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FORMULATION AND EVALUATION OF ANTIMICROBIAL GEL FROM *Plectranthusamboinicus* (LOUR) SPRENG LEAVES EXTRACT

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ABSTRACT
This study explores the formulation and evaluation of an antimicrobial gel derived from *Plectranthusamboinicus* (Lour) Spreng leaves extract. With a rising global incidence of microbial infections, effective treatments are crucial, especially against pathogens like *Staphylococcus aureus* and *Escherichia coli* that cause both local and systemic infections. *Plectranthusamboinicus*, known for its historical use in traditional medicine for its potential antimicrobial and anti-inflammatory properties. The gel formulation involved incorporating the plant extract into a carboxymethyl cellulose (CMC) base, followed by evaluations include physicochemical parameters such as pH (5.8), appearance (brown), viscosity (7215cps), and spreadability (3.9 gm.cm/sec) were assessed to ensure suitability for topical application. Antimicrobial efficacy was evaluated using Minimum Inhibitory Concentration (MIC) and Zone of Inhibition assays against *E. coli* and *S. aureus*, demonstrating inhibition at concentrations above 500 µg/ml. The gel also exhibited significant anti-inflammatory activity compared to diclofenac, a standard anti-inflammatory drug, across various concentrations. Phytochemical analysis via HPLC identified gallic acid as a major component, contributing to the gel's therapeutic potential. *Plectranthusamboinicus* as a promising source

of natural antimicrobial agents, potentially offering safer alternatives to synthetic drugs with reduced side effects.
KEYWORDS: *Plectranthusamboinicus*, Antibacterial, Anti-inflammatory, Gallic Acid

I.INTRODUCTION

Increasing global population and connectivity heighten the risk of microbial infections, affecting skin, lungs, brain, and other body parts. Commonly caused by organisms like *S. aureus*, these infections enter through breaks in the skin, such as insect bites. Skin infections like impetigo and boils are widespread, with systemic infections also displaying skin symptoms. The threat persists due to reduced immunity and increased susceptibility among individuals.

Antimicrobials are vital in modern medicine, reducing and curing diseases, enabling technological advancements in therapies. They hinder microbe growth or destroy them, including antiviral, antibacterial, antifungal, and chemotherapy drugs [1].

Synthetic drugs cause short-term issues like anxiety and seizures, and long-term problems such as memory loss. They can also become less effective due to resistance. Herbal medicines, safer and with fewer side effects, are preferred for chronic conditions and health promotion. Their popularity rises due to dissatisfaction with synthetic treatments.

Plectranthusamboinicus, a plant in the Lamiaceae family, has a rich history in disease treatment and food use. Its botanical name refers to Ambon, an island in Indonesia. The plant spread to East Indies, Africa, and Latin America. Known as "oregano de la Hoja Ancha," it can be found in rainforests of Indonesia and Malaysia. It's popular globally as a low-maintenance house plant.s

Coleus aromaticus, now classified under the genus *Plectranthus*, is a robust perennial herb belonging to the Lamiaceae family (formerly known as Labiatae). It boasts a considerable size, reaching heights between 30 to 90 cm, characterized by its thick, succulent stems and leaves. The plant is highly branched, and its fleshy leaves emit a distinctive, aromatic fragrance. The plant, prevalent throughout India, is not only a native species but is also commonly cultivated in gardens. Its popularity stems from the delightful combination of taste and aroma found in its leaves. Widely used in culinary applications, these leaves serve as excellent flavor enhancers for meat and fish dishes, elevating the overall taste. Furthermore, their utility extends to masking unpleasant odors, making them versatile in various culinary contexts.

Given its unique properties and potential contributions to the culinary world, there exists a significant scope for research on the herb's applications in the food industry. Investigating its various attributes and potential uses could lead to valuable insights and innovations in enhancing flavors and addressing odor-related challenges in food preparation [2].

Classification:

- Division: Magnoliophyta
- Kingdom: Plantae
- Clade: Angiosperms
- Class: Magnoliopsida
- Order: Lamiales
- Family: Lamiaceae
- Genus: Plectranthus
- Species: Coleus aromaticus
- Synonym: Coleus ambonicus Lour.

This classification system provides a hierarchical structure, ranging from broader categories like division and kingdom to more specific ones like genus and species. It helps in organizing and categorizing living organisms based on their evolutionary relationships.

Figure 1: Plectranthusamboinicus (LOUR)

This medicinal plant, often used in syrup, is effective against conditions like flu, bronchitis, and epilepsy. Phytochemical analysis identifies flavonoids, including apigenin, luteolin, and salvigenin [3].

Macroscopic characters

Leaf:

Green, highly aromatic leaves, simple and fleshy, triangular or ovate, with crenate margins and glandular hairs beneath. Max size: 6.5cm x 6cm.

Stem:

Green to pink, aromatic stem, slightly bitter to acrid taste, 70-80cm in length, fleshy stems with rigid hairs or tomentose covering.

Root:

Brown, aromatic roots, slightly bitter taste, extended up to 15-20cm.

Microscopic characters

Leaf: Transverse section reveals dorsiventral features with upper epidermis, differentiated mesophyll, and plano-convex midrib; diacytic stomata present.

Stem: Circular section displays multicellular trichomes, flattened epidermal cells, cortex with chlorenchyma, parenchyma, and collateral vascular bundles; xylem comprises metaxylem and protoxylem.

Root: Matured root exhibits a circular outline with stratified cork, distinct phellogen, secondary phloem with sieve tubes, companion cells, parenchyma, and a bulk of secondary xylem [4].

Phytochemicals

The essential oil of *P. amboinicus* contains key compounds, notably carvacrol (28.65%) and thymol (21.66%), along with a range of other constituents like a-humulene, undecanal, gamma terpinene, p-cymene, caryophyllene oxide, a-terpineol, and beta sellanene. Carvacrol and thymol, major phenolic compounds, contribute to pharmaceutical and culinary properties. The variation in compound levels is attributed to extraction methods, soil, seasons, climate, genetics, and geography.

Hexane extraction yielded the highest oil content (1.40%) compared to steam distillation (0.55%) and supercritical carbon dioxide extraction. Non-volatile constituents include phenolic acids (caffeic, gallic, p-coumaric, rosmarinic, salvianolic, shimbobashiric), flavonoids (chrysoeriol, cirsimaritin, eriodictyol, luteolin, rutin, salvigenin, thymoquinone, quercetin, 5-O-methyl-luteolin, 3,5,7,3’,4’-pentahydroxy flavonone, 4’,5,7-trihydroxy flavone), monoterpene hydrocarbons, sesquiterpene hydrocarbons, and esters [5].

Pharmacological activities

This herb, deeply rooted in tradition, is valued for its effective, low-side-effect wound healing and therapeutic properties. Widely used in formulations, it treats various ailments and is cultivated for anthelmintic activity. Its leaves, consumed raw or as flavoring agents, exhibit promise in addressing respiratory, cardiovascular, oral, skin, digestive, and urinary diseases. HPLC with an isocratic method identifies compounds, while MIC and zone of inhibition assess antibacterial efficacy. UV spectrophotometry, known for accuracy, is employed in pharmaceutical analysis, including quantifying anti-inflammatory drugs [6].

II. MATERIALS AND METHODS

Materials, equipments and chemicals required

Extractof *Plectranthusamboinicus.* [CLSBNSD/2185/141122],Carboxyl methyl cellulose (CMC), Dimethyl sulfoxide (DMSO) [manufactured by RFCL. Ltd. product code-D0170],Nutrient agar [manufactured by HI Media Laboratories Pvt. Ltd.], Muller Hinton agar [manufactured by Micro express], Ciprofloxacin [manufactured by Ives Drugs Pvt. Ltd], E. coli strain [NCIM2687], Staphylococcus aureus strain [NCIM2079], Disodium hydrogen phosphate [manufactured by s d fine-chem. Ltd], Egg albumin. Wattman filter paper, Micropipettes, Petridish, Ointment tile, UV Spectrophotometer, Autoclave, Incubator, Laminar air flow, Hot air oven, HPLC, weighing balance, pH meter, Brookfield Viscometer.

Method of preparation of gel

- **Preparation of CMC Gel:**

CMC (Carboxymethyl Cellulose) was first immersed in water, after which CMC and water were combined at a 3.25% concentration. The mixture was then stirred for 10 minutes at a speed of 800 rpm. This process resulted in the formation of an elastic gel, ensuring thorough mixing and proper hydration of the CMC, which led to its gel-like consistency.

- **Preparation of *P.amboinicus* Gel:**

1g of *P. amboinicus* extract was added to a 3.25% CMC gel using geometric dilution. This mixture was then divided into four parts. First, 0.25g of the extract was mixed with 25g of the gel on an ointment tile using geometric dilution. The remaining parts were then combined using the fusion method. Finally, all four parts were mixed together to prepare 100ml of *P. amboinicus* gel. Other excipients trimethylamine, glycerine and sodium benzoate were also added with continuous stirring.(7)

Evaluation Parameters for gel formulation

1. Physical evaluation

Physical appearance and Homogeneity: The prepared gel was visually examined for colour, homogeneity, and consistency.

pH determination: The pH of the formulations was determined using a digital pH meter at room temperature. This involved immersing the electrode directly into the gel formulation to obtain an accurate reading. Monitoring the pH is crucial as it can impact the stability, efficacy, and compatibility of the formulation with the skin or intended application surface.(8)

Viscosity: The viscosity of the formulations was assessed using a Brookfield Viscometer (DV-2PL) equipped with Spindle no-L6 operating at a speed of 10 revolutions per minute (rpm), and the viscosity measurements were recorded in centipoises (cps) (9)

Spreadability: A predetermined quantity of prepared gel (1g) was placed in between two horizontal plates measuring 20cm x 20cm. A standard weight of 500g was placed on the upper

plate. After a lapse of 20 minutes, the diameter of the resulting circle was measured in centimetres.(10)

2. Determination of gallic acid by HPLC Method:

Deionized water, methanol, and acetic acid were used as the mobile phase and passed through a silica stationary phase at a flow rate of 1 ml/min during the estimation of gallic acid concentration in P. amboinicus using HPLC. A Diode Array Detector was utilized, configured to a wavelength of 270 nm, and measurements at this wavelength were recorded to determine the presence and quantity of gallic acid in the sample.(11)(12)

3. Determination of Antibacterial Activity using Minimum Inhibitory Concentration Test:

In the determination of antibacterial activity using the minimum inhibitory concentration (MIC) , test organisms were cultivated in Muller-Hinton broth due to its consistency in supporting rapid growth of pathogenic bacteria. The microdilution method was employed in standard test tubes, each containing sterilized broth prepared in water. The sample, consisting of 0.2 grams of extract dissolved in 10 ml of Dimethyl sulfoxide (DMSO), was prepared at a concentration of 2 mg/ml. Subsequently, eight test tubes were utilized, each containing 1000 µl of broth. The sample was added to the first tube, with subsequent dilutions prepared for the following tubes to achieve concentrations of 1000 µg/ml, 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.50 µg/ml, and 31.25 µg/ml. Two additional tubes were included as positive (+ve) and negative (-ve) controls. The test tubes were sealed with cotton plugs and incubated under aseptic conditions for 18-24 hours. The -vetube served as a blank without culture, while the +ve tube contained only broth and the bacterial strain, ensuring consistent and reliable conditions for assessing the minimum inhibitory concentration of the sample against the test organisms.(13)(14)

4. Determination of Antibacterial Activity Using Zone of Inhibition Method:

Muller-Hinton Agar was prepared by dissolving 3.8g in 100ml of water, and then Petri dishes, agar, cotton, and 45 discs were sterilized in an autoclave for 15 minutes. In an aseptic area, 1ml of E. coli was introduced into the Petri dish, and 1/4th of the dish was filled with agar, both spread evenly. Three sets of 15 discs each were prepared: one set was poured with 15µl of ciprofloxacin (2mg/ml) as the standard. For the second set, 0.1g of plain gel (CMC+water) was dissolved in 100ml of water, and 30µl was poured onto the discs. The third set involved mixing 1g of gel containing the drug with 100ml of water, and 250µl was poured onto these discs. The dried discs were then placed into the Petri dish containing agar and E. coli, followed by incubation at 37.1°C for 24 hours. After 24 hours, the results were observed.(15)(16)

5. Determination of Anti-Inflammatory Activity Using UV Spectrophotometer:

Diclofenac standard solutions and gel samples were prepared in varying concentrations, ranging from 1000µg/ml to 0.01µg/ml, using serial dilutions in water. A phosphate buffer solution 6.8 Ph was prepared. Additionally, a 1% egg albumin solution was made by mixing egg albumin with water. Test tubes labeled with diclofenac concentrations and gel samples received diclofenac dilution, followed by phosphate buffer and egg albumin solution. After incubation at 37°C for 30 minutes and a brief heat treatment at 70°C for 5 minutes, the samples were cooled for 15 minutes. This preparation aimed to standardize conditions for UV spectroscopy analysis of diclofenac's effects in gel formulations. (17)(18)All the samples were Analysed for absorbance at 660nm and percentage inhibition was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}}$$

III. RESULTS:

1. Evaluation Parameters for gel formulation

The parameters provide important insights into the physical properties and potential performance of the antimicrobial gel formulated from *Plectranthusamboinicus* leaves extract. The acidic pH, brown appearance, high viscosity, and good spreadability are all favorable characteristics for a topical gel intended for antimicrobial application.

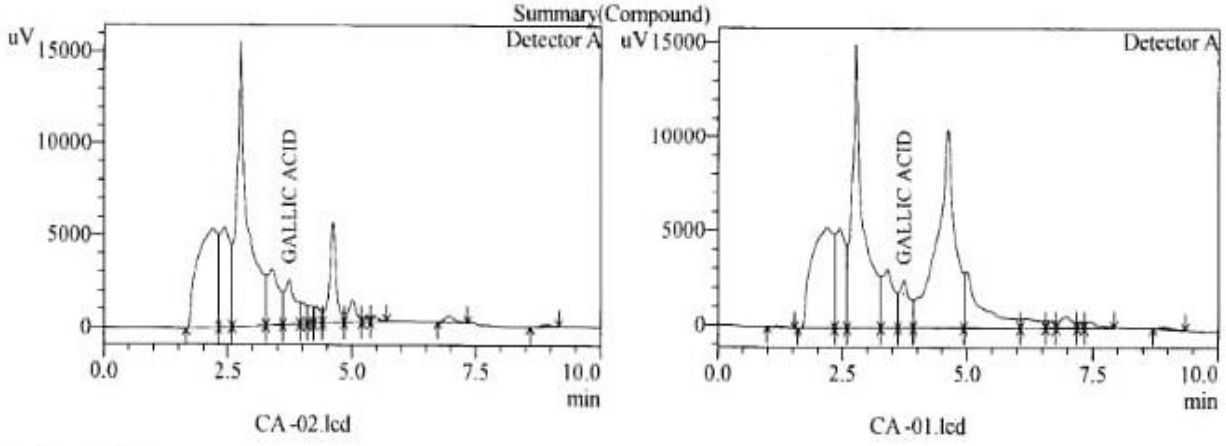
The pH of 5.8 indicates that the gel formulation is slightly acidic. This pH range is typically suitable for topical applications, as it is close to the natural pH of the skin, helping to maintain skin barrier function and compatibility. The viscosity of 7215.5 cps (centipoise), the gel exhibits a relatively high viscosity. This characteristic is important for ensuring that the gel maintains its consistency and spreads evenly. The spreadability of 3.9 gm.cm/sec indicates easily the gel can be spread over the skin surface.The results are shown in table no.1

Table 1: Evaluation parameters for gel formulation (pH, Viscosity and Spreadability)

Evaluation parameters	results
pH	5.8
Appearance	Brown
Viscosity	7215 cps
Spreadability	3.9gm.cm/sec

2. Determination of gallic acid by HPLC Method:

The HPLC analysis determined the presence of gallicacid in the extract was confirmed by the peaks and retention time with the standard are shown in the figureno 2a and 2b



<< Detector A >>
ID#1 Compound Name: GALLIC ACID

Title	Ret. Time	Area	Tailing Factor	Theoretical Plate#
CA-02.lcd	3.387	49125	0.000	192
CA-01.lcd	3.390	50997	0.000	201
Average	3.389	50061	0.000	197
%RSD	0.063	2.644	0.000	3.239

Figure 2a: HPLC of gallic acid in extract

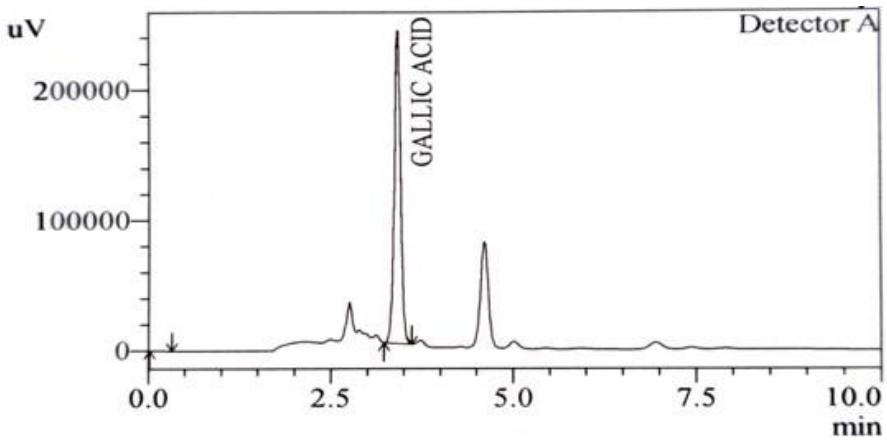


Figure 3b: HPLC of standard gallic acid

3. Determination of Antibacterial Activity using Minimum Inhibitory Concentration Test:
The results suggest that the antimicrobial gel formulated from *Plectranthusamboinicus* leaves extract possesses moderate antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The MIC values indicate that the gel is effective in inhibiting bacterial growth at a concentration of 500 µg/ml, which is promising for its potential use as an antibacterial agent. However, the zone of inhibition results indicates that the gel's efficacy in creating clear zones of inhibition was less pronounced compared to the standard antibiotic, ciprofloxacin.

Table 2: Antibacterial Activity using Minimum Inhibitory Concentration Test

Bacteria	**Minimum Inhibitory Concentration**
Escherichia coli	500µg/ml
Staphylococcus aureus	500µg/ml
Staphylococcus aureus	500µg/ml

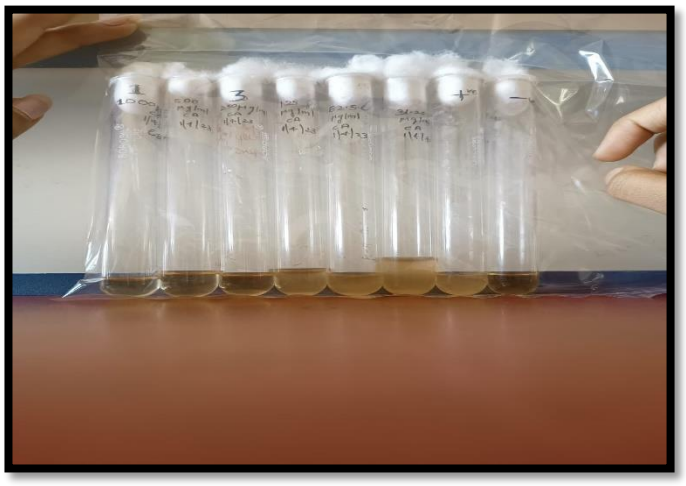


Figure 4: MIC of *Escherichia coli* (-ve)

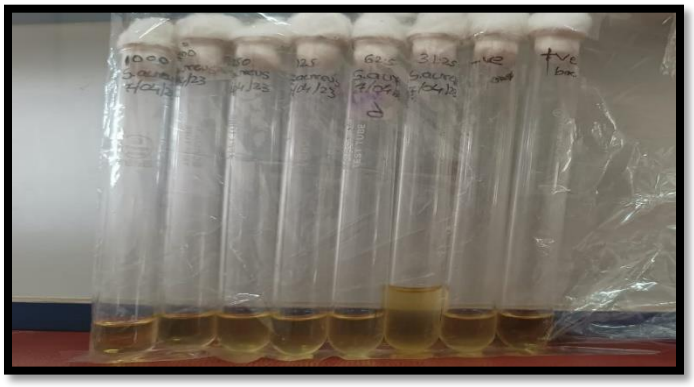


Figure 5*:* MIC of *Staphylococcus aureus* (+ve)

4. Determination of Antibacterial Activity Using Zone of Inhibition Method:

The results showed that Ciprofloxacin, used as the standard, had a ZOI of 12 mm, indicating strong antibacterial activity. The gel containing the drug exhibited a ZOI of less than 12 mm, indicating it has antibacterial properties, but is less effective than Ciprofloxacin. The gel without the drug had a ZOI of less than 5 mm, suggesting minimal antibacterial activity from the gel base alone.

Table 3: Antibacterial Activity Using Zone of Inhibition Method

Sample	**Zone Of Inhibition(mm)**
Ciprofloxacin (std)	12mm
Gel	<12mm
Gel without drug	<5mm

Figure 6: Zone of Inhibition using *E. coli* strain

5. Determination of Anti-Inflammatory Activity Using UV Spectrophotometer:

The formulation of the antimicrobial gel from *Plectranthusamboinicus* (Lour) Spreng leaves extract was evaluated for its anti-inflammatory activity. The inhibition percentages of the gel were compared with those of diclofenac, a standard anti-inflammatory drug, at various concentrations as shown in table

Table 4: Anti-Inflammatory Activity Using UV Spectrophotometer

Concentrations	% Inhibition of Diclofenac	% Inhibition of gel
0.01µg/ml	41.79%	62.68%
0.1µg/ml	44.77%	64.17%
1µg/ml	47.76%	65.6%
10µg/ml	50.74%	68.65%
100µg/ml	58.2%	70.14%
1000µg/ml	61.19%	71.64%

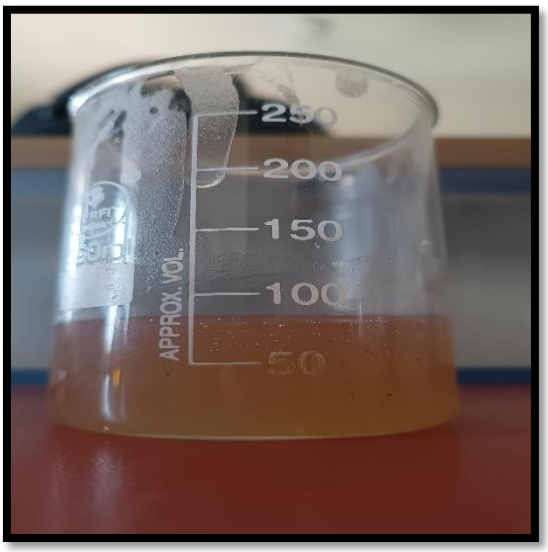


Figure 7: *P. amboinicus* gel

IV.DISCUSSION

Plectranthusamboinicus (Lour.) Spreng, supported by literature on its chemical constituents like asiaticosides, triterpenoids, saponins, and phenolic compounds, known for their potent antimicrobial and anti-inflammatory effects. High-Performance Liquid Chromatography (HPLC) analysis confirmed the presence of gallic acid as a major component in the gel. The concentration of gallic acid is important as it is a known phenolic compound with significant antimicrobial and anti-inflammatory properties, contributing to the overall effectiveness of the gel.The gel formulation displayed several favorable physical characteristics with pH of 5.8 suggests that the gel is slightly acidic, which is compatible with the natural pH of the skin, ensuring that it maintains the skin barrier function without causing irritation. The brown appearance is due to the natural color of the plant extract and does not inherently affect the gel's performance. The high viscosity of 7215 cps ensures that the gel maintains a consistent and

stable form, which is crucial for even application and sustained contact with the skin. Good spreadability, measured at 3.9 gm.cm/sec, indicates that the gel can be easily applied over the skin surface, enhancing therapeutic efficacy.(19)(20)

The Minimum Inhibitory Concentration (MIC) tests demonstrated that the gel is effective in inhibiting the growth of *Escherichia coli* and *Staphylococcus aureus* at a concentration of 500 µg/ml. This suggests that the gel possesses moderate antibacterial activity, making it a promising candidate for treating bacterial infections. However, the Zone of Inhibition (ZOI) method showed that while the gel exhibited antibacterial properties, its efficacy was less than that of the standard antibiotic ciprofloxacin, which had a ZOI of 12 mm. The gel containing the drug showed a ZOI of less than 12 mm, and the gel without the drug showed minimal antibacterial activity (less than 5 mm), indicating that the active antibacterial effect is primarily due to the drug component in the gel.(21)

The anti-inflammatory activity of the gel was evaluated using the egg albumin method and compared with diclofenac, a standard anti-inflammatory drug. The results showed that the gel had a higher percentage inhibition of inflammation across various concentrations, compared to diclofenac. At the highest concentration tested (1000 µg/ml), the gel exhibited 71.64% inhibition, compared to 61.19% for diclofenac. This suggests that the gel has potent anti-inflammatory properties, which could be beneficial for treating inflammatory conditions.

V. CONCLUSION

The antimicrobial gel formulated from *Plectranthusamboinicus* (Lour) Spreng leaves extract demonstrated favorable characteristics for topical application, including a suitable pH (5.8), high viscosity (7215 cps), and good spreadability (3.9 gm.cm/sec). HPLC analysis confirmed gallic acid as a major component, contributing to the gel's therapeutic potential.The gel exhibited moderate antibacterial activity against Escherichia coli and Staphylococcus aureus with an MIC of 500 µg/ml. However, its efficacy in the zone of inhibition assay was lower than that of ciprofloxacin. Additionally, the gel showed significant anti-inflammatory activity, surpassing diclofenac in percentage inhibition across various concentrations. The gel demonstrated a higher percentage inhibition of inflammation, reaching up to 71.64% at the highest concentration (1000 µg/ml), compared to 61.19% for diclofenac. This highlights the gel's potent anti-inflammatory properties, which are beneficial for treating inflammatory conditions.

Overall, the antimicrobial gel formulated from *Plectranthusamboinicus* leaves extract shows as a natural therapeutic agent with moderate antibacterial and significant anti-inflammatory properties. With a potential safer alternative to synthetic drugs, offering reduced side effects and contributing to the ongoing search for effective natural antimicrobial agents and anti-inflammatory treatment.

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