



## COMPARATIVE EVALUATION OF *CLITORIA TERNATEA* MOUTHWASH WITH CHLORHEXIDINE GLUCONATE MOUTHWASH IN PREVENTION OF PLAQUE AND GINGIVITIS - A CLINICAL AND MICROBIOLOGICAL STUDY

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### Abstract

**Introduction:** Plaque accumulation and oral microorganisms are the main predisposing factors to various periodontal diseases and targeting these, therefore, can prove to be an effective way of combating these diseases. Due to many negative effects of conventional forms of treatment, herbal extracts have recently attracted a lot of attention. **Aim:** To evaluate the efficacy of 2% clitoria ternatea extract mouthwash as an antiplaque and antimicrobial agent and compare it with that of 0.2% chlorhexidine gluconate mouthwash in mild to moderate gingivitis. **Materials and methods:** A total of 44 subjects, aged 18 to 40 years visiting the department of periodontology at bharati vidyapeeth (deemed to be) university, dental college and hospital, pune were randomly divided into two groups. Individuals with gingival component of the ramfjord's periodontal disease index score of 1 and 2 and score 3 and 4 on the turesky gilmore-glickman modification of quigley hein plaque index (tqhpi) were included in the study. Group 1 (22) and group 2 (22) were given 0.2% chlorhexidine gluconate mouthwash and 2% clitoria ternatea extract mouthwash respectively. The assessment of plaque index and total microbial count was done at baseline, 7<sup>th</sup> and 14<sup>th</sup> day. **Result:** There was a statistically significant reduction in both clinical and microbiological parameters using both mouthwashes. However, a statistically insignificant difference was observed between the intergroup comparison, which suggests *clitoria ternatea* mouthwash is as effective as chlorhexidine gluconate mouthwash. **Conclusion:** *Clitoria Ternatea* can prove to be an effective and safe alternative to conventional modes of treatment.

**Keywords:** Clitoria ternatea, Chlorhexidine Gluconate, mild to moderate gingivitis, periodontitis

### INTRODUCTION

Gingival and periodontal diseases are affecting most of the population across the world. Dental plaque is a biofilm with layers of microorganisms including aerobic and anaerobic bacteria contained in a matrix that forms on oral surfaces which is considered as etiologic factor for periodontal diseases. Hence, plaque control is necessary where nonsurgical periodontal treatment remains the core component of periodontal therapy. For prevention of periodontal diseases and maintenance of periodontal health, mechanical and chemical methods used in plaque control procedures are of critical importance. Tooth brushing, practising good oral hygiene, and receiving professional prophylaxis are mechanical ways to prevent plaque. Mechanical plaque control techniques are utilised as a mainstay to maintain dental hygiene. Since it takes time and effort to effectively use mechanical plaque management techniques, antimicrobial compounds have been widely used as an adjunct to mechanical cleaning.<sup>1</sup>

Among the plethora of oral hygiene products available, chlorhexidine has been the gold standard owing to its dramatic therapeutic effect, but its various side effects like taste alteration, desquamation of oral mucosa, and extrinsic staining have restricted its usage.<sup>2</sup> Alternative agents based on herbal extracts like Curcumin, Triphala, Aloe vera etc are therefore of particular interest.<sup>3</sup> One such herb, *Clitoria ternatea*, has been studied for its numerous medicinal properties like anti-microbial, anti-inflammatory, analgesic, anti-oxidant, and anti-ulcer properties against periodontal pathogens. The extract of *Clitoria ternatea* is safe, easily available and cost-effective making it, an ideal candidate for plaque control and thereby reducing gingivitis and probable later periodontitis.<sup>4</sup>

Therefore, in this study, an attempt has been made to clinically assess the efficacy of the extract of *Clitoria ternatea* in the form of a mouthwash, in prevention of plaque and gingival inflammation, which is supported by previous microbiological studies.

## MATERIALS AND METHODS

The clinical study was conducted at Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Pune, and the microbiological study to evaluate the total bacterial count was conducted at Surendra Laboratories Pune. The Institutional Ethics Committee (IEC) approved the research proposal to carry out the research project.

### Preparation of mouthwash

The manufacturing of *Clitoria Ternatea* mouthwash was carried out at Stratum Pharmaceuticals, Dehradun.

Formulation of the mouthwash included:

- *Clitoria ternatea* (Aparajita) extract (2%)
- Tween20 (2%) – A surfactant and wetting agent which enhances the spreadability of liquids.
- Sorbitol (20%) - A sweetening agent and moistener.
- Menthol perfume and flavour
- Methyl paraben: A preservative and anti-microbial agent.
- Water (quantity sufficient to produce): Vehicle carrying all the ingredients of the mouthwash.

Individuals visiting the Outpatient Department of Periodontology of Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, Pune were carefully selected based on the inclusion and exclusion criteria.

Selection of Individuals:

### Inclusion criteria:

1. Individuals in the age group of 18- 40 years.
2. Individuals with mild to moderate gingivitis.

### Exclusion criteria:

1. Individuals with a history of periodontal therapy within the last 6 months.
2. Individuals with antibiotic and antiviral therapy or any other type of medication within the last 6 months.
3. Individuals with autoimmune diseases.
4. Individuals with smoking and tobacco chewing habits.
5. Individuals allergic to any of the medications or history of food allergy.
6. Pregnant or lactating women.
7. Individuals with systemic diseases and conditions (e.g.: chronic inflammation, Hepatitis, Rheumatoid arthritis, Atherosclerosis, Diabetes mellitus, etc.)

Each subject provided both verbal and written informed consent.

Each individual underwent recording of relevant case history, clinical oral examination and assessment of clinical parameters was done using proper armamentarium (**Figure 1**). Participants were evaluated for their periodontal status using the Gingival component of the Ramfjord's Periodontal Disease Index and individuals with a score of 1 and 2 were considered in the study.

The subjects were asked to swirl erythrosine disclosing agent (**Figure 2**) and were assessed for plaque by using the Plaque Index (Turesky-Gilmore-Glickman modification of the Quigley Hein 1970) and individuals with a score of 3 and 4 on the Plaque Index were included in the study. (**Figure 3**)

44 participants were randomly assigned into two groups. After assessment of the indices, thorough scaling and polishing was carried out to get the subjects at the baseline. Group 1 received a 0.2% Chlorhexidine mouth rinse (**Figure 4**), while Group 2 received a 2% *Clitoria Ternatea* extract mouth rinse (**Figure 5**). The subjects were instructed on the use of their assigned mouth rinses, and their saliva samples were collected for analysis of bacterial count.

Stimulated saliva samples (10 ml) were collected in sterile containers (**Figure 6**) containing Thioglycolate transport media at baseline and on the 7th and 14th days for evaluation of the antimicrobial effects of the mouthwashes by evaluating the total colony forming units on a nutrient agar culture (**Figure 7**) using an automatic colony counter. Determination of Plaque index was done at baseline, 7<sup>th</sup> and 14<sup>th</sup> day. Subjective and

objective criteria such as taste acceptability, burning sensation, dryness/soreness, ulcer formation, staining of teeth and tongue, and allergies were assessed after 7 and 14 days.

The data collected was statistically analysed. Descriptive statistics were expressed as means and standard deviation. Within each group the comparison of study variables was done using Repeated measures ANOVA test. Intergroup comparison of the study variables was done using Unpaired t test. In the above tests, p values less than or equal to 0.05 was considered statistically significant. All analyses were performed using SPSS version 25.



Figure 1: Armamentarium



Figure 2: Erythrosine disclosing agent



Figure 3: Mild to moderate gingivitis



Figure 4: 0.2% Chlorhexidine gluconate mouthwash



Figure 5: 2 % *Clitoria ternatea* extract mouthwash



Figure 6: Sterile containers

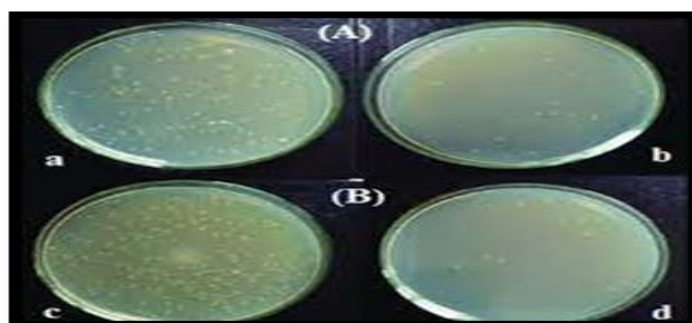


Figure 7: Nutrient Agar culture

## Results

The study aimed to evaluate the effectiveness of *Clitoria ternatea* extract in comparison to chlorhexidine gluconate mouthwash (0.2%) for reducing plaque and bacteria in patients with mild to moderate gingivitis. A total of 44 patients participated, and their saliva samples and plaque scores were collected on day 1, day 7, and day 14. The samples were analysed to assess the antimicrobial and anti-plaque properties of the mouthwashes. Statistical analysis was performed using descriptive statistics, repeated measures ANOVA test, and unpaired t-test. The results were considered statistically significant if p values were less than or equal to 0.05.

### Intergroup Comparison of Mean plaque index score

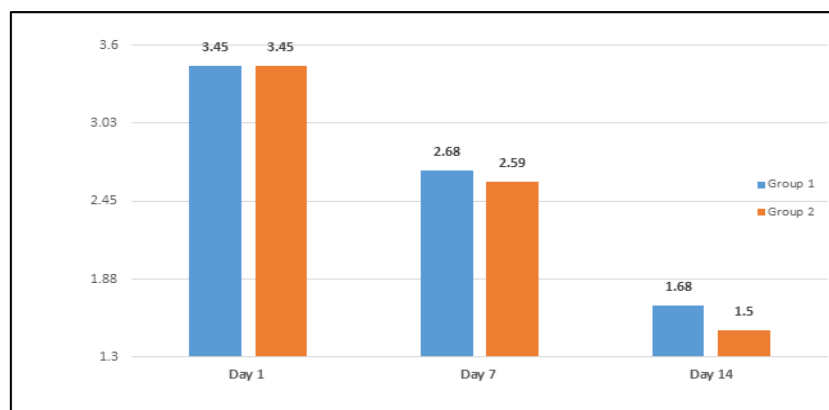
**Table 1:** Comparison of anti-plaque efficacy of Group 1 and Group 2 study participants

Plaque Index score	Group 1 (Chlorhexidine mouth rinse) N = 22	Group 2 ( <i>Clitoria Ternatea</i> extract mouth rinse) N = 22	P value
Day 1	3.45 ± 0.51	3.45 ± 0.51	1.000
Day 7	2.68 ± 0.48	2.59 ± 0.73	0.629
Day 14	1.68 ± 0.65	1.50 ± 0.51	0.307

p-value by Unpaired t test

\*p ≤ 0.05 is statistically significant

(p ≤ 0.001 – highly significant; \*p ≤ 0.01 – significant; \*\*p > 0.05 – non-significant)



**Graph 1:** Comparison of anti-plaque efficacy of Group 1 and Group 2 study participants

**Table 1 and Graph 1** compares the mean plaque score of groups I (0.2% chlorhexidine gluconate mouthwash) and group 2 (2% *Clitoria Ternatea* extract mouthwash) at baseline (day 1), day 7 and 14. For group I, the mean plaque score at baseline is 3.45+0.51, at day 7 is 2.68+0.48 and day 14 it is 1.68+0.65 and for group 2, the mean plaque score at baseline is 3.45+0.51, at day 7 is 2.59+0.73 and day 14 it is 1.5+0.51. This difference is statistically insignificant. This demonstrates that both 0.2% chlorhexidine gluconate mouthwash and 2% *Clitoria Ternatea* extract mouthwash show similar anti-plaque efficacy.

### Intergroup Comparison of total bacterial count:

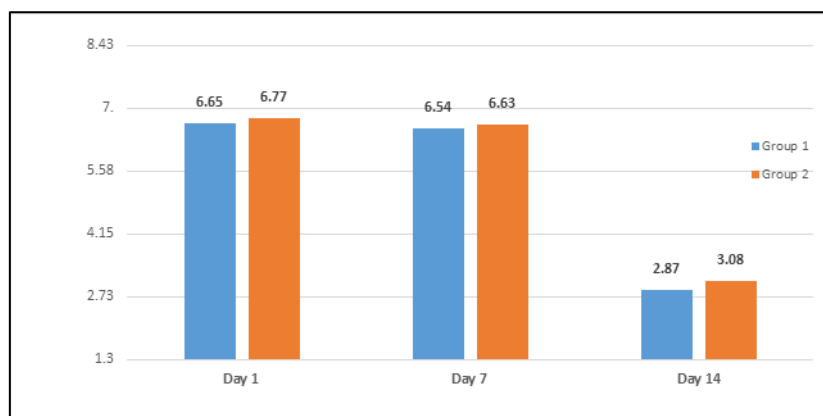
**Table 2:** Comparison of anti-microbial activity of Group 1 and Group 2 study participants

Total bacterial count x 10 <sup>8</sup>	Group 1 (Chlorhexidine mouth rinse) N = 22	Group 2 ( <i>Clitoria Ternatea</i> extract mouth rinse) N = 22	P value
Day 1	6.65 ± 0.40	6.77 ± 0.39	0.324
Day 7	6.54 ± 0.37	6.63 ± 0.37	0.401
Day 14	2.87 ± 0.51	3.08 ± 0.45	0.168

p-value by Unpaired t test

\* $p \leq 0.05$  is statistically significant

( $p \leq 0.001$  – highly significant; \* $p \leq 0.01$  – significant; \*\* $p > 0.05$  – non-significant)



Mean bacterial count  $\times 10^8$

**Graph 2: Comparison of anti-microbial activity of Group 1 and Group 2 study participants**

Table 2 and Graph 2 compares the mean total bacterial count of group I (0.2% chlorhexidine gluconate mouthwash) and group 2 (2% *Clitoria Ternatea* extract mouthwash) at baseline (day 1), day 7 and 14. For group I, the mean total bacterial count at baseline is  $6.65 \pm 0.40 \times 10^8$ , at day 7 is  $6.54 \pm 0.37 \times 10^8$  and day 14 it is  $2.87 \pm 0.51 \times 10^8$  and for group 2, the mean total bacterial count at baseline is  $6.77 \pm 0.39 \times 10^8$ , at day 7 is  $6.63 \pm 0.37 \times 10^8$  and day 14 it is  $3.08 \pm 0.45 \times 10^8$ . This difference is statistically insignificant. This demonstrates that both 0.2% chlorhexidine gluconate mouthwash and 2% *Clitoria Ternatea* extract mouthwash show similar anti-microbial efficacy.

## DISCUSSION

Periodontal diseases affecting the structures surrounding the teeth are often recognized by patients when it has already progressed significantly. Periodontists play a crucial role in the prevention, identification, and treatment of this condition. One common treatment modality involves the use of chemical agents in conjunction with mechanical means. Chlorhexidine (CHX) digluconate, a chemical agent with a long history in dental medicine, has been examined for its ability to control oral microorganisms and plaque formation. However, the side effects associated with it, such as extrinsic tooth staining, unpleasant taste, and a burning sensation has led to a growing interest in the use of natural compounds for oral health.

One such herb is *Clitoria Ternatea*, which has been extensively studied and used in Indian medicine for its neurological benefits, anti-inflammatory properties, antimicrobial effects, and antioxidant properties. It is gaining popularity worldwide as "Blue Tea" due to its medicinal properties.<sup>5</sup>

A study was conducted to explore the efficacy of a 2% *Clitoria Ternatea* extract mouthwash as an antiplaque and antimicrobial agent, comparing it with a 0.2% Chlorhexidine Gluconate mouthwash in individuals with mild to moderate gingivitis. The study was conducted among 44 individuals aged 18-40 at Bharati Vidyapeeth Dental College and Hospital in Pune. Saliva plays a crucial role in oral health as it contains many microorganisms. Saliva facilitates bacterial aggregation and aids in plaque formation. Saliva is also a representative diagnostic specimen for assessing the overall oral microbiota since it contains bacteria from various sites and surfaces of the oral cavity. These associations suggest that it might be reasonable to sample saliva for monitoring supragingival microbiota.<sup>6</sup>

*Streptococcus mutans* has been implicated as a primary causative agent of gingivitis and periodontitis. It first adheres to an acquired pellicle formed on the enamel surface, followed by the synthesis of adhesive glucan from sucrose, which contributes to the accumulation of dental plaque.<sup>7</sup> Detecting plaque can be done using a disclosing agent, such as Erythrosine B (EB) dye, which stains bacteria and indicates their viability.

In the study, plaque scores were recorded at baseline (Day 1), Day 7, and Day 14 after using the respective mouthwashes. The intragroup comparison in Group 1 and Group 2 showed a statistically significant reduction in

plaque values from baseline to Day 14. Intergroup comparisons showed that there was no statistically significant difference between the two mouthwashes implying that both mouthwashes were equally effective in reducing plaque accumulation. A previous study suggested that the inhibitory effects of *Clitoria Ternatea* on plaque accumulation and caries formation are due to the inhibition of bacterial polysaccharide production. It is also supported by another study which suggested that by preventing the formation of fimbriae, which are essential for the bacteria to form biofilms, anthocyanins slowed the growth of Gram-negative bacteria like *E. coli*.<sup>8</sup>

Another study suggests that anthocyanins present in *Clitoria Ternatea* inhibit *P. gingivalis* and the activity of gingipains, which are *P. gingivalis* virulence factors.<sup>9</sup> Flavonoids, inhibit the membrane function and penetrate the lipid bilayer of the gram-negative bacteria, thereby destroying the barrier function of the outer membrane, leads to membrane fusion and causes cell leakage.<sup>10</sup> The flavonoids also inhibit bacterial energy metabolism required for the synthesis of macromolecules (DNA, RNA, and protein).<sup>11</sup>

In the present study, intergroup comparisons showed that there was no statistical difference seen between both the mouthwashes for their anti-microbial capacity which implied that both had similar anti-microbial efficiency. Gowd Pratap et al 2012, in his study, observed significant anti-microbial efficacy of 5% aqueous extract of *Clitoria Ternatea* extract on three oral pathogens, *Streptococcus mutans*, *Lactobacillus casei*, and *Staphylococcus aureus*.<sup>12</sup> Yanti & Yusri (2018) studied the antibacterial and antibiofilm efficacy of *Clitoria Ternatea* anthocyanins.<sup>13</sup> Nguyen GK, et al.(2016) stated that Gram-negative-specific antibacterial cyclotides from the butterfly pea (*Clitoria ternatea*) have Immunomodulating activity.<sup>14</sup> Sankari et al. 2014 in his study observed that flavonoids prevent bacterial cell division and are able to harm the bacterial cell walls and cytoplasm by reducing membrane fluidity of bacterial cells.<sup>15</sup>

## CONCLUSION

Periodontitis is an inflammatory disease characterized by the destruction of periodontal tissues, caused by the interaction between host defense mechanisms and plaque microorganisms. Prevention of periodontal disease is crucial and involves plaque control methods, both mechanical and chemical. Chlorhexidine gluconate has been widely used as a chemotherapeutic agent, but it has side effects, leading to the search for alternative natural compounds. *Clitoria Ternatea*, a tropical herb, has shown antibacterial properties and contains polyphenolic compounds and an active peptide called cliotide, which possess antimicrobial and anti-glycosyltransferase properties.

A study was conducted to evaluate the efficacy of 2% *Clitoria Ternatea* extract mouthwash as an antiplaque and antimicrobial agent, comparing it with 0.2% Chlorhexidine Gluconate mouthwash in individuals with mild to moderate gingivitis. Saliva samples and plaque scores were collected from 44 participants on days 1, 7, and 14 to assess antimicrobial and anti-plaque effects. The total bacterial count in saliva was examined in the laboratory.

### The findings of the study were as follows:

- There was a significant reduction in plaque readings from baseline to day 7 and day 14 in both groups, indicating the efficacy of both mouthwashes in reducing plaque.
- There was no significant difference in plaque readings between the two mouthwash groups, suggesting that *Clitoria Ternatea* extract mouthwash was equally effective as Chlorhexidine gluconate mouthwash.
- The total bacterial count decreased significantly from baseline to day 7 and day 14 in both groups, indicating the antimicrobial efficacy of both mouthwashes.
- There was no significant difference in the total bacterial count between the two mouthwash groups, indicating similar antimicrobial effectiveness.
- In conclusion, the study demonstrated that 2% *Clitoria Ternatea* extract mouthwash is as effective as 0.2% Chlorhexidine gluconate mouthwash in reducing plaque and inhibiting microbial growth in individuals with gingivitis.

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