity and substantivity. However, its long-term use is associated with several side-effects, such tooth staining, altered taste sensation, mucosal irritation, and calculus formation are common drawbacks that limit patient compliance with conventional agents [11,12]. This prompts the growing interest in herbal alternatives.

Herbal medicine has gained substantial attention in recent years due to its natural origin, minimal side-effects, and broad pharmacological properties. Many medicinal plants have demonstrated anti-

inflammatory, antimicrobial, and antioxidant activities, making them promising candidates for adjunctive periodontal therapy [13]. Among these, *Abutilon indicum*, commonly known as *Atibala*, is a traditionally revered medicinal plant known for its wide range of therapeutic applications [14]. Emerging evidence suggests that *Atibala* exhibits potent antibacterial activity against key periodontal pathogens, indicating its potential role in periodontal disease management. Additionally, its strong anti-inflammatory and antioxidant properties may help modulate the host response, thereby reducing gingival inflammation and oxidative stress within periodontal tissues [15]. While Atibala's traditional uses are well-documented in Ayurvedic medicine, contemporary scientific research focusing on its periodontal applications remains limited.

Given its potential benefits, the present study aimed to formulate an *Atibala*-based mouthwash and evaluate its clinical and antimicrobial efficacy in patients with gingivitis. The current study primarily aimed to evaluate the clinical efficacy of *Atibala* mouthwash in reducing gingival inflammation and to compare its effectiveness with 0.12% chlorhexidine. The secondary objective was to assess its antimicrobial activity by examining changes in plaque microbial counts in comparison to 0.12% chlorhexidine mouthwash.

The null hypothesis (H0) states that there is no significant difference in the clinical and antimicrobial efficacy between *Atibala*-based mouthwash and 0.12% chlorhexidine in the management of gingivitis, whereas the alternative hypothesis (H1) posits that *Atibala*-based mouthwash differs significantly in efficacy compared to 0.12% chlorhexidine.

MATERIALS AND METHODS

The present prospective clinical study was conducted to evaluate the clinical and antimicrobial efficacy of an *Atibala*-based mouthwash in patients with gingivitis. The study was conducted at Saveetha Dental College and Hospitals, Chennai, Tamil Nadu, India between June 2024 - September 2024, following approval from the Institutional Ethical Committee (IHEC/SDC/PERIO-2203/25/007). Written informed consent was obtained from all participants before enrolment.

Sample size calculation: The calculation of the sample size was conducted using G*Power Software (Version 3.0), based on the mean and Standard Deviation (SD) of PI (0.7±0.25 in test group and 0.90±0.17 in control group) after three weeks of intervention from a previous study [16]. An alpha value of 0.05 and a statistical power of 80% indicated a requirement of 40 participants.

Study Population: A total of 40 systemically healthy individuals aged 25-35 years with clinically diagnosed gingivitis were recruited. The inclusion criteria included patients with a GI score of 1.1-3 absence of clinical attachment loss or alveolar bone loss, and no history of antibiotic or anti-inflammatory drug use in the past three months. Patients with a history of periodontal therapy in the last six months, systemic diseases affecting periodontal health, smoking habits, or pregnancy were excluded.

Study Procedure

Formulation of Atibala-based Mouthwash: To prepare a 0.6% *Atibala* mouthwash, 10 g of *Atibala* leaf powder was macerated in 100 mL of distilled water and heated at 40-50°C for 10 minutes with occasional stirring to enhance the extraction of bioactive compounds [Table/Fig-1]. The solution was then filtered using Whatman No. 1 filter paper to obtain a clear extract. For the final mouthwash formulation, 60 mL of the prepared *Atibala* extract was diluted in 240 mL of distilled water. To ensure stability and efficacy, 0.025 g of sodium benzoate was added as a preservative, along with three drops (~0.15 mL) of peppermint oil for flavouring and additional antimicrobial benefits. A 5 g of sucrose was incorporated as a sweetening agent, while 0.2 g of sodium lauryl ether sulfate was included as a surfactant to improve solubility and dispersion.

[Table/Fig-1]: Preparation of *Atibala* leaf powder extract.

The solution was stirred until all components were fully dissolved, and the pH was adjusted to 5.5-6.5 for optimal oral compatibility. Finally, the mouthwash was stored in sterile amber-coloured bottles at room temperature to maintain stability and efficacy.

Intervention and study groups: Participants were randomly assigned into two groups:

- Group A (Control group n=20): Chlorhexidine (0.12%) mouthwash (Clohex Plus® mouthwash, Dr. Reddy's Lab Ltd., Hyderabad, India).
- Group B (Test Group n=20): *Atibala*-based mouthwash.

Both the group participants underwent full-mouth ultrasonic scaling before starting the mouthwash regimen by the Principal Investigator (PJ). Participants were instructed to use 10 mL of the assigned mouthwash in 1:1 dilution with water twice daily for one minute for a period of one month without any mechanical plaque removal interventions apart from routine brushing. Compliance was monitored through follow-up calls.

Clinical Evaluation: Baseline and one month post-intervention clinical parameters were recorded by a calibrated examiner (AR) blinded to the study groups. The following indices were assessed: GI (Löe and Silness, 1963) [17]; PI (Silness and Löe, 1964) [18].

Microbiological Analysis: The supragingival plaque samples were collected from the buccal surfaces of tooth with help of sterile Gracey curette (Hu-Friedy®, Chicago, US), at baseline and one month post-intervention. The plaque samples were carried in phosphate buffer solution for microbiological study. The plaque samples were assessed for total microbial count, expressed as Colony Forming Units per milliliter (CFU/mL). Sample collection was done by AR. Throughout the study duration, no adverse effects or allergic reactions to the test or control formulations were reported by any of the participants.

STATISTICAL ANALYSIS

The clinical and microbiological data were compiled and analysed using the Statistical Package for Social Sciences Software, Version 23.0 (IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro-Wilk test, which confirmed a parametric distribution. Descriptive statistics were expressed as mean±SD. For inter-group comparisons, the independent t-test was used, whereas Intragroup comparisons were performed using the paired t-test. A p-value <0.05 was considered statistically significant.

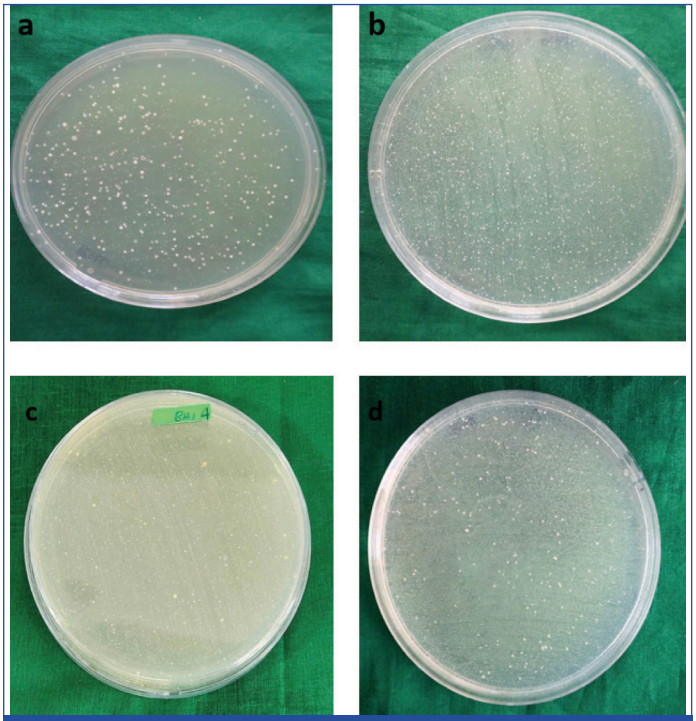
RESULTS

The Intragroup comparison demonstrated a statistically significant reduction in GI, PI, and total microbial count from baseline to the one-month follow-up in both groups ($p<0.05$) for all parameters. In Group A, the GI decreased from 2.45±0.32 to 0.60±0.21, the PI reduced from 2.09±0.28 to 0.67±0.19, and the microbial count dropped from 197.22±39.05 CFU/mL to 31.02±9.01 CFU/mL. Similarly, in Group B, the GI declined from 2.39±0.30 to 0.57±0.22, the PI decreased from 2.05±0.27 to 0.65±0.20, and the microbial count reduced from 195.82±41.01 CFU/mL to 30.16±9.77 CFU/mL. These findings indicate a notable improvement in oral health within each group following the intervention [Table/Fig-2]. The plaque samples were assessed for total microbial count, expressed as CFU/mL. Both groups show a visible reduction in CFU count after one month, indicating antimicrobial efficacy [Table/Fig-3]. Comparison of clinical and microbiological parameters between Group A (Chlorhexidine mouthwash) and Group B (Atibala-based mouthwash) at baseline. Parameters assessed include GI, PI, and total microbial count (CFU/mL). Values are expressed as mean±SD. No statistical significance was observed between the two groups in all the parameters ($p>0.05$) [Table/Fig-4].

The inter-group comparison at the one-month follow-up revealed no statistically significant difference between Group A and Group B. The GI values at one month were 0.60±0.21 in Group A and 0.57±0.22 in Group B ($p=0.66$), while the PI values were 0.67±0.19 and 0.65±0.20, respectively ($p=0.74$). The microbial count was 31.02±9.01 CFU/mL in Group A and 30.16±9.77 CFU/mL in Group B ($p=0.77$). Since all p-values were greater than 0.05, the differences between the two groups were not statistically significant, indicating that both interventions were equally effective in improving gingival health and reducing microbial load [Table/Fig-3,5].

Group	Mean±SD GI		t-value	p-value[a]	Mean±SD PI		t value	p-value[a]	Mean±SD Total microbial colony (CFU/mL)		t value	p-value[a]
	Baseline	1 month			Baseine	1 month			Baseline	1 month		
A	2.45±0.32	0.60±0.21	22.41	0.0002*	2.09±0.28	0.67±0.19	20.36	0.0001*	197.22±39.05	31.02±9.01	18.54	0.0002*
B	2.39±0.30	0.57±0.22	21.87	0.0002*	2.05±0.27	0.65±0.20	19.92	0.0001*	195.82±41.01	30.16±9.77	17.57	0.0003*

[Table/Fig-2]: Intragroup comparison (Paired t-test).
[a]paired t-test; *statistically significant ($p<0.05$); GI: Gingival index; PI: Plaque index; CFU/mL: Colony forming unit per millilitre



[Table/Fig-3]: The microbial growth: (a) Group-A baseline; (b) Group-B baseline; (c) Group-A at 1 month; and (d) Group-B at 1 month.

Variable	Mean±SD Group A	Group B	t value	p-value[b]
GI	2.45±0.32	2.39±0.30	0.61	0.54
PI	2.09±0.28	2.05±0.27	0.46	0.64
Total microbial count (CFU/mL)	197.22±39.05	195.82±41.01	0.11	0.91

[Table/Fig-4]: Intergroup comparison at baseline (Independent t-test).
[b]independent t-test, * statistically significant ($p<0.05$); GI: gingival index; PI: Plaque index; CFU/mL: Colony forming unit per millilitre.

Variable	Mean±SD Group A	Group B	t-value	p-value[b]
GI	0.60±0.21	0.57±0.22	0.44	0.66
PI	0.67±0.19	0.65±0.20	0.32	0.74
Total microbial count (CFU/mL)	31.02±9.01	30.16±9.77	0.29	0.77

[Table/Fig-5]: Inter-group comparison at 1 month follow-up (Independent t-test).
b- independent t-test, * statistically significant ($p<0.05$); GI: Gingival index; PI: Plaque index; CFU/mL: Colony forming unit per millilitre.

DISCUSSION

The present study aimed to evaluate the clinical and antimicrobial efficacy of an *Atibala*-based herbal mouthwash in managing gingivitis, in comparison with the widely used 0.12% chlorhexidine mouthwash. Both groups demonstrated a significant reduction in gingival inflammation and plaque accumulation over the study period. Notably, the performance of *Atibala*-based mouthwash was comparable to chlorhexidine. However, the null hypothesis is accepted. The findings of Pattanashetti JI et al., reported strong antibacterial activity of *Abutilon indicum* extracts against periodontal pathogens, such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum* [19].

Herbal mouthwashes have gained popularity as viable alternatives to conventional chemical agents due to concerns over adverse effects such as tooth staining, taste alteration, and mucosal irritation commonly associated with chlorhexidine [20]. In this context, the efficacy demonstrated by *Atibala* underscores its potential as a safer, plant-derived adjunct in routine oral hygiene practices.

The observed effects may be attributed to the phytoconstituents present in *Abutilon indicum*, such as flavonoids, tannins, alkaloids, glycosides, and essential oils [21]. These compounds have been previously reported to exert antimicrobial, anti-inflammatory, and antioxidant actions, which are critical in the management of periodontal diseases. Specifically, flavonoids have been shown to inhibit a wide range of bacterial activities, including biofilm formation and cytoplasmic membrane disruption, while also modulating host immune responses [22].

In addition, flavonoids have demonstrated the capacity to suppress pro-inflammatory cytokines like IL-1β, IL-6, TNF-α, and PGE2, thereby reducing tissue breakdown and promoting healing [23]. Such anti-inflammatory effects are particularly relevant in gingivitis, where inflammation is a predominant feature. Moreover, flavonoids stimulate osteoblast differentiation and periodontal ligament cell function, further suggesting their role in regenerative periodontal therapies [24].

Comparative studies also reinforce these findings. Gowtham R et al., and Mishra DN et al., documented broad-spectrum antimicrobial

efficacy of *A. indicum*, attributed to its bioactive constituents [25,26]. Similarly, Wakale S and Kandepalli M demonstrated significant inhibition zones against oral pathogens, comparable to those observed with chlorhexidine [27]. Ferdioz JA and Roy A and Andhare AA and Shinde RS further validated the antibacterial potency of *A. indicum* via aqueous-alcoholic extracts [28,29]. Additionally, Manandhar S et al., showed its effectiveness against both Gram-positive and Gram-negative bacteria [30]. These findings mirror the results of the present study, strengthening the clinical rationale for integrating *Atibala*-based formulations into periodontal care.

Furthermore, the antioxidant properties of *A. indicum* may play a dual role in controlling both microbial insult and host-mediated tissue damage by attenuating oxidative stress in gingival tissues [31]. Research into *A. indicum*-mediated nanoparticles has shown promise in enhancing these effects, with studies reporting superior antibacterial and antioxidant activity due to improved bioavailability and targeted delivery [32-34].

Limitation(s)

Despite the promising findings of the current study, certain limitations must be acknowledged. The present study was conducted over a limited duration, and the long-term effects of *Atibala*-based mouthwash on gingival and periodontal health remain unexplored. Additionally, while the sample size was adequate for preliminary evaluation, larger studies encompassing diverse populations would be necessary for broader validation. Another limitation is that the study primarily focused on clinical parameters without delving into molecular-level mechanisms, such as how the phytoconstituents modulate the host immune response and microbiome balance. Future studies should incorporate advanced methodologies, such as genomic and proteomic analyses, to better understand the molecular interactions of Atibala's bioactive compounds within the oral environment. Furthermore, comparative studies assessing its long-term efficacy against standard chemical mouthwashes in different periodontal conditions would provide a clearer understanding of its clinical applicability. Further exploration of formulation stability and optimisation of concentration could enhance its therapeutic potential and practical utility in periodontal care.

CONCLUSION(S)

The study demonstrated that *Atibala*-based mouthwash was as effective as chlorhexidine in reducing gingival inflammation, plaque accumulation, and microbial load in patients with gingivitis, underscoring its potential as a natural adjunct in periodontal care. Further research is warranted to investigate its long-term effects and underlying molecular mechanisms.

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