Original Article

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Antioxidant, Antimicrobial, and Anti-inflammatory Properties of *Clitoria Ternatea* Gel as a Caries Removal Agent: An In Vitro Analysis

Dentistry Section

PRAGATI PAREEK[1], R RAMESH[2]

ABSTRACT

Introduction: Dental caries is a common oral health issue in children. *Clitoria ternatea*, which is gaining emerging focus due to the bioactive and biocompatible properties of its plant-based compounds, offers a promising natural alternative for Chemomechanical Caries Removal (CMCR) as a non invasive approach.

Aim: To evaluate the in-vitro bioactivity of *Clitoria ternatea* gel in caries removal, focusing on its antioxidant, antimicrobial and anti-inflammatory properties.

Materials and Methods: This in-vitro study was conducted over a three-month period in July 2024 at the Blue Laboratory, SIMATS University, Chennai, Tamil Nadu, India, in collaboration with Saveetha Dental College and Hospital. The research involved the formulation and analysis of *Clitoria ternatea* gel, evaluating its phytochemical composition, antioxidant activity, antimicrobial efficacy and anti-inflammatory properties. The study was performed on a sample of 12 gel extracts. The statistical tests used included independent paired t-tests, correlation analysis, effect size analysis using Cohen's d and Hedges' correction and multivariate analysis.

Results: The *Clitoria ternatea* gel demonstrated significant antioxidant, antimicrobial and anti-inflammatory effects. In the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) assay, antioxidant activity increased from 39.75 µg/mL at 100 µg/mL to 65.06 µg/mL at 500 µg/mL, while the Nitric Oxide (NO) assay showed similar improvements, increasing from 7.49 µg/mL to 39.99 µg/mL. The gel exhibited effective antimicrobial activity against cariogenic bacteria; however, it was significantly less effective than the control ($p<0.001$). Additionally, it demonstrated a concentration-dependent anti-inflammatory effect, with activity rising from 16.36 at 100 µg/mL to 58.78 at 500 µg/mL, along with strong correlations with standard treatments.

Conclusion: The *Clitoria ternatea* gel demonstrates strong potential as a natural CMCR agent, offering effective antioxidant, antimicrobial and anti-inflammatory properties. This formulation could serve as a biocompatible and minimally invasive option for removing dental caries.

Keywords: Carisolv, Dental caries susceptibility, Paediatric dentistry

INTRODUCTION

Dental caries affects approximately 3.5 billion people worldwide, as highlighted in the Global Oral Health Status Report 2022, making it a highly prevalent condition [1]. Dental caries result from the progressive demineralisation of tooth structure, stemming from an imbalance in the dental biofilm that encourages the growth of cariogenic bacteria [1,2]. This shift is often attributed to a carbohydrate-rich diet, particularly with frequent intake of fermentable sugars [3,4]. Consequently, the balance between demineralisation and remineralisation processes within the dental hard tissues becomes skewed, favouring mineral loss [5]. Over time, this mineral deficit leads to the formation of carious lesions, potentially advancing to the dentin layer [6,7].

Carious dentine has two layers: the outer infected layer, which lacks dentinal tubules and features degraded collagen and bacterial contamination and the inner affected layer, which is demineralised but retains intact collagen with minimal bacteria. Modern caries management favours preserving this viable dentine, avoiding overtreatment [8,9]. Caries gel selectively dissolves infected dentine, preserving tooth structure and minimising pulp exposure with minimal discomfort [10,11].

Chemomechanical Caries Removal (CMCR) agents offer a minimally invasive alternative for managing carious dentine [12]. By targeting and dissolving the infected dentine tissue, these agents facilitate the gentle removal of softened tissue while preserving the integrity of the underlying dentine [13]. Early CMCR formulations used sodium hypochlorite for tissue dissolution, while the 2005 development of enzyme-based agents like Papacárie provided a more controlled, less invasive approach to caries management [14,15].

Recently, attention has turned to natural, plant-based compounds as alternative CMCR agents due to their bioactive properties and biocompatibility [16]. *Clitoria ternatea* Linn., also known as butterfly pea or Aparajita, is a member of the Fabaceae family, widely recognised in Ayurvedic medicine for its various medicinal benefits [17,18]. Native to tropical Asia and widely naturalised in India, China and South America, this plant is traditionally utilised to treat various ailments due to its wide array of pharmacological activities [19]. The plant contains active compounds with analgesic, anti-inflammatory, antimicrobial and other properties, making its flowers, leaves, roots, seeds and stems widely used in traditional medicine [20].

Given the increasing interest in harnessing natural compounds for medical and dental applications, *Clitoria ternatea* has emerged as a promising candidate due to its bioactive compounds, including its antioxidant, antimicrobial and anti-inflammatory properties, which make it a potential caries removal agent for dental caries [21]. By effectively targeting cariogenic bacteria, these properties offer a holistic, biocompatible alternative to other CMCR agents [22].

Despite the established pharmacological effects of *Clitoria ternatea*, there is a notable gap in the literature concerning its specific application in dental care, particularly as a gel formulation for caries removal. Existing literature has focused on its general medicinal properties rather than its use in oral health applications [23]. Thus, the present study addresses this need by providing a comprehensive evaluation of *Clitoria ternatea* gel as a caries removal agent, examining its phytochemical composition, antioxidant activity, antimicrobial efficacy and anti-inflammatory properties. The novelty of this research lies in its focus on the specific application of *Clitoria ternatea* gel for dental caries removal, offering a less invasive and

potentially more comfortable alternative to traditional mechanical drilling methods.

MATERIALS AND METHODS

The present in-vitro study was conducted in July 2024 at the Blue Laboratory, SIMATS University, Chennai, Tamil Nadu, India, in collaboration with Saveetha Dental College and Hospital, after obtaining approval from the Institutional Human Ethical Committee Review Board (Approval No. SRB/SDC/PEDO-2305/24/326).

Study Procedure

1. Preparation of *Clitoria ternatea* gel: Initially, 7.2 mL of *Clitoria ternatea* extract (0.6 mL per sample), the active ingredient, was derived from dried butterfly pea flowers (Brand A D FOOD & Herbs). These flowers were shade-dried, washed thoroughly and finely powdered. The powder was dissolved in 180 mL of glycerin with mild heating to produce Solution A.

A pre-measured quantity of Hydroxypropyl Methylcellulose (HPMC) K100M and Carbopol 934, sufficient for 12 samples, was dispersed in 900 mL of distilled water. This mixture was stirred continuously for 30 to 45 minutes using a magnetic stirrer to create a smooth, lump-free suspension, resulting in Solution B. Propylparaben and citric acid, adjusted for the batch size, were then incorporated into Solution B. Next, Solution A was gradually added to Solution B while stirring to achieve a uniform dispersion. A 10% sodium hydroxide solution was added dropwise to neutralise the gelling agents and obtain the desired gel consistency. Finally, distilled water was added to bring the total volume to 1200 mL at room temperature. The final gel formulations were then assessed for consistency, stability and other relevant parameters [Table/Fig-1a] [24].

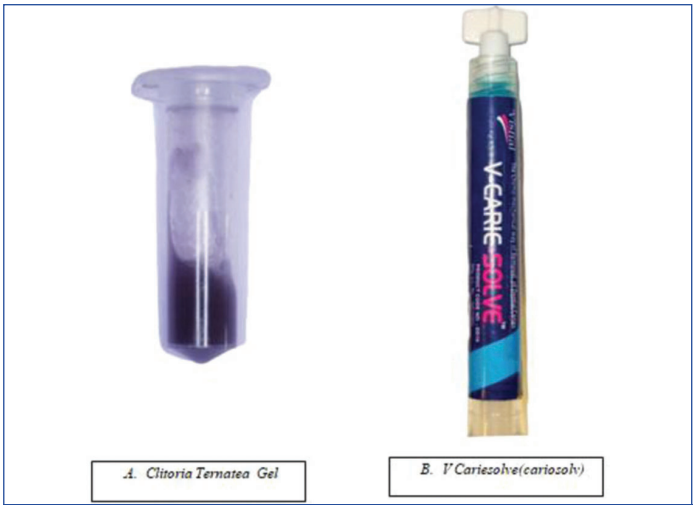
2. Phytochemical screening: The phytochemical evaluation of *Clitoria ternatea* gel (pH 2, concentration 10 µg/mL) was performed using qualitative tests to identify its bioactive constituents. Tannins were examined by mixing 1 mL of the gel with 1 mL of 5% ferric chloride solution. Saponins were tested by combining 1 mL of the gel with an equal volume of distilled water, followed by vigorous shaking for 15 minutes. Alkaloids were identified by adding 2 mL of concentrated hydrochloric acid and Mayer's reagent to 1 mL of the gel. Glycosides were tested by combining 1 mL of the gel with 3 mL of chloroform and 10% ammonium solution. For terpenoids, 1 mL of the gel was mixed with 2 mL of chloroform and concentrated sulfuric acid. Phenols were detected by adding 2 mL of distilled water and 10% ferric chloride solution to 1 mL of the gel. Steroids were confirmed by adding 2 mL of chloroform and 1 mL of sulfuric acid to 1 mL of the gel. Carbohydrates were identified by adding two drops of 20% alcoholic α-naphthol solution to 2 mL of the gel, followed by concentrated sulfuric acid. The presence of starch was confirmed by mixing 2 mL of iodine solution containing potassium iodide with 2 mL of the gel [25].

3. Antioxidant activity:

- **2,2-DPPH free radical scavenging activity:** The DPPH radical scavenging activity of *Clitoria ternatea* gel was evaluated using a previously established method with slight modifications. Briefly, 1.0 mL of DPPH solution was mixed with 1.0 mL of *Clitoria ternatea* gel at varying concentrations (100, 200, 300, 400 and 500 µg/mL). The mixture was incubated at room temperature for 50 minutes and absorbance was measured at 517 nm. Carisolv, under the brand name V Cariesolve [Table/Fig-1b], was used as the control at the same concentrations. The percentage of DPPH radical scavenging was calculated using the formula:

DPPH radicals scavenged (%)={(Control OD - Sample OD) / Control OD}×100. [26,27].

- **Nitric Oxide (NO) assay: Nitric Oxide (NO) radical scavenging activity:** The NO radical scavenging activity of *Clitoria ternatea* gel was assessed using a previously established method with slight modifications. The reaction mixture (3 mL) consisted of sodium nitroprusside (10 mM, 2 mL), phosphate-buffered saline (0.5 mL) and *Clitoria ternatea* gel at varying concentrations (100, 200, 300, 400 and 500 µg/mL) (0.5 mL). The mixture was incubated at 25°C for 150 minutes.



[Table/Fig-1]: Shows the violet coloured prepared Clitorea Teranata gel; and b) shows the control group Carisolv.

After incubation, 0.5 mL of the reaction mixture containing nitrite was mixed with 1 mL of sulfanilic acid reagent (0.33% in 20% acetic acid) and left to stand for five minutes for diazotisation. Then, 1 mL of naphthyl ethylenediamine dihydrochloride was added, mixed and allowed to stand for 30 minutes at 25°C, forming a pink-coloured chromophore in diffused light. Carisolv, at the same concentrations, was used as the control. The absorbance was measured at 550 nm and the percentage of NO radical scavenging was calculated using the formula:

Nitric Oxide (NO) radical scavenged (%)={(Control OD – Sample OD) / Control OD}×100 [28].

4. Anti-inflammatory activity: To assess the anti-inflammatory effects of *Clitoria ternatea* gel, a volume of 1 mL of *Clitoria ternatea* gel or Carisolv (used as the control) at different concentrations (100, 200, 300, 400 and 500 µg/mL) was mixed with 1 mL of an aqueous solution of bovine serum albumin (5%) and incubated at 27°C for 15 minutes. Protein denaturation was induced by heating the mixture in a water bath at 70°C for 10 minutes, followed by cooling at room temperature. The absorbance was measured at 660 nm and each test was performed in triplicate. The percentage of protein denaturation inhibition was calculated using the formula:

% inhibition={(Control OD - Sample OD) / Control OD}×100 [27].

5. Antimicrobial activity: The antimicrobial activity of *Clitoria ternatea* gel was evaluated against *Streptococcus mutans*, *Enterococcus faecalis* and *Lactobacillus acidophilus* using the agar well diffusion method. Twelve samples were tested on Mueller-Hinton agar, where bacterial suspensions (200 µL) were spread on separate plates and wells were filled with *Clitoria ternatea* gel at a concentration of 10 µg/mL. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured and photographed. Carisolv gel {Vishal – Caries Removal Innovative Enzyme Solution Offering Less Invasive, Valuable Extraction (V – CARIE-SOLVE)} served as the positive control [29].

STATISTICAL ANALYSIS

The statistical analyses performed in this study included paired t-tests, which were used to compare the antioxidant activity of *Clitoria ternatea* gel across different assays, as well as to evaluate its antimicrobial and anti-inflammatory effects. Correlation analysis was conducted to examine the relationship between gel concentration and bacterial inhibition, as well as between the gel and standard treatments in terms of anti-inflammatory activity. Independent

paired t-tests were applied to assess the mean inhibition zones for various bacterial strains treated with the gel. Additionally, effect size estimation using Cohen's d was calculated to determine the magnitude of the antimicrobial response.

RESULTS

1. Phytochemical Screening

Tannins were identified using the ferric chloride test, which produced a violet colour. The foam test confirmed the presence of saponins by forming a stable 1 cm foam layer. Alkaloids were detected in Mayer's test, indicated by either a green colour or a white precipitate. Glycosides were confirmed in the chloroform and ammonium solution test, where a pink colour appeared. Terpenoids were identified by a red-brown colour at the interface during the chloroform and sulfuric acid test. Phenols were detected in the ferric chloride test by a blue or green colour. Steroids were confirmed in the chloroform and sulfuric acid test by the formation of a reddish-brown ring. Carbohydrates were identified using the α-naphthol test, marked by a red-violet ring, while the presence of starch was confirmed in the iodine test by a blue colouration.

2. Antioxidant Activity

The in-vitro antioxidant activity of *Clitoria ternatea* gel for caries removal was evaluated using DPPH [Table/Fig-2] and nitric acid assays [Table/Fig-3] across concentrations of 100-500 µg/mL. Both assays demonstrated a concentration-dependent increase in antioxidant activity, with the DPPH assay showing standard values ranging from 52.66 to 67.72 µg/mL and sample values ranging from 39.75 to 65.06 µg/mL.

S. No.	Concentration (µg/mL)	DPPH assay (Standard)	DPPH assay (*Clitoria ternatea* gel)	p-value
1	100	52.6625±0.20127	39.7525±0.03957	<0.01
2	200	53.5050±0.08196	43.9725±0.03957	<0.01
3	300	54.3342±0.04010	50.0017±0.04019	<0.01
4	400	59.0633±0.03869	53.6125±0.03957	<0.01
5	500	67.7233±0.03869	65.0600±0.04068	<0.01

[Table/Fig-2]: Intergroup comparison of DPPH assay between standard and *Clitoria ternatea* gel.
*p-value <0.05 was considered statistically significant

S. No.	Concentration (µg/mL)	Nitric acid assay (Standard)	Nitric acid assay (*Clitoria ternatea* gel)	p-value
1	100	18.5950±0.03606	7.4950±0.03606	<0.01
2	200	25.5825±0.03957	14.1625±0.03957	<0.01
3	300	34.8825±0.03957	25.0000±0.04068	<0.01
4	400	67.4475±0.04413	32.5025±0.03957	<0.01
5	500	74.4100±0.04068	39.9983±0.04019	<0.01

[Table/Fig-3]: Intergroup comparison of nitric acid assay between standard and *Clitoria ternatea* gel.
*p-value <0.05 was considered statistically significant

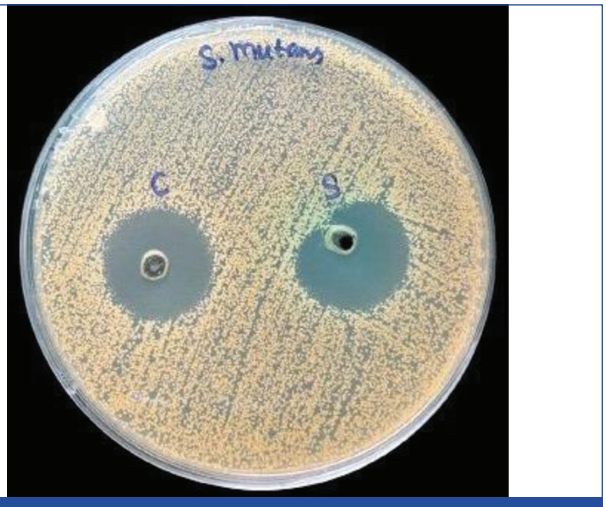
Similarly, in the nitric acid assay, antioxidant activity increased from 18.59 to 74.41 µg/mL in the standard and from 7.49 to 39.99 µg/mL in the sample. Low Standard Error of the Mean (SEM) values across all measurements confirmed data precision.

The intergroup comparison of the DPPH assay between the standard and *Clitoria ternatea* gel demonstrated a significant reduction in DPPH activity in the gel group across all tested concentrations (100-500 µg/mL) as shown in [Table/Fig-2]. The mean DPPH values in the gel group ranged from 39.7525±0.03957 to 65.0600±0.04068, whereas the standard group values ranged from 52.6625±0.20127 to 67.7233±0.03869, with a p-value of <0.01 for all concentrations, indicating statistically significant differences.

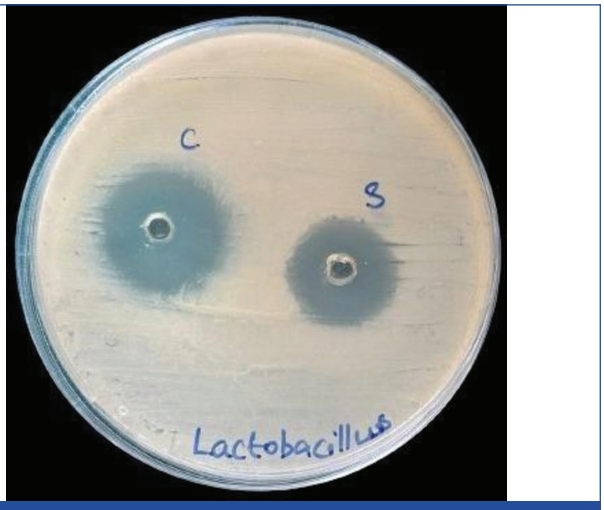
Similarly, the nitric acid assay revealed a notable reduction in activity in the *Clitoria ternatea* gel group when compared to the standard as presented in [Table/Fig-3]. The mean values for the gel group ranged from 7.4950±0.03606 to 39.9983±0.04019, while the standard group values ranged from 18.5950±0.03606 to 74.4100±0.04068, with all p-values being <0.01, confirming significant differences between the two groups.

3. Antimicrobial Activity

The antimicrobial activity of *Clitoria ternatea* gel was evaluated against *Streptococcus mutans* [Table/Fig-4], *Lactobacillus acidophilus* [Table/Fig-5] and *Enterococcus faecalis* [Table/Fig-6]. The intergroup comparison of mean inhibition zones between the control and *Clitoria ternatea* gel for the three bacterial strains is outlined in [Table/Fig-2].



[Table/Fig-4]: Mean inhibition zones for *Streptococcus mutans* using control (Carisolv Gel) and *Clitoria ternatea* Gel.

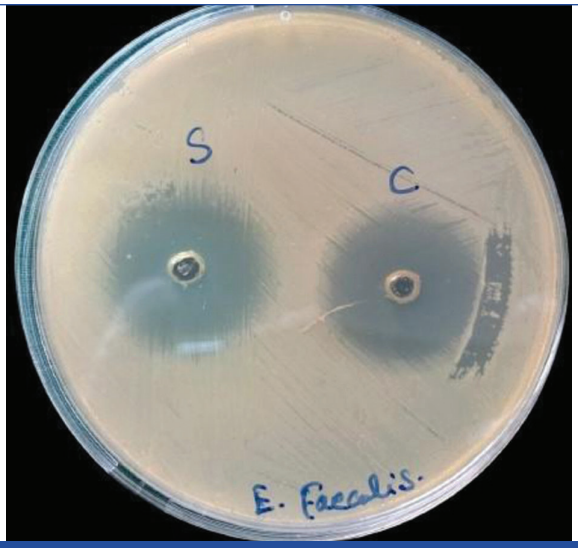


[Table/Fig-5]: Mean inhibition zones for lactobacillus using control (Carisolv Gel) and *Clitoria ternatea* Gel.

For *Streptococcus mutans*, the mean inhibition zone measured 22.013±0.048 mm in the control group and 18.004±0.044 mm in the gel group, with a significant mean difference of 4.0089 (p<0.01). In the case of *Lactobacillus acidophilus*, the control group showed a mean inhibition zone of 24.005±0.048 mm, whereas the gel group measured 19.005±0.042 mm, reflecting a mean difference of 5.0000 (p<0.01). For *Enterococcus faecalis*, the mean inhibition zone was 25.001±0.048 mm for the control and 22.008±0.046 mm for the gel, with a mean difference of 2.9925 (p<0.01). All differences were statistically significant [Table/Fig-7].

4. Anti-inflammatory Activity

The comparison between the standard and *Clitoria ternatea* gel across various concentrations (100 to 500 µg/mL) revealed significantly higher anti-inflammatory activity in the gel group, with p-values <0.001 for all comparisons. The consistently higher mean



[Table/Fig-6]: Mean inhibition zones for *Enterococcus faecalis* using control (Carisolv Gel) and *Clitoria ternatea* Gel.

S. No.	Zone of inhibition		Mean difference	p-value
	Control	Gel		
Streptococcus mutans	22.013±0.048	18.004±0.044	4.0089	<0.001
Lactobacillus	24.005±0.048	19.005±0.042	5	<0.001
Enterococcus faecalis	25.001±0.048	22.008±0.046	2.9925	<0.001

[Table/Fig-7]: [Intergroup comparison of mean inhibition zones between control and *Clitoria ternatea* gel (p<0.001).
*p-value <0.05 was considered statistically significant

values for *Clitoria ternatea* gel, combined with statistically significant p-values, indicate its superior anti-inflammatory potential across all tested concentrations [Table/Fig-8].

S. No.	Concentration (µg/mL)	Anti-inflammatory activity		Correlation (r)	p-value
		Standard	*Clitoria ternatea* gel		
1	100	7.96±0.199	16.36±0.019	0.882	<0.001
2	200	16.8±0.068	24.84±0.014	0.882	<0.001
3	300	27.4±0.067	41.8±0.009	0.882	<0.001
4	400	33.6±0.062	50±0.005	0.998	<0.001
5	500	47.7±0.049	58.78±0.005	0.998	<0.001

[Table/Fig-8]: Intergroup comparison between the standard and gel preparations.
*p-value <0.05 was considered statistically significant

The correlation values (r) indicate a strong positive relationship between the anti-inflammatory activity of the standard and *Clitoria ternatea* gel at different concentrations. For 100, 200 and 300 µg/mL, the correlation value is 0.882, reflecting a moderately strong correlation. At 400 and 500 µg/mL, the correlation value increases to 0.998, indicating a near-perfect positive correlation. This suggests that, while both treatments show increasing anti-inflammatory activity with concentration, *Clitoria ternatea* gel consistently demonstrates superior efficacy, especially at higher concentrations.

The multivariate analysis demonstrated a statistically significant effect of treatment on antioxidant, anti-inflammatory and antimicrobial activities (Wilks' Lambda=0.315, F=42.87, p<0.001; Pillai's Trace=0.685, F=45.32, p<0.001). The *Clitoria ternatea* gel exhibited significantly higher anti-inflammatory activity compared to the standard (p<0.001), indicating its potential effectiveness as a CMCR agent. However, its antimicrobial efficacy was lower, as reflected by smaller inhibition zones for S. mutans, L. bacillus and E. faecalis (p<0.001). These results suggest that, while the gel is promising for non invasive caries removal, further optimisation may enhance its antimicrobial properties for improved clinical application [Table/Fig-9].

S. No.	Variable	F-value	p-value	Interpretation
1	Antioxidant activity (DPPH Assay)	45.23	<0.001	Significant difference between standard and *Clitoria ternatea* gel, with gel showing less antioxidant activity as compared to control
2	Anti-inflammatory activity	52.76	<0.001	Significant correlation between concentration and activity; *Clitoria ternatea* gel exhibited stronger anti-inflammatory effects.
3	Antimicrobial activity (S. mutans)	38.12	<0.001	*Clitoria ternatea* gel showed reduced inhibition zone compared to standard, indicating lower antimicrobial efficacy.
4	Antimicrobial activity (L. bacillus)	41.89	<0.001	Significant reduction in inhibition zone for *Clitoria ternatea* gel compared to standard.
5	Antimicrobial activity (E. faecalis)	34.56	<0.001	*Clitoria ternatea* gel had lower antimicrobial action than the standard treatment.

[Table/Fig-9]: Multivariate analysis of antimicrobial, antioxidant and anti-inflammatory activities of *Clitoria ternatea* Gel.

DISCUSSION

The present study demonstrates a concentration-dependent increase in antioxidant activity, suggesting that the gel's antioxidant properties help protect oral tissues by neutralising harmful free radicals, thereby reducing oxidative stress and facilitating caries removal during dental procedures. Nanoparticle-based antimicrobial strategies in oral care, combined with advanced caries removal agents, have the potential to shape the future of dentistry by protecting oral tissues, neutralising harmful free radicals, reducing oxidative stress and enhancing the effectiveness of caries removal during dental procedures [30].

These study findings are consistent with research by Jeyaraj EJ et al., which also reported notable antioxidant activity in *C. ternatea* flower extracts obtained through both solvent and water extractions [31]. Their study highlighted kaempferol hexosyl-rhamnosyl-rhamnoside as a key bioactive compound contributing to the antioxidant activity. Similarly, Shiau SY et al., found strong antioxidant effects from *C. ternatea* flower extracts, with the highest activity resulting from hot water extraction, supporting the gel's antioxidant potential for oral applications [32]. Additionally, Adisakwattana S et al., investigated the antioxidant effects of anthocyanins, specifically delphinidin derivatives, in *C. ternatea* flower-based beverages, demonstrating its potential for oxidative protection beyond oral applications, extending to food preservation [33].

Furthermore, Jaafar NF et al., optimised extraction techniques to maximise phenolic content and antioxidant activity in *C. ternatea* flowers, showing effectiveness at 37% ethanol concentration and 45°C, which suggests potential for food and nutraceutical uses [34]. Collectively, these studies support the broad applicability of *C. ternatea* as an antioxidant, reinforcing the value of the gel's antioxidant properties in dental care while also highlighting the flower's versatility for use in food, health and nutraceutical products.

The antimicrobial properties of *Clitoria ternatea* gel have been investigated in several studies, highlighting its potential in both general and dental healthcare. For example, Jeyaraj EJ et al., explored the antibacterial effects of anthocyanin-rich flower extracts, which showed activity against *Bacillus cereus*, *Bacillus subtilis* and *Escherichia coli* [35]. The antibacterial efficacy of *Clitoria ternatea* against oral pathogens like *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis* supports its potential as a caries-preventive agent. Since these bacteria are major contributors to dental caries, the gel's ability to inhibit their growth highlights its promising role in dental care [36]. This aligns with the current study's findings, reinforcing the potential application of *Clitoria ternatea* gel in managing oral microbial infections and improving oral health outcomes.

Deorankar P et al., investigated ethanolic and aqueous root extracts of *Clitoria ternatea* and found the ethanol extract to have broader antimicrobial activity against various bacteria, such as Staphylococcus aureus and *E. coli*, as well as fungi like *Aspergillus niger* and *Candida albicans* [37]. This suggests that the plant's root extracts have a wider spectrum of action than the gel, which is more focused on oral pathogens. Similarly, the research by Islam MA et al., demonstrated the biofilm-inhibiting and antibacterial properties of methanolic extracts from the leaves and flowers, highlighting the plant's ability to combat antibiotic-resistant bacteria [38].

While these studies reveal the diverse antimicrobial potential of *Clitoria ternatea*, the gel's specific effectiveness against oral bacteria positions it as a valuable tool in dental applications. The strong antibacterial effects observed in the present study, particularly against *S. mutans*, *Lactobacillus* and *E. faecalis*, support its potential as a CMCR agent. The gel's ability to inhibit bacterial growth in carious lesions suggests that it could offer a less invasive and more targeted approach to treating dental caries, potentially enhancing traditional caries removal methods.

The current study on *Clitoria ternatea* gel demonstrates its significant potential as an anti-inflammatory agent, with promising applications in both dental care and skin healing. In comparison to studies by Snehil S and Vicky B and Harsh S and Rachit K, which also investigated the anti-inflammatory effects of *Clitoria ternatea* in gel formulations, the results of this study reinforce the plant's therapeutic benefits. Snehil S and Vicky B's research highlighted the sustained release and anti-inflammatory activity of gels containing *Clitoria ternatea* and *Salvia officinalis*, showing significant effects in reducing inflammation without cytotoxicity [39,40]. Similarly, Harsh S and Rachit K's study on emulgel formulations with *Clitoria ternatea* and thyme oil found dose-dependent reductions in inflammatory markers, showcasing the plant's role in managing inflammation [40]. These findings align with the current study, which also demonstrates a dose-dependent anti-inflammatory effect, especially at higher concentrations of the gel. Specifically, acidic formulations of *Clitoria ternatea* gel may serve as an effective chemomechanical agent for caries removal, reducing inflammation in carious lesions and surrounding tissues, which is critical for enhancing patient comfort and improving the overall outcome of dental treatments.

In the present study, *Clitoria ternatea* gel was formulated with an acidic pH (pH 2) to evaluate its potential in preventing dental caries by enhancing its anti-inflammatory properties. This approach contrasts with the traditional alkaline formulation used for treating wounds. The gel contains bioactive compounds such as flavonoids, thymol and carvacrol, which are known for their anti-inflammatory effects. These compounds contribute to the gel's therapeutic potential in oral health applications, aligning with previous research highlighting the plant's anti-inflammatory benefits [41].

Clitoria ternatea gel demonstrates significant potential as a CMCR agent for children's dental care, offering antioxidant, antimicrobial and anti-inflammatory benefits that reduce oxidative stress, promote tissue healing and combat oral pathogens like *Streptococcus mutans* and *Lactobacillus acidophilus*. The gel's anti-inflammatory effects make it a natural alternative for treating oral conditions such as gingivitis and ulcers. Its vibrant blue colour not only engages children but also calms their anxiety, improving patient cooperation during dental visits. Additionally, the gel's versatility could extend beyond dental care, offering therapeutic benefits in managing skin inflammation and promoting wound healing.

Limitation(s)

The present study was conducted in-vitro and the results may not directly apply to clinical practice, highlighting the need for in-vivo studies and Randomised Controlled Trials (RCTs) for further validation. The long-term safety and cytotoxic effects of *Clitoria ternatea* gel were not evaluated, necessitating biocompatibility testing. Although the gel exhibited notable antimicrobial activity, its effectiveness against a wider range of oral pathogens remains unexplored. Additionally, the study did not assess the depth of caries penetration or compare its performance with conventional CMCR agents. The small sample size also limits the broader applicability of the findings. Future research should focus on clinical trials, cytotoxicity evaluations and comparative studies to establish the gel's potential for oral healthcare use.

CONCLUSION(S)

Clitoria ternatea gel shows strong clinical promise as a minimally invasive treatment for caries management, making it an essential option for patients seeking gentler dental care. The gel's antioxidant properties protect oral tissues by reducing oxidative damage, which may enhance healing and lower post-treatment discomfort. Its antimicrobial action effectively targets cariogenic bacteria, including *Streptococcus mutans*, *Lactobacillus acidophilus* and *Enterococcus faecalis*, potentially reducing bacterial load and the risk of caries progression. The gel's anti-inflammatory properties can help reduce discomfort and promote healing during non invasive caries removal procedures, such as CMCR, by minimising caries progression in the affected dentine tissues. By selectively removing only infected tissue and preserving healthy dentine, *Clitoria ternatea* gel may improve clinical outcomes, enhance patient comfort and decrease the need for invasive procedures. Future clinical studies are recommended to optimise application methods, assess long-term efficacy and evaluate patient satisfaction.

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PARTICULARS OF CONTRIBUTORS:
1. Postgraduate Student, Department of Paediatric Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India.
2. Assitant Professor, Department of Paediatric Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. R Ramesh,
Assitant Professor, Department of Paediatric Dentistry, Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai-600077, Tamil Nadu, India.
E-mail: rameshr.sdc@saveetha.com

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