

FORMULATION AND EVALUATION OF HERBAL ANTIMICROBIAL GEL CONTAINING APRAJITA *CLITORIA TERNATEA L.*

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ABSTRACT

Skin infections caused by pathogenic microorganisms remain a major healthcare concern, and the increasing prevalence of antimicrobial resistance has highlighted the need for safer and more effective topical therapies. Herbal medicines have gained considerable attention because of their broad therapeutic potential, low toxicity, and improved patient acceptability. The present study aimed to formulate and evaluate a herbal antimicrobial gel containing the ethanolic extract of *Clitoria ternatea* L. (Aprajita). Fresh flowers of *Clitoria ternatea* were collected, authenticated, shade-dried, powdered, and extracted using ethanol. The extract was incorporated into a Carbopol-based gel using suitable excipients to obtain a stable topical formulation. The prepared gel was evaluated for its physicochemical properties, including appearance, homogeneity, pH, viscosity, spreadability, extrudability, washability, and stability. Antimicrobial activity was assessed

against selected pathogenic microorganisms using a standard microbiological method. The formulated gel exhibited acceptable physical characteristics, good consistency, suitable pH for topical application, satisfactory spreadability, and excellent stability throughout the study period. The formulation demonstrated promising antimicrobial activity, indicating the presence of bioactive phytoconstituents in the ethanolic extract of *Clitoria ternatea*. These findings suggest that the developed herbal gel has potential as a safe and effective topical antimicrobial formulation for the management of superficial skin infections. Further preclinical and clinical investigations are recommended to establish its therapeutic efficacy and long-term safety.

KEYWORDS: *Clitoria ternatea* L., Aprajita, ethanolic extract, herbal gel, antimicrobial activity, topical formulation, phytochemical.

INTRODUCTION

Skin infections caused by pathogenic microorganisms remain a significant public health concern worldwide. The increasing prevalence of antimicrobial resistance has reduced the effectiveness of many conventional antibiotics, creating an urgent need for safer and more effective alternative therapies. Herbal medicines have gained considerable attention because they contain diverse bioactive compounds with antimicrobial, antioxidant, and anti-inflammatory properties while generally exhibiting fewer adverse effects than synthetic drugs.^[1]

Clitoria ternatea L. (Aprajita), a medicinal plant belonging to the Fabaceae family, has been widely used in traditional systems of medicine for the management of various ailments. Different parts of the plant, particularly the flowers, are rich in phytoconstituents such as flavonoids, anthocyanins, phenolic compounds, tannins, and alkaloids. These bioactive constituents have been reported to possess antimicrobial, antioxidant, wound-healing, and anti-inflammatory activities, making the plant a promising candidate for topical therapeutic formulations.

Topical drug delivery systems, especially gels, offer several advantages over conventional dosage forms. Gels are non-greasy, easy to apply, provide better patient compliance, and facilitate uniform drug distribution on the skin. Their high water content produces a cooling effect, enhances comfort during application, and promotes prolonged contact between the

active ingredient and the affected area. These characteristics make gels an appropriate vehicle for delivering herbal extracts intended for the treatment of superficial microbial infections.^[2]

Ethanol extraction is commonly employed to obtain a broad spectrum of pharmacologically active phytochemicals from medicinal plants. The ethanolic extract of *Clitoria ternatea* has demonstrated inhibitory activity against several pathogenic microorganisms in previous studies, suggesting its potential as a natural antimicrobial agent. Incorporating this extract into a gel formulation may enhance its topical applicability while maintaining the biological activity of its phytoconstituents.^[3]

The present study focuses on the formulation and evaluation of a herbal antimicrobial gel containing the ethanolic extract of *Clitoria ternatea*. The developed formulation is evaluated for its physicochemical characteristics, including appearance, pH, viscosity, spreadability, homogeneity, and stability, along with its antimicrobial activity against selected microbial strains. The findings of this study may contribute to the development of an effective, safe, and plant-based topical antimicrobial preparation that could serve as a potential alternative to conventional synthetic product for the management of superficial microbial infection.^[4]

MATERIAL AND METHODS

1.1. Plant material collection and Authentication

Fresh flowers of *Clitoria ternatea* (Aprajita) were collected from healthy, mature plants during their flowering period in the local region of Bareilly, Uttar Pradesh, India. Only fresh and undamaged flowers were selected to ensure the quality of the plant material. After collection, the flowers were rinsed thoroughly with distilled water to remove dust and other foreign matter. They were then shade-dried at room temperature under adequate ventilation to preserve their bioactive constituents. Once completely dried, the flowers were ground into a fine powder using a mechanical grinder. The powdered material was stored in a clean, airtight container in a cool and dry environment until it was used for extraction and subsequent formulation studies.^[5]

The collected flower of *clitoria ternatea* L. were authenticated by the department of Botany, Mahatma Jyotiba Phule Rohilkhand University(MJPRU) Bareilly, Uttar Pradesh India. The Botanical identity of plant was confirmed by a qualified taxonomist based on standard taxonomic characteristics. The authentication was issued under Reference number-[RU/PS/PI/2025/314] date-[25/11/2025] The authenticated plant material was subsequently for the preparation of the ethanolic extract and formulation of herbal antimicrobial gel.^[6]

1.2. Preparation of Extract

The collected flowers of *Clitoria ternatea* L. (Aprajita) were washed with clean water, shade-dried, and ground into a coarse powder using a grinder. The powdered material was stored in an airtight container until use. About 100 g of the powder was placed in a Soxhlet apparatus and extracted with 95% ethanol. The extraction was continued until the solvent became colourless, indicating complete extraction of the plant constituents. The extract was then filtered to remove plant particles, and the ethanol was evaporated using a rotary evaporator to obtain a concentrated extract. The concentrated extract was further dried to obtain a semisolid mass, transferred to an airtight amber-coloured container, and store it.^[7]

METHODS

Formulation Procedure

Table 1: Formulation of ingredients *clitoria ternatea* herbal gel.

Ingredients	Quantity
Ethanollic extract	2.5g
Carbopol 934	0.5g
Propylene glycol	5g(humectant)
Methyl paraben	0.1g (preservative)
Triethanolamine	q.s
Distilled water	q.s

Carbopol 934 was slowly dispersed in the required quantity of purified water with continuous stirring to avoid the formation of lumps.^[8]

- The dispersion was allowed to stand for 24 hours to ensure complete swelling of the polymer and formation of a smooth gel base.^[9]
- Methyl paraben was dissolved separately and added to the gel base as a preservative.
- The ethanollic extract of *Clitoria ternatea* (Aprajita) was mixed with propylene glycol until a uniform solution was obtained.
- The extract mixture was added gradually to the hydrated Carbopol gel with continuous stirring to achieve uniform mixing.^[10]
- Triethanolamine was added dropwise to adjust the pH and convert the dispersion into a clear and homogeneous gel.
- The prepared gel was stirred gently until a smooth and uniform consistency was obtained.
- Finally, the gel was transferred into clean, airtight containers, properly labelled, and stored at room temperature for further evaluation.^[11]

EVALUATION

1. Physical evaluation

The herbal gel was evaluated for colour, consistency and odour. They appear light greenish yellow and opaque free from grittiness and homogenously dispersed.^[12]

2. PH

The pH of all the formulation herbal gel was measured by using pH paper.

3. Viscosity

Viscosity of herbal gel was determine by using Brookfield rotational viscometer at 100 rpm using spindle no. 64.^[13]

4. Homogeneity

Inhabitancy of any aggregates in the gel formulation was visually inspected and the homogeneity was approved.

5. Spreadability

The spreadability of gel formulations was determined by measuring the spreading diameter of 1g of between two horizontal plates.

6. Grittiness

All the formulations were evaluated microscopically for the presence of any appreciable particulate matter which was seen under light microscope. Hence, obviously the gel preparation fulfills the requirement of freedom from particulate matter form grittiness as desired for any topical preparation.^[14]

Antimicrobial activity

The antimicrobial potential of the prepared herbal gel was assessed by the disc diffusion technique against *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The formulation demonstrated a dose-dependent antibacterial effect, with the greatest zone of inhibition recorded at 200 mg/mL. The most pronounced activity was observed against *S. aureus* (11.17 ± 1.04 mm), followed by *E. coli* (10.00 ± 0.50 mm), *P. aeruginosa* (9.67 ± 0.29 mm), and *S. epidermidis* (9.33 ± 0.29 mm). Ciprofloxacin, used as the positive control, produced the highest inhibition, while methanol showed no antibacterial effect. Overall, the findings indicate that the formulation exhibits moderate antibacterial properties.^[15]

RESULT

Preliminary phytochemical screening

Table 2: Phytochemical screening.

Phytochemical Constituent	Results
Alkaloids	(+)
Flavonoids	(+)
Tannis	(+)
Saponins	(+)
Steroids	(+)
Phenol	(+)
Glycosides	(+)

Preliminary phytochemical screening of the ethanolic flower extract of *Clitoria ternatea* revealed the presence of alkaloids, flavonoids, tannins, saponins, steroids, and glycosides, which may be responsible for its antimicrobial and therapeutic activities.

Evaluation Results of Formulation Herbal Antimicrobial Gel

Table 3: Evaluation of herbal antimicrobial gel.

Parameter	Observation/Result
Physical Appearance	Smooth, homogeneous, blue-colored gel with characteristics odour
PH	6.21
Viscosity	50000cps
Spreadability	6.25g.cm/sec
Skin Irritation Test	No erythema, edema, or irritation observed
Washability	Easily washable with water
Stability study	No significant change in color, pH, homogeneity, or viscosity during storage

Discussion

The formulated herbal antimicrobial gel was evaluated for various physicochemical and performance parameters to determine its suitability as a topical formulation. The results indicated that the gel possessed acceptable characteristics for dermal application.

The gel exhibited a smooth, homogeneous, blue-colored appearance with a characteristic odor, indicating uniform distribution of ingredients and good aesthetic acceptability. Homogeneity is an important quality parameter as it ensures consistent drug distribution throughout the formulation.

The pH of the gel was found to be 6.21, which is close to the normal pH range of human skin (4.5–6.5). This suggests that the formulation is compatible with the skin and is unlikely to cause irritation or discomfort upon topical application.

The viscosity of the gel was 50,000 cps, indicating adequate consistency and rheological behavior. Appropriate viscosity helps the gel remain at the site of application for a longer duration, thereby enhancing contact time and therapeutic effectiveness. The spreadability value of 6.25 g·cm/sec demonstrated that the gel could be easily spread on the skin surface with minimal effort. Good spreadability is desirable for uniform application and patient compliance.

The skin irritation study revealed no signs of erythema, edema, or irritation, confirming the safety and biocompatibility of the formulation for topical use. This result indicates that the herbal ingredients and excipients used in the formulation are non-irritant in nature.

The gel showed excellent washability, as it could be easily removed with water. Easy washability enhances user convenience and improves patient acceptance of the formulation.

The stability study demonstrated that there were no significant changes in color, pH, homogeneity, or viscosity during the storage period. These findings indicate that the formulation remained physically and chemically stable under the tested storage conditions, suggesting a satisfactory shelf life.

Antimicrobial Activity

Table 4: Antibacterial activity of sample CTG/01, Ciprofloxacin (+ve control), Methanol (-ve control) against *S. epidermidis* using Disc Diffusion method.

Sample	Concentration (mg/ml)	R1(mm)	R2(mm)	R3(mm)	Mean $\pm$ SD (mm)
Herbal gel	200	9.0	9.5	9.5	9.33 $\pm$ 0.29
Herbal gel	100	8.0	8.0	9.0	8.33 $\pm$ 0.58
Herbal gel	50	7.0	8.0	9.0	8.00 $\pm$ 1.00
Concentration(mg/ml)	250ppm	30	30	30	30.00 $\pm$ 0.00
Blank Gel/Vehicle(-ve control)	-	Nil	Nil	Nil	Nil

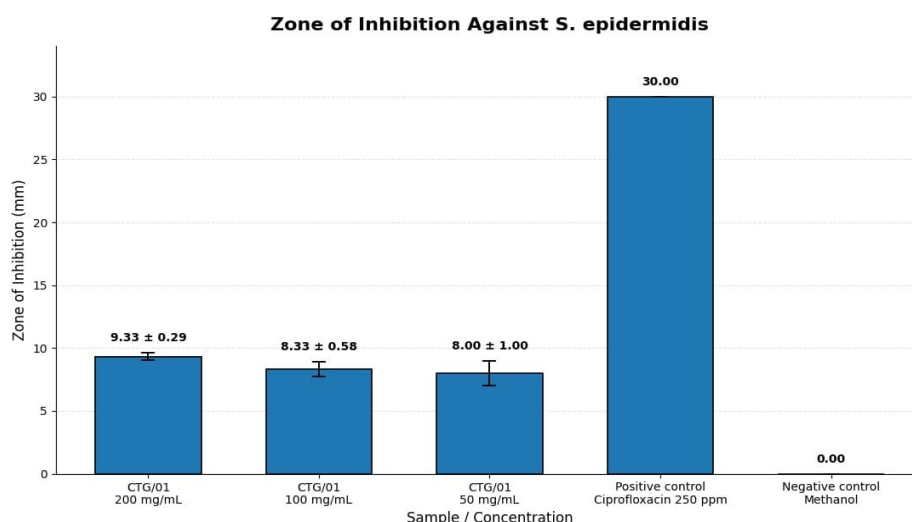


Figure: Graph represents the Antimicrobial Activity of Formulated Herbal Gel against *S. epidermidis*.

DISCUSSION

At a concentration of 200 mg/mL, the herbal gel showed the highest zone of inhibition (9.33 ± 0.29 mm), indicating effective inhibition of microbial growth. When the concentration was reduced to 100 mg/mL and 50 mg/mL, the mean zones of inhibition decreased to 8.33 ± 0.58 mm and 8.00 ± 1.00 mm, respectively. This trend suggests that the antimicrobial activity of the herbal gel is directly related to the concentration of the active phytoconstituents present in the formulation. The positive control, Ciprofloxacin (250 ppm), produced a significantly larger zone of inhibition (30.00 ± 0.00 mm), confirming the sensitivity of the test microorganism and validating the experimental procedure. The greater efficacy of Ciprofloxacin is expected because it is a potent broad-spectrum antibiotic with a purified active ingredient. In contrast, the blank gel (vehicle control) showed no zone of inhibition, indicating that the gel base itself possesses no antimicrobial activity. Therefore, the observed antimicrobial effect of the herbal gel can be attributed solely to the bioactive constituents present in the plant extract.

Table 5: Antibacterial activity of sample CTG/01, Ciprofloxacin (+ve control), Methanol (-ve control) against *Escherichia coli* using Disc Diffusion method.

Sample	Concentration (mg/ml)	R1	R2	R3	Mean $\pm$ SD (mm)
Herbal Gel	200	10.5	10.0	9.5	10.00 ± 0.50
Herbal Gel	100	9.5	9.5	9.0	9.33 ± 0.29
Herbal Gel	50	9.0	9.0	9.0	9.00 ± 0.00
Ciprofloxacin (+ve Control)	250ppm	25	25	26	25.33 ± 0.58

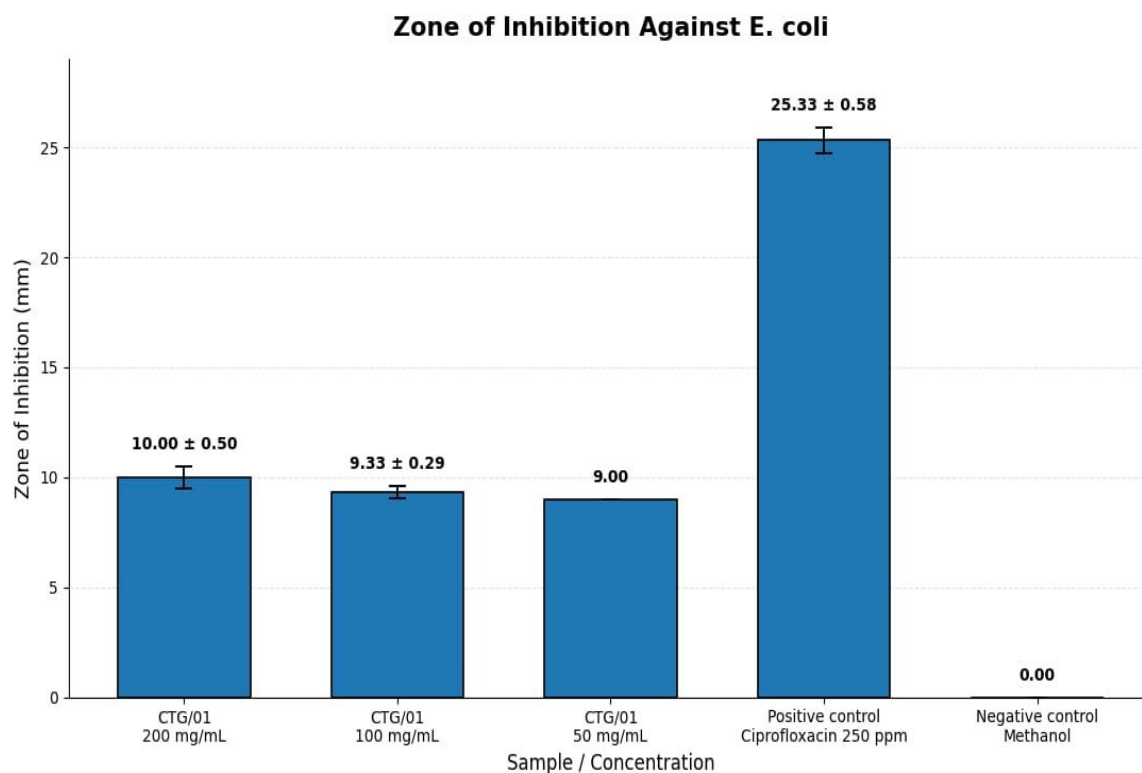


Figure: Graph represents the Antimicrobial Activity of Formulated Herbal Gel Against *Escherichia coli*.

Discussion

The herbal gel showed a concentration-dependent increase in antimicrobial activity. At the highest concentration (200 mg/mL), the gel produced the largest zone of inhibition (10.00 ± 0.50 mm), indicating greater antimicrobial effectiveness. At concentrations of 100 mg/mL and 50 mg/mL, the zones of inhibition were 9.33 ± 0.29 mm and 9.00 ± 0.00 mm, respectively. Although the increase in activity with concentration was moderate, the results suggest that higher concentrations of the herbal extract enhance the antimicrobial potential of the formulation. The positive control, Ciprofloxacin (250 ppm), exhibited a substantially larger zone of inhibition (25.33 ± 0.58 mm), demonstrating superior antimicrobial activity. This result was expected because Ciprofloxacin is a highly effective broad-spectrum antibiotic containing a purified active pharmaceutical ingredient. The significantly higher inhibition zone obtained with Ciprofloxacin validates the susceptibility of the test organism and confirms the reliability of the experimental method.

Table 6: Antibacterial activity of sample CTG/01, Ciprofloxacin (+ve control), Methanol (-ve control) against *Pseudomonas aeruginosa* using Disc Diffusion method.

Sample	Concentration (mg/ml)	R1	R2	R3	Mean $\pm$ SD (mm)
Herbal gel	200	9.5	10.0	9.5	9.67 ± 0.29
Herbal gel	100	8.5	9.0	9.0	8.83 ± 0.29
Herbal gel	50	8.0	8.5	8.0	8.17 ± 0.29
Ciprofloxacin (+ve Control)	250ppm	30	30	30	30.00 ± 0.00
Blank Gel/Vehicle (-ve Control)	Nil	Nil	Nil	Nil	Nil

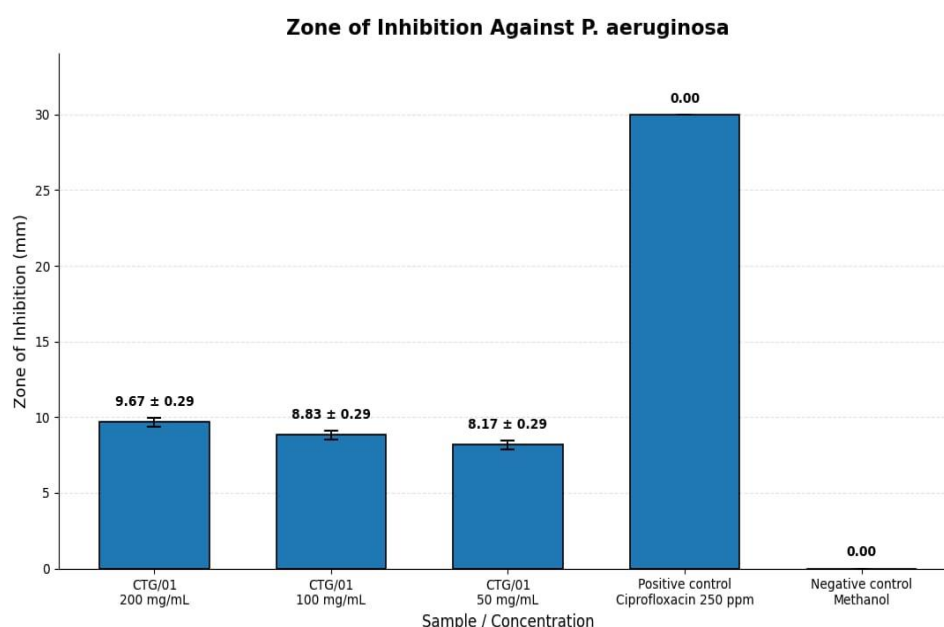


Figure: Graph represent the Antimicrobial Activity of Formulated Herbal Gel Against *Pseudomonas aeruginosa*.

Discussion

The herbal gel exhibited a concentration-dependent antimicrobial effect. The highest concentration (200 mg/mL) produced a mean zone of inhibition of 9.67 ± 0.29 mm, indicating the maximum antimicrobial activity among the tested concentrations. At 100 mg/mL, the gel showed a zone of inhibition of 8.83 ± 0.29 mm, while at 50 mg/mL, the inhibition zone was 8.17 ± 0.29 mm. The gradual increase in inhibition zone with increasing concentration suggests that the antimicrobial activity is influenced by the amount of bioactive phytoconstituents present in the formulation.

The positive control, Ciprofloxacin (250 ppm), exhibited a significantly larger zone of inhibition (30.00 ± 0.00 mm) compared to the herbal gel. This result was expected because

Ciprofloxacin is a potent broad-spectrum antibiotic with a highly purified active ingredient and well-established antimicrobial efficacy. The strong inhibition produced by the standard drug also confirms the sensitivity of the test microorganism and validates the experimental procedure.

Table 7: Antibacterial activity of sample CTG/01, Ciprofloxacin (+ve control), Methanol (-ve control) against *Staphylococcus aureus*_Disc Diffusion method.

Sample	Concentration (mg/ml)	R1(mm)	R2(mm)	R3(mm)	Mean $\pm$ SD (mm)
Herbal Gel	200	12.0	10.0	11.5	11.17 $\pm$ 1.04
Herbal Gel	100	10.0	10.0	10.5	10.16 $\pm$ 0.29
Herbal Gel	50	10.0	10.5	9.5	9.83 $\pm$ 0.76
Ciprofloxacin (+ve Control)	250ppm	26.5	26.5	26.0	26.17 $\pm$ 0.29
Blank Gel/Vehicle (-ve Control)	-	Nil	Nil	Nil	Nil

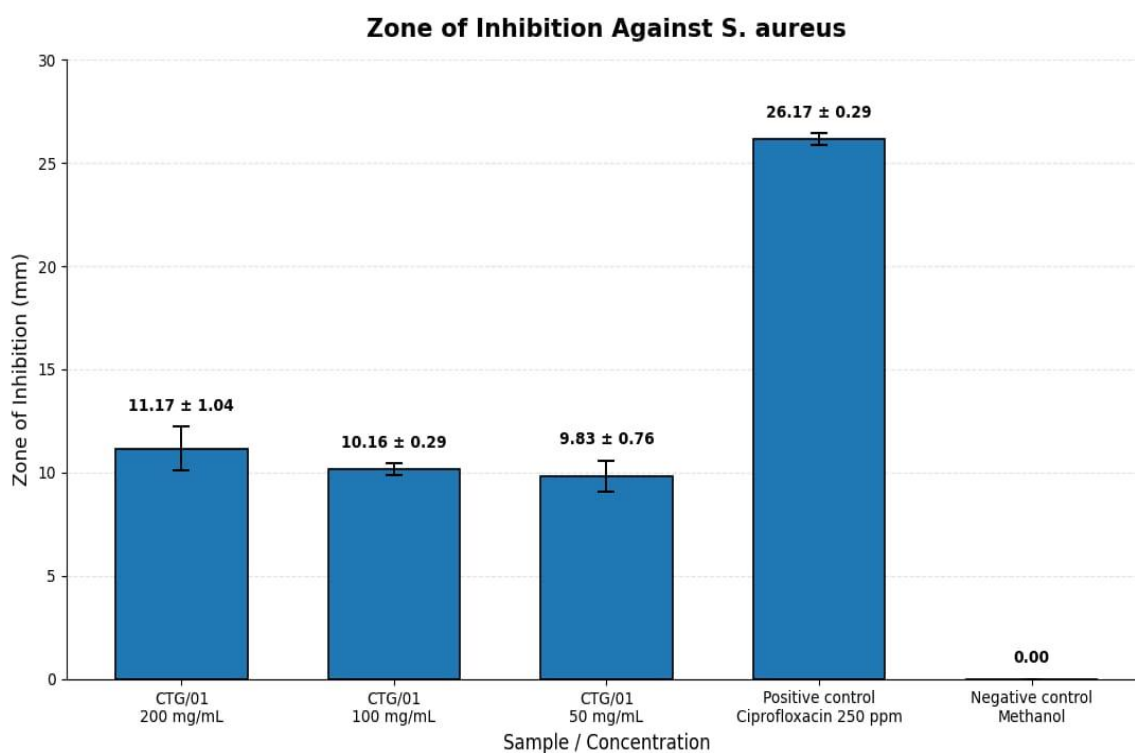


Figure: Graph represent the Antimicrobial Activity of Formulated Herbal Gel Against *Staphylococcus aureus*.

DISCUSSION

Among the tested concentrations, the herbal gel at 200 mg/mL showed the highest antimicrobial activity, producing a mean zone of inhibition of 11.17 $\pm$ 1.04 mm. At 100

mg/mL, the zone of inhibition was 10.16 ± 0.29 mm, while at 50 mg/mL, it was 9.83 ± 0.76 mm. The increase in inhibition zone with increasing concentration indicates a concentration-dependent antimicrobial effect, suggesting that higher amounts of phytoconstituents enhance microbial growth inhibition.

The standard drug, Ciprofloxacin (250 ppm), exhibited a considerably larger zone of inhibition (26.17 ± 0.29 mm) than the herbal gel at all tested concentrations. This result is expected because Ciprofloxacin is a potent synthetic antibiotic with strong bactericidal activity. The consistent inhibition zone produced by the positive control confirms the validity and reliability of the antimicrobial assay.



Figure: Antibacterial activity of sample CTG/01 of concentrations: 200mg/ml, 100mg/ml and 50mg/ml, against *S. epidermidis* using Disc Diffusion method; +ve control - Ciprofloxacin used at 250ppm, -ve control Methanol.



Figure: Antibacterial activity of sample CTG/01 of concentrations: 200mg/ml, 100mg/ml and 50mg/ml, against *E. coli* using Disc Diffusion method; +ve control - Ciprofloxacin used at 250ppm, -ve control Methanol.



Figure: Antibacterial activity of sample CTG/01 of concentrations: 200mg/ml, 100mg/ml and 50mg/ml, against *P. aeruginosa* using Disc Diffusion method; +ve control - Ciprofloxacin used at 250ppm, -ve control Methanol.



Figure: Antibacterial activity of sample CTG/01 of concentrations: 200mg/ml, 100mg/ml and 50mg/ml, against *S. aureus* using Disc Diffusion method; +ve control - Ciprofloxacin used at 250ppm, -ve control Methanol.

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