ANTI-INFLAMMATORY AND ANTI-MYCOBACTERIAL POTENTIAL OF *BARLERIA CRISTATA* LINN. (AERIAL PART)

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ABSTRACT

OBJECTIVE: The present investigation aimed to assess the anti-inflammatory effects as well as antimycobacterial properties using extracts obtained from *Barleria cristata* through ethanol and petroleum ether extraction techniques.

MATERIALS AND METHOD

Tuberculosis Anti-TB test by MABA Assay Test Organism:

Strain Used: The study utilized the *Mycobacterium tuberculosis* H37Rv strain (vaccine type), corresponding to ATCC accession number 27294.

Chemicals and Reagents:

The study made use of Middlebrook 7H9 culture medium, Alamar Blue dye for viability assessment, 10% solution of Tween 80, sterile deionized water, and conventional anti-tuberculosis drugs. (Isoniazid, Ethambutol, Pyrazinamide, Rifampicin, Streptomycin)

Evaluation of anti inflammatory potential was performed through the application of the HRBC (Human Erythrocyte) membrane stability assay: Alsever's solution, employed in this method, was formulated by combining 2% glucose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in purified water. Additional reagents included isotonic saline (0.85%), a 10% v/v isotonic saline solution, phosphate buffer at 0.15 M concentration with a pH of 7.4, and a hypotonic saline solution containing 0.36% sodium chloride**.**

RESULTS: The extracts obtained using ethanol and petroleum ether from *Barleria cristata* demonstrated considerable anti-inflammatory and antimycobacterial activities. In the HRBC (Human Red Blood Cell) membrane stabilization assay, ethanolic extract showed improved inflammation-reducing potential, especially at higher doses. Conversely, the petroleum ether extract displayed stronger antimycobacterial action, with a lowest concentration needed to inhibit growth (MIC) being 6.25 micrograms per milliliter, as compared to 12.5 micrograms per milliliter observed in the case of ethanolic extract.

CONCLUSIONS: Both petroleum ether and ethanolic extracts of Barleria cristata exhibit medicinal potential, showing effectiveness against tuberculosis and inflammation, respectively, for targeted therapeutic applications.

KEYWORDS: *Barleria cristata* Linn, Ethanolic extract, Petroleum ether extract, Anti-inflammatory activity, Anti Mycobacterial activity

INTRODUCTION

Inflammation is a complex physiological reaction of blood vessels in response to damaging factors such as infectious agents, chemical irritants or cell injury. It functions as a protective response aimed at eliminating the root cause of tissue damage and promoting its repair and regeneration. [2] Inflammation is initiated by triggering and secretion of major inflammatory mediators, such as prostaglandins, leukotrienes, NO (nitric oxide), and cellular adhesion proteins. These mediators arise as a result of enzyme activation, particularly iNOS (inducible nitric oxide synthase) and COX (cyclooxygenase), which are essential in the onset, continuation, and advancement of both short-term and long-term inflammatory responses.[3,4,5,6]

Tuberculosis (TB), caused by its primary pathogen, is among the most widespread infectious diseases, According to data from the World Health Organization (2004) and the TB Alliance (2011), approximately around one-third of the global population has been impacted by TB.

TB primarily affects theSE lungs, a condition known as pulmonary TB; however, it can also involve other organs such as the kidneys, spine, and brain, in which case it is termed extrapulmonary tuberculosis. It spreads through tiny droplets released into the air when an infected individual sneezes or coughs. However, it does not transmit through physical contact, sharing food, or touching contaminated surfaces.

Tuberculosis can occur in two forms:

1. During latent TB infection (LTBI), the tuberculosis-causing bacteria persist in a dormant state and do not lead to any clinical signs. Individuals with LTBI are not infectious.

2. Active TB Disease – In this form, the bacteria are active and multiplying, leading to Symptoms often involve a continuous cough, noticeable weight decline, high fever, and night sweats. This type can spread from person to person and requires urgent medical care. [7]

The Barleria genus is classified under the *Acanthaceae* plant group, which is commonly distributed in open woodland environments. It is indigenous to the tropical zones of Asia and Africa. With around 300 unique species, Barleria is recognized as the third-largest genus within this plant family. In India, 32 species were reported by Balkwill, while Karthikeyan and colleagues noted one subspecies, six varieties, and 29 species.[8,9] Although commonly known as 'Philippine violet,' the plant is neither indigenous to the Philippines nor botanically related to true violets, despite the violet hue of its flowers.

Barleria cristata is an ornamental, evergreen subshrub characterized by its erect, hairy stems and typically grows to a height of 3–4 feet. It is now widely cultivated across tropical and subtropical zones, including parts of India, Southeast Asia, and southern China. In the United States, it is suitable for growth in regions like Florida, southern Texas, Louisiana,

Arizona, and California. However, it is also considered potentially invasive, particularly in disturbed areas and along roadways.[10,11,12]

Phytochemical studies of Barleria cristata reveal, It contains bioactive constituents including triterpenoids, flavonoids, iridoids, phenolic substances, and phenylethanoid glycosides. The plant is known for its diverse pharmacological properties, including hepatoprotective, antiplasmodial, antimicrobial, antifungal, antidiabetic, anti-inflammatory, and antioxidant activity.[13,14,15,16]

Inflammation-reducing potential was evaluated through the application of the HRBC (Human Erythrocyte) membrane stability assay where increased membrane stability corresponded to greater anti-inflammatory efficacy. Antimycobacterial effects were examined by applying various concentrations of each extract against *Mycobacterium* species, with the resulting sensitivity indicating their potential effectiveness.

RESEARCH DESIGN AND MATERIALS

Collection and Authentication

Barleria cristata Linn was collected from a nearby medicinal plant garden situated in Kakapalayam. Its identity was verified and certified by Dr. K. N. Sunil Kumar a scientific officer affiliated with the Siddha Central Research Institute, situated within the Anna Government Hospital Campus, Arumbakkam, Chennai – 600106.

Plant Material Handling and Preparation

Fresh plant foliage was gathered and initially washed extensively under flowing the running water to eliminate any external contaminants such as dirt or dust particles. It was then thoroughly washed with distilled water to ensure it was clean. The cleaned foliage was subsequently chopped into smaller pieces followed by allow to naturally dried in the shade over a period of approximately 7 to 10 days. Once completely dehydrated, the plant material was milled in to the uniform powder using an electric milling device. The resulting leaf powder was subsequently kept in a sealed container at room temperature until they were used for extraction procedures.

Extraction Procedure for Plant Powder

The dried and powdered plant material underwent sequential extraction, beginning with petroleum ether and followed by ethanol, to isolate compounds based on their varying solubility in each solvent. The resulting extracts were subsequently filtered and reduced to dryness using a rotary evaporator. The concentrated residues were appropriately labeled as BCW-PE for the petroleum ether extract and BCW-ET for the ethanol extract. These extracts were preserved in sealed glass containers and kept in a desiccator for subsequent use.

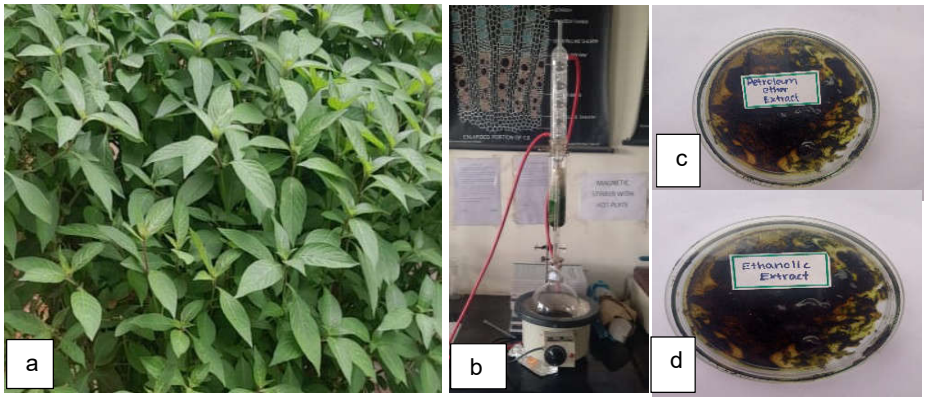


Figure:1 Diagrammatic Presentation of Materials and Methods (a)Barleria Cristata Linn (Aerial parts), (b)Extraction Process (Soxhlet), (c) Petroleum ether Extract, (d)Ethanolic Extract.

PHARMACOLOGICAL ACTIVITIES

1. ANTI-MYCOBACTERIAL ACTIVITY
2. ANTI-INFLAMMATORY ACTIVITY

1. ANTI- MYCOBACTERIAL ACTIVITY

METHODOLOGY

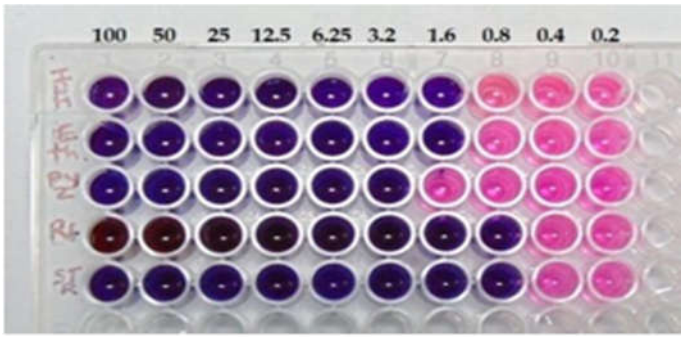
1. The antimycobacterial potential of the tested compound was evaluated against *Mycobacterium tuberculosis* using the Microplate Alamar Blue Assay (MABA).
2. This assay is considered safe, employs a thermally stable indicator and align well with both the proportional testing approach and the BACTEC radiometric system.
3. To reduce evaporation of the medium, 200 µL of autoclaved distilled water was dispensed into the peripheral wells of a 96-well plate before introducing samples into the test wells for the incubation process.
4. Each well of the microplate was loaded with 100 µL of Middlebrook 7H9 broth and gradient dilutions of the tested substance were carried out within the wells.
5. The test samples were analyzed at across a concentrations range of 100 µg/mL to 0.2 µg/mL.
6. The microplates were then covered with parafilm and kept at 37°C for a duration of 5 days.
7. Following the initial incubation, 25 µL of a newly prepared reagent containing equal portions of Alamar Blue and 10% Tween 80 was dispensed into each test well. The microplates were then kept for a further 24-hour incubation.
8. A blue coloration in the wells indicated absence of bacterial proliferation, whereas a pink color was taken as evidence of microbial growth.
9. The minimum inhibitory concentration (MIC) was determined as the smallest amount of test substance that maintained the blue appearance, indicating complete inhibition of bacterial activity.

<u>The standard anti-TB drugs were tested at the following concentrations:</u>

- **Isoniazid** – 1.6 µg/mL
- **Ethambutol** – 1.6 µg/mL
- **Pyrazinamide** – 3.125 µg/mL
- **Rifampicin** – 0.8 µg/mL

- **Streptomycin** – 0.8 µg/mL

Figure:2 Image of the Reference Drug



2. **AntiInflammatoryActivity**

Membrane stabilization method

The anti-inflammatory effect was examined through the HRBC (Human Red Blood Cell) membrane stabilization method, as outlined by Shinde et al. (1999).

Principle

Stabilization Assay of Human Erythrocyte Membranes (HRBC Method)

When human red blood cells (HRBCs) are exposed to harmful agents like hypotonic solutions, they undergo membrane rupture, leading to hemolysis and oxidative changes in hemoglobin (Feirrali et al., 1992). Because the structure of HRBC membranes closely resembles that of lysosomal membranes, their stabilization is considered an effective indicator of anti-inflammatory activity (Mounnissamy et al., 2008).

Reagents Used:

1. A solution of **Alsever's reagent** was formulated by mixing 2% glucose, 0.8% trisodium citrate, 0.05% citric acid and 0.42% common salt into the deionized water
2. **Isotonic Saline (0.85%, pH 7.2)** – Commercially available.
3. **Isotonic Saline (10% v/v)** – Commercially available.
4. **pH-buffered saline (0.15 molar, pH 7.4) –** Obtained as a pre-formulated commercial product.
5. **Hypotonic Saline (0.36%)** – Commercially available.
6. **Reference Drug** – Diclofenac sodium was used as the standard anti-inflammatory agent

Preparation of Red blood cell

Samples of blood were obtained from healthy volunteers with their prior informed consent. The obtained blood was immediately mixed with an equivalent quantity of sterile Alsever's reagent, which served as an anticoagulant. This blend was then centrifuged at 3000 rpm for a duration of 5 minutes. The separated erythrocytes underwent repeated rinsing with 0.85% saline solution adjusted to pH 7.2 and a

10% volume-per-volume cell mixture was then prepared using this same saline solution. This final formulation was referred to as the HRBC suspension.

For the membrane stabilization study, each test mixture included 1.0 milliliter of phosphate-buffered saline (0.15 molar concentration, pH adjusted to 7.4), 2.0 milliliter of hypotonic saline solution (0.36%), 0.5 milliliter of the human red blood cell mixture along with 1.0 milliliter of the sample across different dosage levels (200, 400, 600, 800, and 1000 µg/mL). In the control group, 2.0 mL of deionized water replaced the hypotonic saline.

All tubes were maintained at 37°C for half an hour, then spun at 3000 revolutions per minute for five minutes. The optical density of the supernatant was read at wavelenght 560 nm to determine the degree of erythrocyte lysis and membrane protection.

The formula below was applied to calculate the hemolysis percentage:

Percentage of Hemolysis (%) = (optical density of Sample / optical densit of Control) × 100

RESULTS OVERVIEW AND ANALYSIS

ANTI MYCOBACTERIAL EFFICACY

Table 1: Investigation into the Anti-Mycobacterial efficacy of BCL PE Extract

SI NO.	Concentration of Sample (µg/mL)	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
01	Barleria Cristata Linn (Plant) Extract Ethanolic Extract	+	+	+	+	-	-	-	-
02	Barleria Cristata Linn (Plant) Extract Petroleum Ether Extract	+	+	+	+	+	-	-	-

Note:

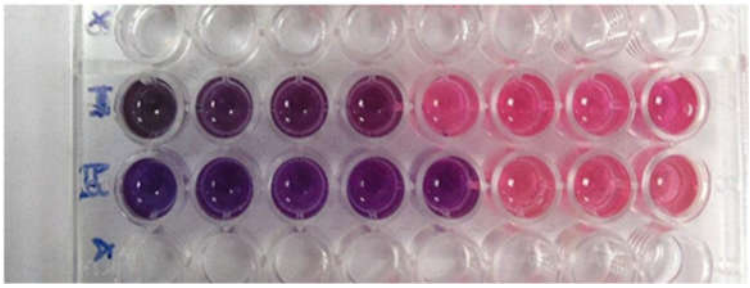
Positive (+): Sensitive

Negative (-): Resistance

Table: 2 MIC Table:

S. No	Sample	MIC Value
01	*Barleria Cristata* Linn (Plant) Extract Ethanolic Extract	12.5 µg/ml
02	*Barleria Cristata* Linn (Plant) Extract Petroleum Ether Extract	6.25 µg/ml

Photograph:



ANTI INFLAMMATORY EFFICACY

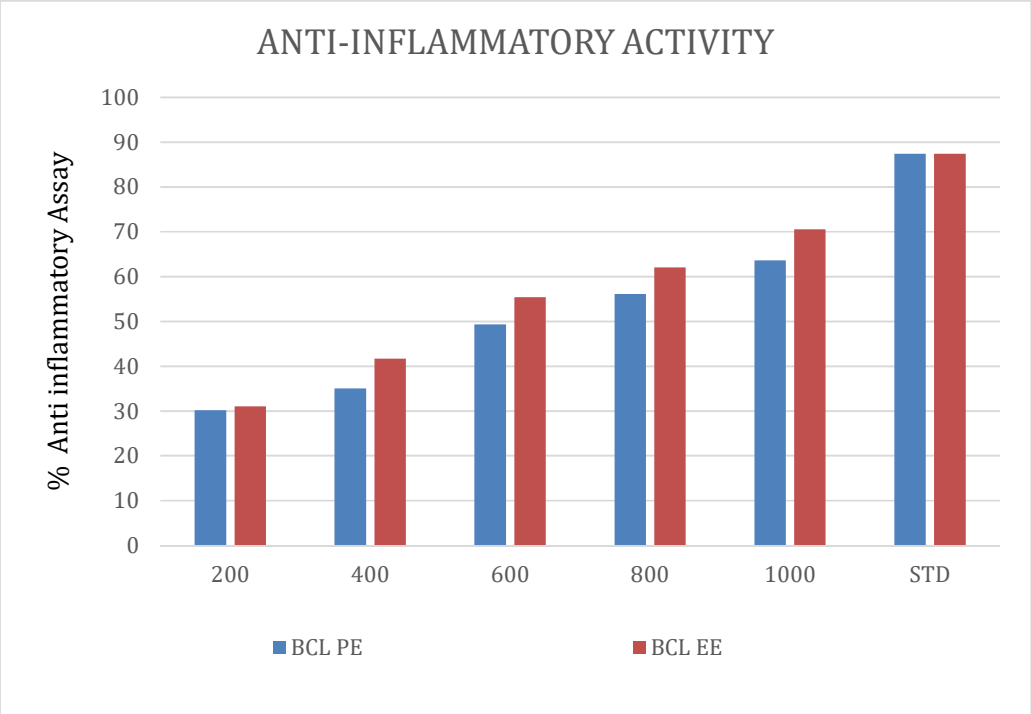
Table 1: Investigation into the Anti-inflammatory Action of BCL PE Extract

Concentration (µg/ml),	Optical density (560nm)	HRBC Membrane Stabilization (%)
200 µg/ml	0.298	**30.19**
400 µg/ml	0.354	**35.06**
600 µg/ml	0.487	**49.34**
800 µg/ml	0.554	**56.12**
1000 µg/ml	0.628	**63.62**
Standard	0.863	**87.43**
Control	0.987	-

Table 2: Assessment of Anti-inflammatory Effects of BCL EE Sample

Concentration (µg/ml)	Optical density (560nm)	HRBC Membrane Stabilization (%)
200 µg/ml	0.306	**31.00**
400 µg/ml	0.412	**41.74**
600 µg/ml	0.547	**55.42**
800 µg/ml	0.612	**62.00**
1000 µg/ml	0.697	**70.61**
Standard	0.863	**87.43**
Control	0.987	-

Graph: 1: Anti inflammatory effect of Samples (BCL PE and BCL EE)



CONCLUSION

This research was conducted to explore the inflammation-reducing and anti-tuberculosis activities exhibited by *Barleria cristata* Linn. through the application of two distinct solvent extracts: petroleum ether extract (PEE) and ethanolic extract (EE).

ANTI MYCOBACTERIUM ACTIVITY

For the evaluation of anti-tubercular activity, both extracts demonstrated significant inhibitory effects against *Mycobacterium* species. The petroleum ether extract proved to be more effective, showing a displaying a lowest effective concentration of 6.25 µg/mL, while the ethanolic extract showed a value of 12.5 µg/mL. These findings indicate that the petroleum ether extract likely contains more active phytochemicals with stronger antimycobacterial properties.

ANTI-INFLAMMATORY EFFECTS

During the evaluation of anti-inflammatory properties, both extracts exhibited a dose-responsive membrane-protective effect on human erythrocytes (HRBC). Ethanolic extract showed slightly enhanced activity, achieving 70.61% membrane protection at 1000 µg/mL, whereas the petroleum ether extract provided 63.62% protection at the same dosage. The enhanced anti-inflammatory response observed with the ethanol-based extract could be attributed to its higher levels of bioactive polar constituents, including flavonoids and phenolic compounds, which are widely recognized for their anti-inflammatory properties.

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Conflict of interest

The authors do not have any conflict of interest

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This study involved the use of established animal cell lines. No live animals were used. Ethical approval was not required as the cell lines were obtained from a recognized cell repository and used according to standard laboratory practices.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required

Clinical Trial Registration

This research does not involve any clinical trials.

Authors' Contribution

The sole author was responsible for the conceptualization, methodology, data collection, analysis, writing, and final approval of the manuscript.

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