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Research

A Comparative and Synergistic study of *Ficus religiosa* and *Lantana camara* plant extracts on Antibacterial activity against Gram+ve and Gram-ve bacteria.

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Abstract

This study investigates the comparative and synergistic antibacterial activity of *Ficus religiosa* and *Lantana camara* plant extracts against two clinically significant bacterial strains: *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). Both plants are traditionally used in herbal medicine and are known to possess bioactive compounds such as flavonoids, tannins, alkaloids, and essential oils with documented antimicrobial properties. Methanolic and aqueous extracts of the leaves and bark (for *Ficus religiosa*) and leaves and flowers (for *Lantana camara*) were prepared using standard extraction methods. Antibacterial activity was assessed using the agar well diffusion method, and the zone of inhibition was measured to evaluate efficacy. Results indicated that both plants exhibited significant antibacterial effects individually, with *Ficus religiosa* showing stronger activity against *Staphylococcus aureus* and *Lantana camara* demonstrating notable inhibition against *E. coli*. Interestingly, when used in combination, the extracts exhibited a synergistic effect, resulting in enhanced antibacterial activity against both pathogens. These findings suggest the potential of these plant extracts, particularly in combination, as natural antibacterial agents and support further investigation for possible use in developing herbal-based antimicrobial formulations.

Keywords: Antibacterial activity, *Escherichia coli*, *Ficus religiosa*, *Lantana camara*, Medicinal plants, Phytochemicals, Plant extracts, Synergistic effect.

INTRODUCTION

The rise in antimicrobial resistance has significantly challenged modern medicine, necessitating the search for novel and more effective antimicrobial agents, particularly from natural sources. Medicinal plants have been historically utilized for their therapeutic properties, and recent scientific advances have renewed interest in their potential as alternatives or adjuncts to conventional antibiotics. In this context, the present study explores the comparative and synergistic antibacterial activity of *Ficus religiosa* and *Lantana camara*-two ethnomedicinally important plant species-against two clinically significant bacterial pathogens: *Escherichia coli* (a Gram-negative bacterium) and *Staphylococcus aureus* (a Gram-positive bacterium).[1] *Ficus religiosa*, commonly known as the sacred fig or "Peepal tree," is widely recognized in traditional medicine for its antimicrobial, anti-inflammatory, and wound-healing properties. Similarly, *Lantana camara*, although considered invasive in some ecosystems, is known for its broad spectrum of bioactive compounds with antibacterial and antifungal effects. The presence of phytochemicals such as flavonoids, alkaloids, tannins, and saponins in both plants is believed to contribute to their antimicrobial properties.[2,3] This study aims to evaluate and compare the individual antibacterial efficacy of each plant extract as well as investigate any potential synergistic effects when used in combination. Special emphasis is placed on *E. coli* and *S. aureus* due to their medical relevance and differing cell wall structures, which influence susceptibility to plant-derived compounds. By assessing zone of inhibition and determining the minimum inhibitory concentration (MIC), this investigation contributes to the growing body of evidence supporting plant-based antimicrobials in combating resistant bacterial infection.[4,5]

Bacterial disease

Bacterial diseases are infections caused by pathogenic bacteria-microorganisms that can invade the body, multiply, and produce toxins or harmful immune responses.[6] These diseases range from mild to life-threatening conditions and affect various parts of the body including the respiratory tract, gastrointestinal system, skin, urinary tract, and bloodstream. Bacterial diseases remain a major public health concern globally, especially with the rise of antibiotic resistance.[7]

Bacteria are single-celled prokaryotic organisms that come in various shapes-cocci (spherical), bacilli (rod-shaped), and spirilla (spiral). While many bacteria are harmless or even beneficial, pathogenic species can cause serious diseases in humans and animals. They reproduce rapidly and can be transmitted through air, water, food, physical contact, or vectors.[8]

Common Bacterial Diseases:

- Tuberculosis (TB)
- Typhoid fever
- Cholera
- Pneumonia
- Urinary tract infections
- Staphylococcal Infections.[9]

Antibacterial agents markedly available

Table 1: Marketed drugs for antibacterial activity.[10]

Generic Name	Brand name	Uses
Amoxicillin	Amoxil, Mox	Respiratory, dental, and urinary tract infections
Ciprofloxacin	Cipro, Ciplox	UTIs, GI infections, respiratory infections
Azithromycin	Zithromax, Azithral	Throat infections, pneumonia, STDs
Cefixime	Suprax, Zifi	Typhoid, UTIs, respiratory infections
Doxycycline	Doxicip, doxy-1	Acne, malaria, STDs, zoonoses
Rifampicin	Rifandin, R-cin	Tuberculosis, leprosy
Meropenem	meronem	Severe hospital-acquired infections

MATERIALS AND METHODS

Collection and identification of plant materials
Fresh and healthy leaves of *Ficus religiosa* and *Lantana camara* were collected from local area of Dundigal.

Authentication
The ariel parts of plants were collected nearby surroundings of Marri Laxman Reddy Institute of Pharmacy, at Dundigal, Hyderabad. Both the plants were authenticated by Mrs. R. Naveena, Assistant professor in the department of Pharmacognosy at Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad. The plants herbarium was submitted in the college for future reference purpose.



Fig 1*: Ficus religiosa*



Fig 2: *Lantana camara*

Processing of *Lantana camara*
Washing: The leaved and barks are washed with water to remove any microbial contamination.[11]
Drying: The cleaned leaves are kept for drying process for about one week under shade.



Fig 3: Dried leaves of *Lantana camara*

Grinding: The dried leaves are kept for grinding using grinder at laboratory level.



Fig 4: Grinded powder of *Lantana camara*

Maceration: The powder obtained is kept for maceration with three different solvents [pet ether, water, methanol] for 24hrs. [12]



Fig 5: Maceration with water, methanol and Pet ether

Processing of *Ficus religiosa:*
Washing: The leaved and barks are washed with water to remove any microbial contamination.
Drying: The cleaned leaves are kept for drying process for about one week under shade.



Fig 6: Dried leaves of *Ficus Religiosa*

Grinding: The dried leaves are kept for grinding using grinder at laboratory level.



Fig 7: Grinded powder of *Ficus religiosa*

Maceration: The powder obtained is kept for maceration with three different solvents [pet ether, water, methanol] for 24hrs.







Fig 8: Maceration with methanol **Fig 9**: **Maceration with water** **Fig 10: Maceration with pet ether**

Filtration

To obtain the solvent extract of two plants it is kept for filtration process after maceration process.





Fig 11: **Filtrate of *Lantana camara*** **Fig 12**: **Filtrate of *Ficus religiosa***

Evaporation

The evaporation method involves removing the solvent from a plant extract after extraction (usually methanol, pet ether, or water) to obtain a concentrated crude extract**.**[13]

Phytochemical screening

The chemical extracts were screened with different phytochemical constituents with different chemical elements using standard procedure: Alkaloids, saponin glycosides, Cardiac glycosides, flavonoids, Tannins, Phenols, Steroids and triterpenoids, Amino acids, Carbohydrates, volatile oils, proteins. [14]

Antibacterial Screening

Zone of inhibition

The Zone of Inhibition Assay, also known as the Agar Well Diffusion Method, is a widely used technique to evaluate the antibacterial activity of plant extracts. The materials required for this procedure include the plant extracts of *Lantana camara* and *Ficus religiosa*, labeled as Sample- 1 and Sample-2, respectively. [15] Agar plates serve as the medium for bacterial growth, while bacterial cultures of *Escherichia coli* (a Gram-negative strain) and *Staphylococcus aureus* (a Gram-positive strain) are used as test organisms. Additional materials include sterile cotton swabs for spreading the bacterial inoculum, a sterile cork borer or micropipette for preparing wells in the agar, and an incubator set at 37°C to promote bacterial growth. Positive controls (such as standard antibiotics) and negative controls (such as solvents) are also included to validate the results and ensure experimental

reliability. [16]

Steps

Preparation of Inoculum

The preparation of inoculum is a crucial step in antibacterial testing. In this process, *Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus)* are first cultured in nutrient broth for 18 to 24 hours to ensure optimal bacterial growth. Following incubation, the turbidity of the bacterial suspension is adjusted to match the 0.5 McFarland standard, which corresponds to an approximate concentration of 1.5×10^8 colony-forming units per millilitre (CFU/mL). This standardization ensures uniform bacterial density, allowing for consistent and reliable results in subsequent antimicrobial assays. [17-19]

Inoculation of Plates

The inoculation of plates is carried out after preparing the bacterial inoculum. First, sterilized Mueller-Hinton Agar is poured into Petri dishes and allowed to solidify at room temperature. Once the agar has set, a sterile cotton swab is dipped into the standardized bacterial suspension, and the culture is evenly spread across the entire surface of the agar plate to ensure a uniform bacterial lawn. This step is essential for accurately assessing the antimicrobial activity of test substances in the following stages of the assay.[20-23]

Well Preparation

The well preparation step involves creating uniform wells in the solidified agar to hold the test samples. Using a sterile cork borer, wells approximately 6 mm in diameter are carefully punched into the agar surface. Each well is then properly labeled to distinguish between different plant extracts and control samples (positive and negative). This setup allows for clear identification and comparison of the antimicrobial effects exhibited by each sample during the assay. [24-27]

Application of Extracts

In the next step, the prepared wells are carefully filled with 50– 100 µL of the test samples. Sample-1, containing the extract of *Lantana camara*, and Sample-2, containing the extract of *Ficus religiosa*, are introduced into their respective labelled wells. Additionally, a positive control (usually a standard antibiotic) and a negative control (the solvent used for extraction) are also included to validate the results. These controls are added in balanced volumes to ensure consistency and to enable accurate comparison of the antibacterial activity exhibited by the plant extracts. [28]

Incubation

After loading the wells with the plant extracts and control solutions, the prepared agar plates are incubated at 37°C for 24 hours. This incubation period allows the bacteria to grow uniformly across the surface of the agar, while the test substances diffuse from the wells into the surrounding medium. Any antibacterial activity present in the samples will inhibit bacterial growth, leading to the formation of clear zones around the wells, which are assessed in the next step. [29]

Measurement of Zone of Inhibition

Following the 24-hour incubation period, the agar plates are examined for zones of inhibition, which appear as clear, circular areas surrounding the wells where bacterial growth has been suppressed. The diameter of each zone is carefully measured in millimetres using a ruler, providing a quantitative assessment of the antibacterial activity of each plant extract and control. Larger zones indicate stronger antimicrobial effects, while the absence of a zone suggests little to no activity. These measurements are critical for comparing the efficacy of the tested substances. [30]

RESULTS AND DISCUSSION

Phytochemical tests of arial parts of *Ficus religiosa*

The phytochemical analysis of the aerial parts of *Ficus religiosa* using water, methanol, and petroleum ether extracts revealed the presence of several important phytoconstituents. Alkaloids were detected in all three extracts, though the specific tests showed varying results across solvents. Cardiac glycosides were present in water and methanol extracts but absent in petroleum ether. Saponin glycosides were found in the water and petroleum ether extracts, while flavonoids and mucilage were absent in all. [31] Tannins and phenolic compounds were detected mainly in methanol and to some extent in water, but were absent in petroleum ether for tannins. Steroids and triterpenoids showed consistent presence across all solvents except for a negative result in the Salkowski's test for the petroleum ether extract. Amino acids and carbohydrates were observed primarily in water and methanol extracts, while volatile oils and proteins were present in all three. These findings confirm the presence of a broad

range of bioactive compounds in *Ficus religiosa*, supporting its traditional medicinal use. [32]

Phytochemical tests of areal parts of *Lantana camara*

The phytochemical screening of the aerial parts of *Lantana camara* using water, methanol, and petroleum ether extracts revealed the presence of various bioactive compounds. Alkaloids were detected differently across the solvents: the water extract showed positive results for Dragendorff's and Mayer's tests, methanol was positive for Mayer's test only, while petroleum ether showed a positive result in the Tannic acid test.[33] Cardiac glycosides were present only in the water extract. Saponin glycosides were also found exclusively in the water extract. Flavonoids were detected in the methanol extract, while mucilage was absent in all three. Tannins and phenolic compounds were strongly present in water and methanol extracts and partially in petroleum ether. Steroids and triterpenoids were confirmed in all extracts by the Liebermann- Burchard test, though the Salkowski's test was negative for both methanol and petroleum ether. Amino acids were mainly found in the water extract, while carbohydrates were present in all extracts, with reducing sugars detected only in methanol.[34] Volatile oils were confirmed in all three solvents, and proteins were present in water and methanol extracts but absent in petroleum ether. These findings highlight the rich phytochemical profile of *Lantana camara*, supporting its potential medicinal value.[35]

Table 2: Phytochemical tests of arial parts of *Ficus religiosa* [36]

Phytoconstituents and chemical tests	Positive indication	water	Methanol	Pet ether
Alkaloids:				
Dragendorff's test:	Reddish brown ppt	Positive	Positive	Positive
Mayer's test:	Cream color ppt	Positive	Negative	Negative
Tannic acid test:	Buff color ppt	Negative	Negative	Positive
Cardiac glycosides:				
Legal's test:	Blood red color	Positive	Positive	Negative
Baljet's test:	Orange color	Positive	Positive	Negative
Saponin glycosides:				
Foam test**:**	Formation of foam	Positive	Negative	Positive
Mucilage:				
Ruthenium red test:	Pink color	Negative	Negative	Negative
Flavonoids: Shinoda test:	Crimson red / green to blue color	Negative	Negative	Negative
Tannins:				
Ferric chloride test:	Blue or Green color	Negative	Positive	Negative
Gelatin test:	White ppt	Positive	Positive	Negative
Phenolic compounds:				
Ferric chloride test:	Blue or green color	Negative	Positive	Positive
lead acetate test:	White ppt	Positive	Positive	Positive
Steroids and triterpenoids:				
Liebermann Burchard test:	Brown ring	Positive	Positive	Positive
Salkowski's test:	Red colour	positive	Positive	Negative
Amino acids:				
Ninhydrin test:	Violet colour	Positive	Positive	Negative
Millon's test:	White ppt	Negative	Positive	Negative
Carbohydrates:				
Molisch's test:	Violet colour	Positive	Positive	Positive
Barfoed's test:	Brick red colour	Negative	Positive	Negative
Volatile oils: Sudan red 3 test:	Appearance of oil globules	Positive	Positive	Positive
Proteins:				
Biuret test:	Violet colour	Positive	Positive	Positive

Table 3: Phytochemical tests of arial parts *Lantana camara* [37-39]

Phytoconstituents and chemical tests	Positive indication	water	Methanol	Pet ether
Alkaloids:				
Dragendorff's test:	Reddish brown ppt	Positive	Negative	Negative
Mayer's test:	Cream color ppt	Positive	Positive	Positive
Tannic acid test:	Buff color ppt	Negative	Negative	Positive
Cardiac glycosides:				
Legal's test:	Blood red color	Positive	Negative	Negative
Baljet's test:	orange color	Positive	Negative	Negative
Saponin glycosides:				
Foam test**:**	Formation of foam	Positive	Negative	Negative
Mucilage:				
Ruthenium red test:	Pink color	Negative	Negative	Negative
Flavonoids:	Crimson red/ green			
Shinoda test:	to blue color	Negative	Positive	Negative
Tannins:				
3. Ferric chloride test:	Blue or Green color	Positive	Positive	Positive
Gelatin test:	White ppt	Positive	Positive	Negative
Phenolic compounds:				
3. Ferric chloride test:	Blue or green color	Positive	Positive	Positive
lead acetate test:	White ppt	Positive	Positive	Positive
Steroids and triterpenoids:				
3. Liebermann Burchard test:	Brown ring Red color	Positive	Positive	Positive
Salkowski's test:		positive	Negative	Negative
Amino acids:				
Ninhydrin test:	Violet color	Positive	Positive	Negative
Millon's test:	White ppt	Positive	Negative	Negative
Carbohydrates:				
Molisch's test:	Violet color	Positive	Positive	Positive
Barfoed's test:	Brick red color	Negative	Positive	negative
Volatile oils:	Appearance of			
Sudan red 3 test:	oilglobules	Positive	Positive	Positive
Proteins:				
Biuret test:	Violet color	Positive	Positive	Negative

Anti-bacterial screening

Zone of inhibition of plant extracts and its combination on Gram Positive Bacteria- *Staphylococcus aureus*

The antibacterial activity of *Lantana camara*, *Ficus religiosa*, their combinations in various ratios, and the standard antibiotic Amoxicillin was evaluated against *Staphylococcus aureus* using the agar well diffusion method. Ethanol, used as a control, showed no zone of inhibition, confirming its inactivity. Individually, *Lantana camara* exhibited moderate antibacterial activity with a 1.6 cm zone of inhibition, while *Ficus religiosa* showed weaker activity with a 0.6 cm zone. Amoxicillin displayed a 1.4 cm zone, validating the test method.[40] Among the combinations, the 8:2 and 2:8 ratios (Samples b and h) demonstrated the highest antibacterial activity with 2 cm zones of inhibition, surpassing even the standard drug. Other combinations showed varying degrees of effectiveness, with the equal ratio (5:5) also exhibiting good synergistic potential. These results suggest that specific ratios of *Lantana camara* and *Ficus religiosa* extracts have enhanced antibacterial activity, indicating promising synergistic effects**.**

Zone of inhibition of plant extracts and its combination on Gram Negative Bacteria-E. coli

The antibacterial activity of *Lantana camara*, *Ficus religiosa*, their combinations in various ratios, and the standard antibiotic Amoxicillin was tested against *E. coli* using the agar well diffusion method. Ethanol, used as a control, showed no inhibitory effect. Among individual extracts, *Lantana camara* demonstrated the strongest antibacterial activity with a 2.0 cm zone of inhibition, followed by *Ficus religiosa* with 1.4 cm, while Amoxicillin produced a 1.2 cm zone. In combination studies, the 9:1 and 8:2 ratios (Samples a and b) showed notable antibacterial effects (1.4 cm zones), slightly exceeding that of the standard drug. However, as the proportion of

Ficus religiosa increased in the mixtures, the antibacterial activity declined, with the 1:9 ratio showing the weakest effect (0.2 cm). Overall, *Lantana camara* alone and its dominant combinations with *Ficus religiosa* exhibited promising antibacterial potential against *E. coli*, while higher concentrations of *Ficus religiosa* reduced efficacy. Gram-negative bacteria culture. [41]

Table 4: Results For Gram positive bacteria

S.no.	Sample	Composition	Taken Ratios	Radius of zone of inhibition	Diameter of Zone of inhibition
1	Blank:	Ethanol	--	0	0
2	Sample-1	*Lantana camara*	--	0.8cm	1.6
3	Sample-2	*Ficus religiosa*	--	0.3cm	0.6
4	Standard:	Amoxicillin	--	0.7cm	1.4
5	Sample -a	*L.camara: F. Religiose*	9:1	0.5cm	1
6	Sample-b	*L.camara: F. Religiose*	8:2	1.0cm	2
7	Sample-c	*L.camara: F. Religiose*	7:3	0.8cm	1.6
8	Sample-d	*L.camara: F. Religiose*	6:4	0.8cm	1.6
9	Sample-e	*L.camara: F. Religiose*	5:5	0.9cm	1.8
10	Sample-f	*L.camara: F. Religiose*	4:6	0.5cm	1
11	Sample-g	*L.camara: F. Religiose*	3:7	0.6cm	1.2
12	Sample-h	*L.camara: F. Religiose*	2:8	1.0cm	2
13	Sample-i	*L.camara: F. Religiose*	1:9	0.5cm	1

Table 5: Results For gram negative bacteria

Sl.no.	Sample	Composition	Taken Ratios	Radius of zone of inhibition	Diameter of Zone of inhibition
1	Blank:	Ethanol	--	0	0
2	Sample-1	*Lantana camara*	--	1.0	2
3	Sample-2	*Ficus religiosa*	--	0.7	1.4
4	Standard:	Amoxicillin	--	0.6	1.2
5	Sample -a	*Lcamara: F. Religiose*	9:1	0.7	1.4
6	Sample-b	*L.camara: F. Religiose*	8:2	0.7	1.4
7	Sample-c	*L.camara: F. Religiose*	7:3	0.4	0.8
8	Sample -d	*L.camara: F. Religiose*	6:4	0.6	1.2
9	Sample-e	*L.camara: F. Religiose*	5:5	0.2	0.4
10	Sample-f	*L.camara: F. Religiose*	4:6	0.2	0.4
11	Sample-g	*L.camara: F. Religiose*	3:7	0.5	1
12	Sample-h	*L.camara: F. Religiose*	2:8	0.2	0.4
13	Sample-i	*L.camara: F. Religiose*	1:9	0.1	0.2

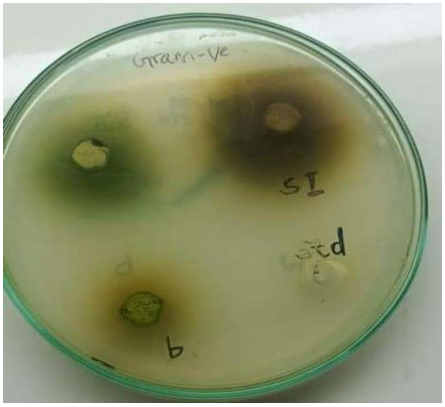

Fig 13: Zone of inhibition of Plant extracts with sample-1, sample- 2, standard drug and sample-b on gram-negative bacteria

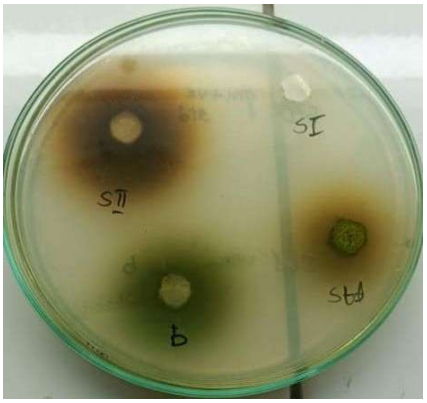

Fig 14: Zone of inhibition of Plant extracts with sample-1, sample- 2, standard drug and sample-b on gram-positive bacteria



Fig 15: Zone of inhibition of Plant extracts with sample-h, sample- d, Sample-i and Blank on gram-positive bacteria

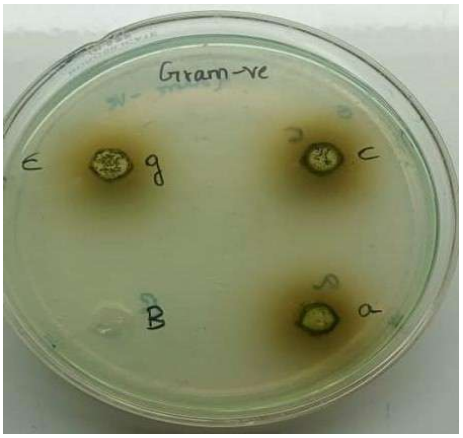

Fig 16: Zone of inhibition of Plant extracts with sample-g, sample- c, Sample-a and Blank on gram-negative bacteria

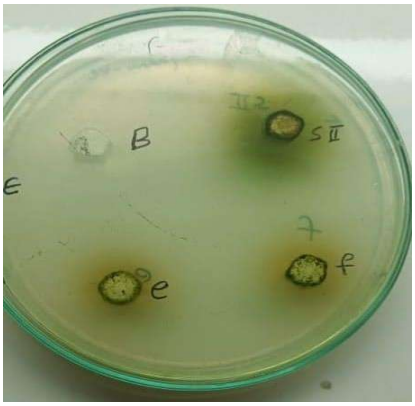

Fig 17: Zone of inhibition of Plant extracts with sample-2, sample- e, Sample-f and Blank on gram-negative bacteria

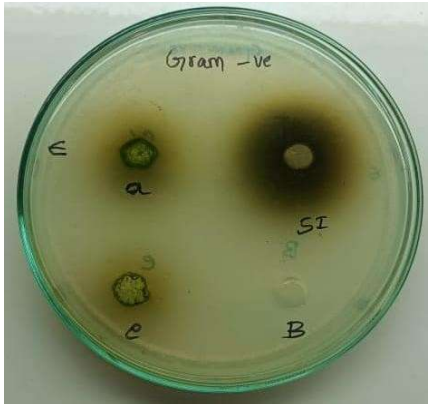

Fig 18: Zone of inhibition of Plant extracts with sample-1, sample- a, Sample-e and Blank on gram-negative bacteria

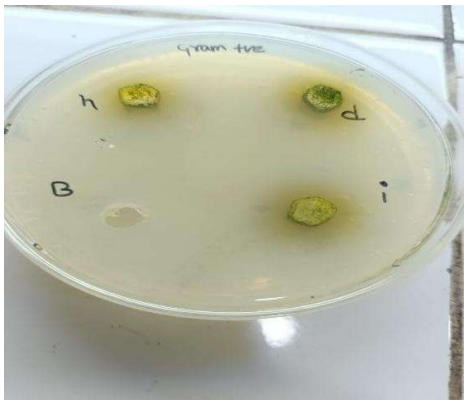

Fig 19: Zone of inhibition of Plant extracts with sample-h, sample- d, Sample-i and Blank on gram-positive bacteria



Fig 20: Zone of inhibition of Plant extracts with sample-g, sample- c, Sample-a and Blank on gram-positive bacteria



Fig 21: Zone of inhibition of Plant extracts with sample-1, sample- a, sample-e and Blank on gram-positive bacteria



Fig 22: Zone of inhibition of Plant extracts with sample-2, sample-e, sample-f and Blank on gram-positive bacteria

CONCLUSION

The phytochemical screening of the aerial parts of *Lantana camara* and *Ficus religiosa* revealed the presence of several bioactive compounds known for their antimicrobial potential. These results suggest that *Lantana camara* is primarily responsible for the antibacterial action due to its richer and more active phytochemical profile. However, in certain proportions, *Ficus religiosa* contributes synergistically to enhance the overall antibacterial effect, especially against Gram-positive bacteria. The study concludes that both plants possess significant antibacterial activity, with *Lantana camara* being more effective on its own, and specific combinations of the two offering enhanced synergistic potential. These findings support the traditional use of these plants and highlight their promise for developing effective herbal antibacterial formulations.

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