Chapter 11

Analysis of secondary metabolites flowers of *Blepharis maderaspatensis* (L.) B. Heyne ex Roth

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ABSTRACT

The study presents a comprehensive phytochemical analysis of *Blepharis maderaspatensis* flowers, employing qualitative screening and thin-layer chromatography (TLC) of extracts. The results demonstrated the presence of a diverse array of bioactive compounds, including tannins, saponins, phenols, reducing sugars, and alkaloids. The TLC analysis revealed distinct Rf values, indicative of varying polarities among the detected compounds. These findings provide a robust foundation for further investigation into the medicinal properties and potential therapeutic applications of *B. maderaspatensis.*

Keywords: Bioactive compounds, herbs, pharmacology

INTRODUCTION

The emergence and dissemination of antimicrobial resistance (AMR) has become a pressing global health concern, threatening the efficacy of antibiotics and posing as a significant challenge for the treatment of infectious diseases (Kumar and Jena, 2014; Sahoo *et al.,* 2021; Singh *et al.,* 2022; Salam *et al.,* 2023; Hossain *et al.,* 2025).

The World Health Organization (WHO) has declared AMR a major threat to global health security, emphasizing the urgent need for innovative strategies to combat this growing problem (Coque *et al.,* 2023). One promising approach is the exploration of plant-derived compounds, which have been used for centuries in traditional medicine and have shown potential as antimicrobial agents (Tripathy *et al.,* 2015; Palei *et al.,* 2024; Jena *et al.,* 2025).



Figure 1: Flower of *B. maderaspatensis*

Blepharis maderaspatensis, a plant species with a rich history of traditional use (Dirar *et al.,* 2021; Figure 1), has been selected for phytochemical analysis to identify potential bioactive compounds with antimicrobial properties. Present study aims to investigate the phytochemical composition of *B. maderaspatensis* flowers,

with focus on identifying compounds that may contribute to their antimicrobial activity. By exploring the phytochemical profile of this plant, this research seeks to contribute to the discovery of novel antimicrobial agents and provide insights into the potential therapeutic applications of *B. maderaspatensis***.**

METHODOLOGY

The flowers of *B. maderaspatensis* were collected from Khordha district of Odisha, India (Figure 2). The plant species was identified by the authors (Figure 1). The Soxhlet extraction method was adopted using n-hexane, ethanol, and distilled water for phytochemical analysis. Detection of nine secondary metabolites were conducted using standard methods (Devi *et al.,* 2023; Marndi *et al.,* 2024; Sony *et al.,* 2024; Sreedevi et al., 2024; Das *et al.,* 2025; Thakur *et al.,* 2025).



Figure 2: Collected *B. maderaspatensis* flowers for experimental work

Test for tannin: 1 ml of flower extract was taken in a test tube and 3-5 drops of 10% lead acetate solution were added into it. The gelatinous precipitate formation confirmed the presence of tannin.

Test for saponin: About 1 ml of distilled water was added to 1 ml of the flower extract and shaken well. The formation of persistent froth was observed confirming the presence of saponin.

Test for flavonoids: About 1 ml of the flower extract was taken. 2 ml of NaOH solution and 3-5 drops of dilute HCl were added to it. The colour initially turned to an intense yellow colour with NaOH solution and later became colourless which confirmed the presence of flavonoids.

Test for terpenoids: To 1 ml of the flower extract, 5-6 drops of chloroform were added. Then 4-6 drops of concentrated sulphuric acid were added into it. The reddish-brown interface confirmed the presence of terpenoids.

Test for phenols: About 1 ml of the flower extract was taken. A few drops of 5% ferric chloride solution were added. The dark bluish-black appearance confirmed the presence of phenolic compounds.

Test for reducing sugars: About 1 ml of the flower extract was taken. 2 drops of Fehling's solution A followed by Fehling's solution B were added and kept in the water bath for a few minutes. The presence of red-orange precipitate confirmed the presence of reducing sugars.

Test for steroids: To 1 ml of the flower extract, 1 ml of chloroform followed by 1 ml of concentrated sulphuric acid were added to it. The appearance of upper red and lower yellow with green fluorescence provides the presence of steroids.

Test for alkaloids: About 1 ml of the flower extract was taken. 3-4 drops of Dragendroff's reagent were added to it. The formation of a reddish-brown precipitate confirmed the presence of alkaloids.

Test for carbonyl compounds: About 1 ml of flower extract was taken and 3-4 drops of 2,4-Dinitrophenylhydrazine (DNPH) reagent were added. The formation of yellow crystals confirmed the presence of carbonyl compounds.

TLC (Thin layer chromatography) analysis: TLC plates were prepared on 6 cm glass slides using silica gel powder. Slides were washed with clinical laboratory detergent and dried. Clean and dried slides were wiped with ethyl acetate for removing surface adherents. 3 g of silica was taken in 20 ml of distilled water and slurry was prepared by constant stirring, and finally was poured over the slides and slides were left undisturbed till the drying of silica layer. Then the slides were activated at 50^{o} C for 15-20 minutes before running the TLC. Methanol: chloroform (1:1), ethyl acetate: chloroform (1:9) and ethyl acetate: distilled water: acetic acid (8:1:1) were used as mobile phases. Then spots were marked, RF (Retention Factor) values were recorded and visualized under UV chamber (Mishra and Bhatnagar, 2024).

RESULTS AND DISCUSSION

The phytochemical analysis of *B. maderaspatensis* flowers revealed the presence of various bioactive compounds, including tannins, saponins, phenols, reducing sugars, and alkaloids (Table 1; Figures 3-5). These compounds have been reported to possess antimicrobial properties, suggesting that *B. maderaspatensis* may be a valuable source of natural antimicrobial agents. The presence of these compounds in the plant may contribute to its traditional use in treating various ailments, including infections. The TLC analysis showed distinct Rf values for the detected compounds, indicating varying polarities among the bioactive molecules (Table 2; Figure 6). This diversity in polarity may be attributed to the different chemical structures and functional groups present in the compounds. The TLC results also suggest that the plant contains a complex mixture of bioactive compounds, which may work synergistically to exert their antimicrobial effects. The findings of this study have significant implications for the discovery of novel antimicrobial agents.

The bioactive compounds identified in *B. maderaspatensis* may serve as leads for the development of new antimicrobial drugs, which could help address the growing problem of antimicrobial resistance. Further studies are needed to isolate and characterize the bioactive compounds, evaluate their antimicrobial activity, and investigate their potential therapeutic applications. The results suggest that the bioactive compounds present in the flowers have varying polarities, and the choice of mobile phase can significantly affect their separation and detection. The TLC analysis detected multiple spots in each mobile phase, indicating the presence of multiple bioactive compounds in the plant. Mohan *et al.,* (2010) reported that *B. maderaspaten*sis whole plant extracts contain different bioactive compounds such as alkaloids, terpenoids, flavonoids, quinones, phenols and glycosides. The leaf extracts of *B. maderaspatensis* contain various secondary metabolites such as alkaloids, flavonoids, phenols, tannins, saponins, glycosides, etc. (Neelambika *et al.,* 2014).

Table 1: Qualitative phytochemical screening of *Blepharis maderaspatensis* flowers using different extracts

Bioactive components	Extracts		
	n-Hexane	Ethanol	Aqueous
Tannin	Not detected	Detected	Detected
Saponin	Detected	Detected	Detected
Flavonoids	Not detected	Not detected	Not detected
Terpenoids	Not detected	Not detected	Not detected
Phenols	Detected	Detected	Detected
Reducing sugar	Not detected	Detected	Detected
Steroids	Not detected	Not detected	Not detected
Alkaloids	Not detected	Detected	Detected
Carbonyl compounds	Not detected	Not detected	Not detected

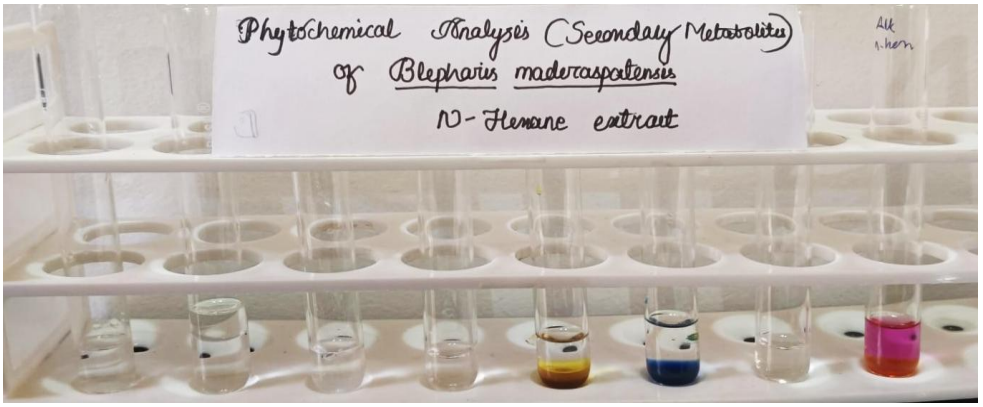


Figure 3: Detection of secondary metabolites of *B. maderaspatensis* flowers using n-hexane extract

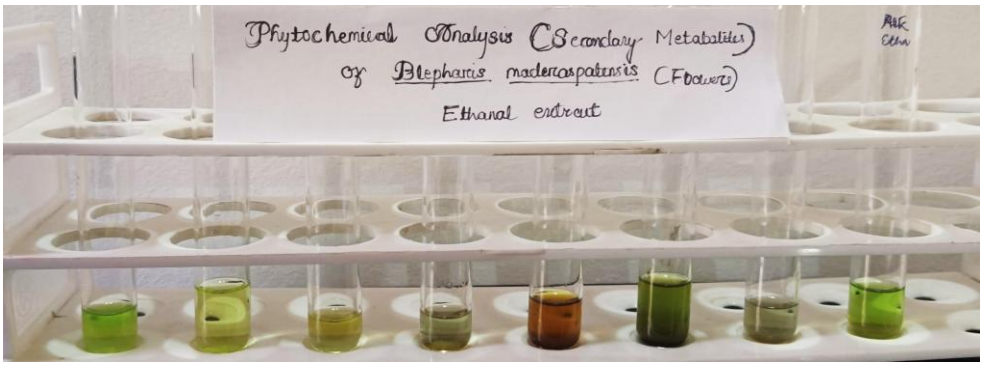


Figure 4: Detection of secondary metabolites of *B. maderaspatensis* flowers using ethanol extract



Figure 5: Detection of secondary metabolites of *B. maderaspatensis* flowers using aqueous extract

Table 2: TLC analysis of *B. maderaspatensis* flowers using different mobile phases

Mobile phases	Extracts	Rf value of spots			
		S_1	S_2	S_3	S_4
Chloroform: methanol (1:1)	Acetone	0.85	0.89	0.92	0.95
Ethyl acetate: chloroform (1:9)	Acetone	0.86	0.89	Nil	Nil
Ethyl acetate: water: acetic acid (8:1:1)	Acetone	0.90	0.94	Nil	Nil

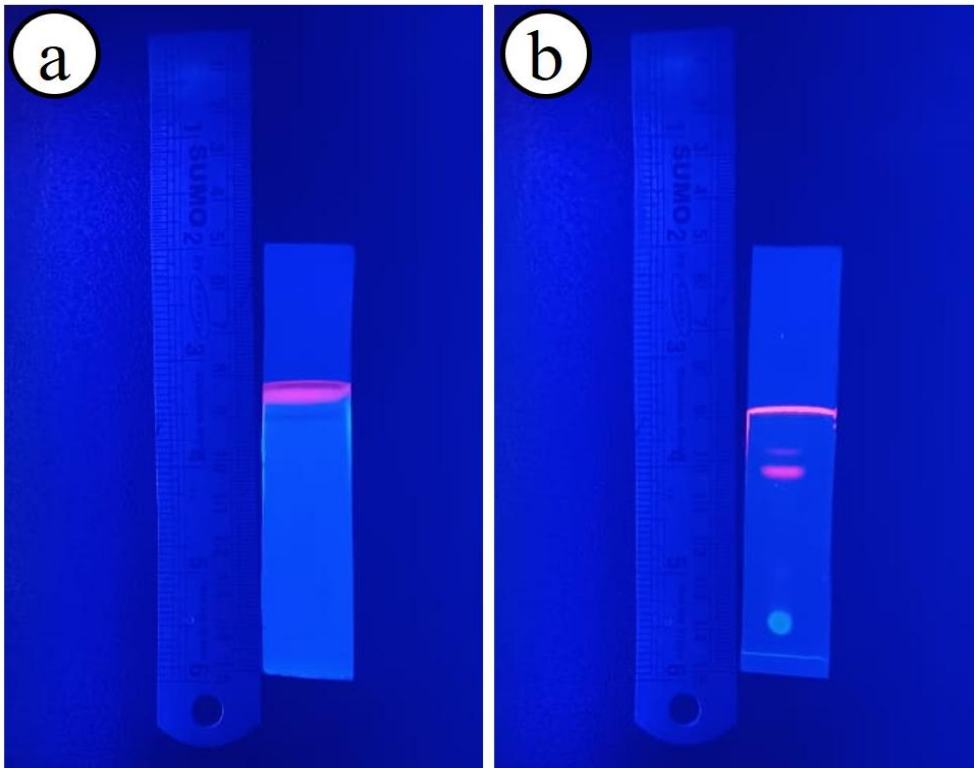


Figure 6: Spots on TLC plates under UV light; a) (Chloroform: methanol); b) Ethyl acetate: chloroform) using acetone extract of *B. maderaspatensis* flowers

CONCLUSISON

Present study demonstrates the presence of various bioactive compounds in *B. maderaspatensis* flowers, including tannins, saponins, phenols, reducing sugars, and alkaloids, which may contribute to its antimicrobial properties. The findings of this research provide a foundation for further investigation into the therapeutic potential of *B. maderaspatensis* and highlight its potential as a source of novel antimicrobial agents. Further studies are warranted to explore the pharmacological activities and potential applications of this plant.

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