

Antimicrobial Activity of *Eclipta alba* (L.) and *Sphagneticola trilobata* (L.) Pruski

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Abstract

Rising antimicrobial resistance has renewed interest in medicinal plants as sources of new antimicrobial agents. This study evaluated the antimicrobial activity of alcoholic extracts of *Eclipta alba* (L.) and *Sphagneticola trilobata* (L.) Pruski (Asteraceae) using the agar cup diffusion method. The extracts were tested at 25, 50, 75 and 100 mg/mL against two bacteria, *Escherichia coli* (MCC 2412) and *Staphylococcus aureus* (NCIM 5022), and two fungi, *Malassezia furfur* (MTCC 1374) and *Candida albicans* (MCC 1151). Both extracts inhibited all four organisms, and zones of inhibition generally widened as concentration increased. The maximum antibacterial zones were 11 mm (*E. alba*) and 11.3 mm (*S. trilobata*) against *E. coli*, and 9.3 mm and 11.3 mm against *S. aureus*. Antifungal zones were smaller, reaching 9 mm (*E. alba*) and 8.83 mm (*S. trilobata*) against *C. albicans*, and 7.6 mm and 6.3 mm against *M. furfur*. These findings provide a preliminary indication of the antibacterial and antifungal potential of both plants. Confirmation and further characterization of these effects require the determination of minimum inhibitory, bactericidal, and fungicidal concentrations, and identification of the active constituents.

Keywords: *Eclipta alba*; *Sphagneticola trilobata*; Antimicrobial activity; Agar cup diffusion; Antimicrobial resistance.

I. INTRODUCTION

Medicinal plants have long been used to manage various ailments, including infectious diseases, and remain important sources of bioactive compounds for pharmaceutical, cosmetic, and nutraceutical applications (Mahato & Sharma, 2018). Traditional medicine continues to contribute to primary healthcare worldwide, including among tribal communities in India. Despite their widespread use, the antimicrobial properties of many plant-derived compounds require further scientific investigation (Namita & Mukesh, 2012; Munuswamy *et al.*, 2013; Wylie & Merrell, 2022).

Plants produce secondary metabolites, including alkaloids, phenolic compounds, and terpenoids, that possess diverse biological activities and may enhance antibiotic effectiveness against infectious pathogens (Chassagne *et al.*, 2021). Their investigation is particularly relevant given the growing threat of antimicrobial resistance (AMR), which is exacerbated by antibiotic overuse and improper disposal. AMR-associated deaths could reach 10 million annually by 2050 if effective interventions are not implemented (Thakur *et al.*, 2024). Therefore, medicinal plants warrant further investigation as potential sources of antimicrobial agents.

Sphagneticola trilobata (L.) Pruski, also belonging to Asteraceae, is a perennial herb native to South America and introduced into India, where it grows in moist habitats. It can reach 70 cm in height and forms dense ground-covering mats. In traditional Indian medicine, it has been used to manage backache, muscle cramps, rheumatism, wounds, swelling, arthritic pain, fever, and malaria (Singhal *et al.*, 2021).

Eclipta alba (L.), commonly known as Bhringraj or false daisy, belongs to the family Asteraceae. This annual herb is widely used in traditional medicine across tropical and subtropical regions, including India, Brazil, Thailand, and China. Its major phytoconstituents include coumestan derivatives such as wedelolactone, as well as apigenin, oleanolic acid, and sitosterol (Sapat *et al.*, 2026).

The present study aims to evaluate the antimicrobial activity of alcoholic extracts of *Eclipta alba* (L.) and *Sphagneticola trilobata* (L.) Pruski to investigate their potential as sources of antimicrobial agents.

II. MATERIALS AND METHODS

Collection and Authentication of Plant Material

Sphagneticola trilobata (L.) Pruski (Accession No. PK-32) and *Eclipta alba* (L.) (Accession No. 2769) were collected from local market in Mumbai, India, and authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai.

Preparation of Extract

Extracts were prepared using the cold maceration technique, in which 10 g of powdered plant material was soaked in 50 mL of alcohol overnight. The mixture was filtered through Whatman No. 1 filter paper, and the resulting filtrate was evaporated at 40°C to obtain the concentrated extract (Dharajiya *et al.*, 2012).

Antimicrobial activity

Antimicrobial activity of alcoholic extracts of *Sphagneticola trilobata* (ST06) and *Eclipta alba* (EP05) species was evaluated using the agar cup diffusion method against selected bacterial and fungal test organisms.

Antibacterial activity

Bacterial test organisms, MCC 2412 and NCIM 5022, were cultured in Luria-Bertani (LB) broth and incubated at 37°C for 24 hours. The bacterial cultures were adjusted using a 0.5 McFarland standard (approximately (10^8) CFU/mL) and subsequently diluted to obtain a final cell density of (1×10^6) CFU/mL. The prepared bacterial suspensions were used for antibacterial activity assessment by the agar cup diffusion method. Three to four wells were prepared in each agar plate. The test wells were loaded with 0.1 mL of the respective alcoholic plant extracts, while the remaining wells were used for positive and negative controls. The plates were incubated at 37°C for 24 hours.

Antifungal activity

Fungal test organisms, MTCC 1374 and MCC 1151, were cultured in Dixon broth and incubated at 30°C for 48 hours. The fungal cultures were adjusted using a 0.5 McFarland standard (approximately (10^6) CFU/mL) and diluted to obtain a final cell density of (1×10^4) CFU/mL. Antifungal activity was evaluated using the agar cup diffusion method. Three wells were prepared in each agar plate, with 0.1 mL of the respective alcoholic plant extract added to the test well and the remaining two wells used for positive and negative controls. The plates were incubated at 30°C for 48 hours.

III. RESULTS

Antibacterial activity

Both extracts inhibited the growth of *E. coli* (MCC 2412) and *S. aureus* (NCIM 5022), and the zones of inhibition increased with extract concentration (Fig 1 and 2). Against *E. coli*, zones for *E. alba* rose from 1.83 mm at 25 mg/mL to 11 mm at 100 mg/mL. Zones for *S. trilobata*

rose from 1.83 mm to 11.3 mm over the same range, so the two extracts were closely matched (Fig. 1). Against *S. aureus*, zones for *E. alba* ranged from 2 to 9.3 mm and those for *S. trilobata* from 1.7 to 11.3 mm, so the *S. trilobata* extract produced the larger zone at the highest concentration (Fig. 2).

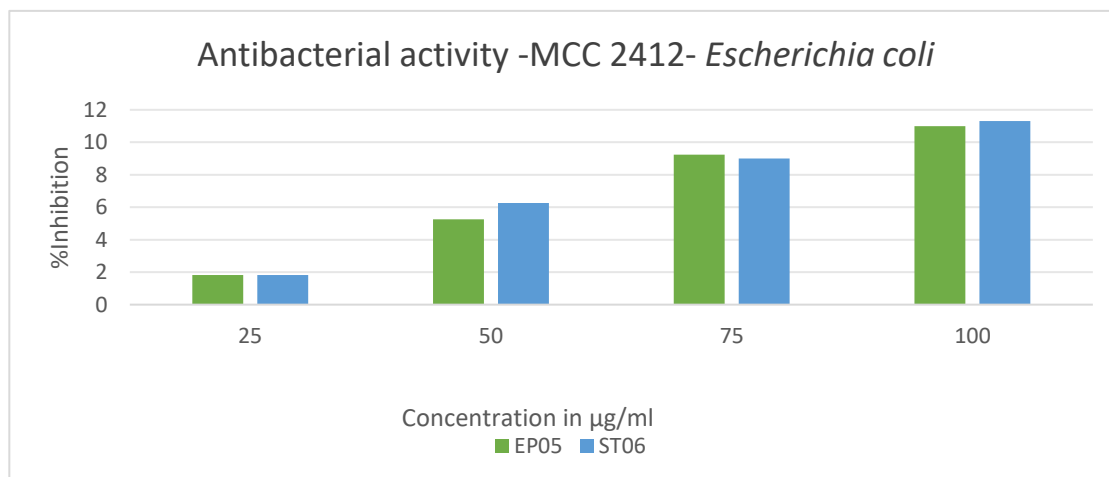


Fig. 1. Antibacterial activity of alcoholic extracts of *Eclipta alba* (EP05) and *Sphagneticola trilobata* (ST06) against *Escherichia coli* (MCC 2412) at different extract concentrations.

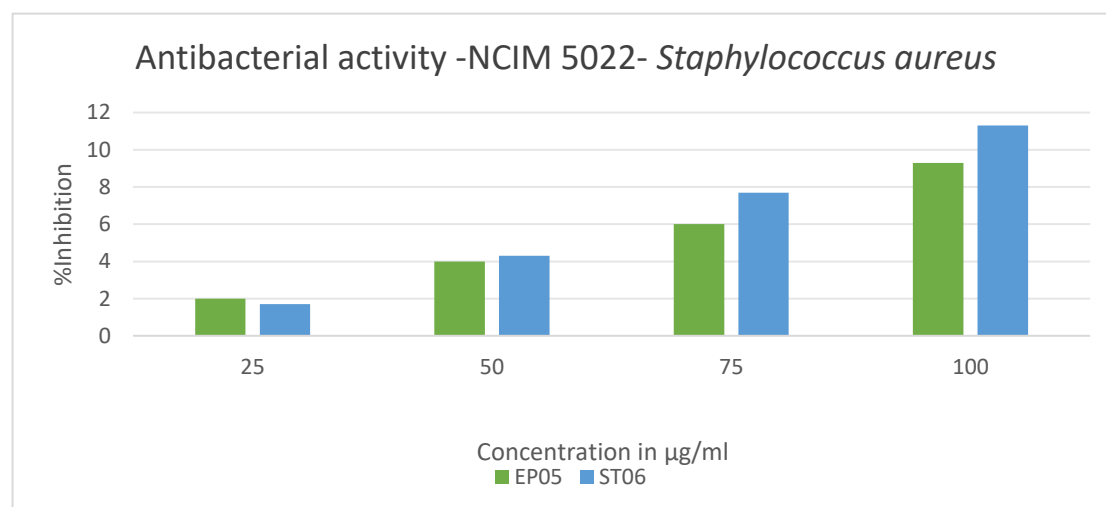


Fig. 2. Antibacterial activity of alcoholic extracts of *Eclipta alba* (EP05) and *Sphagneticola trilobata* (ST06) against *Staphylococcus aureus* (NCIM 5022) at different extract concentrations.

Antifungal activity

Both extracts also inhibited *M. furfur* (MTCC 1374) and *C. albicans* (MCC 1151), although the zones were smaller than those seen with the bacteria (Fig 3 and 4). Against *M. furfur*, zones for *E. alba* ranged from 2 to 7.6 mm and those for *S. trilobata* from 2.2 to 6.3 mm. The *E. alba* zone reached its maximum at 75 mg/mL (7.6 mm) and was slightly smaller at 100 mg/mL (7

mm), so the response was not strictly proportional to concentration (Fig. 3). Against *C. albicans*, zones for *E. alba* ranged from 1.3 to 9 mm and those for *S. trilobata* from 1.5 to 8.83 mm, with near-equal activity at 100 mg/mL (Fig. 4).

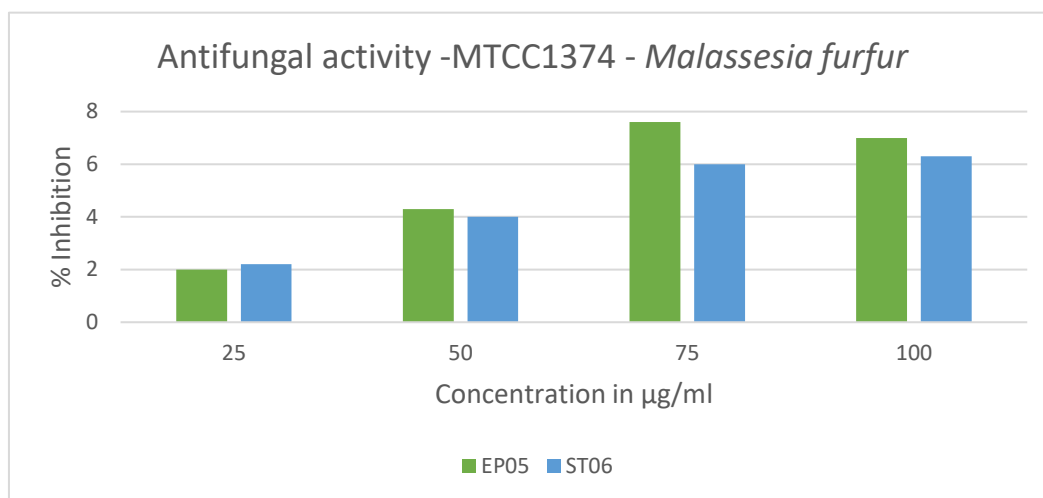


Fig. 3. Antifungal activity of alcoholic extracts of *Eclipta alba* (EP05) and *Sphagneticola trilobata* (ST06) against *Malassezia furfur* (MTCC 1374) at different extract concentrations.

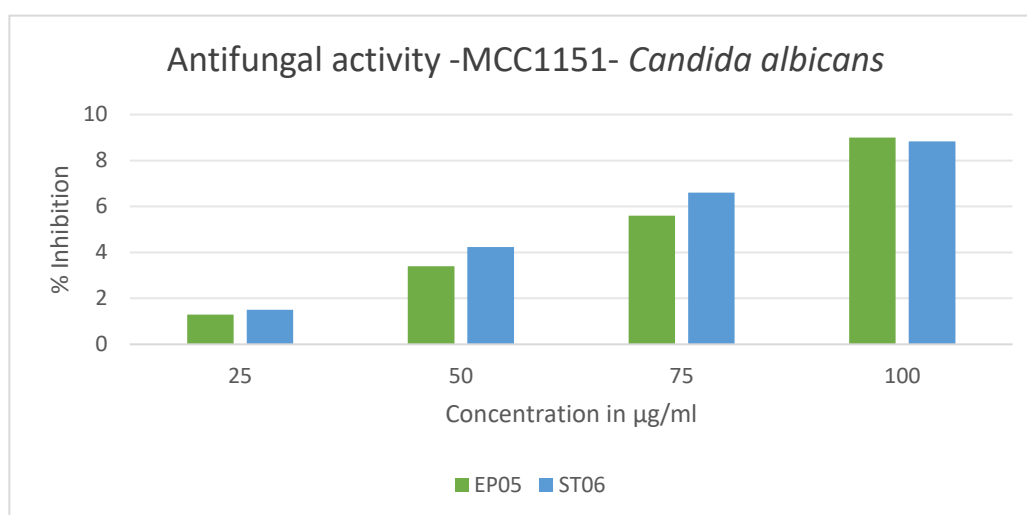


Fig. 4. Antifungal activity of alcoholic extracts of *Eclipta alba* (EP05) and *Sphagneticola trilobata* (ST06) against *Candida albicans* (MCC 1151) at different extract concentrations.

IV. DISCUSSION

Alcoholic extracts of *E. alba* and *S. trilobata* inhibited both bacterial and fungal test organisms. Maximum zones were 11.3 mm for the bacteria and 9 mm for the fungi, and zones generally widened with concentration. These zones are modest, and activity was somewhat stronger against the bacteria than against the fungi. Other work on these plants shows that the response depends on the organism. Leite *et al.* (2019) reported that a hydroalcoholic leaf extract of *S.*

trilobata inhibited several Gram-positive and Gram-negative bacteria, but not *Pseudomonas aeruginosa*, *Salmonella typhimurium* or *Klebsiella pneumoniae*. Sharma *et al.* (2022) found that a methanolic leaf extract of *E. alba* acted against sensitive *E. coli*, *P. aeruginosa* and methicillin-resistant *S. aureus*, but not against every strain tested. The activity seen against *E. coli*, *S. aureus*, *C. albicans* and *M. furfur* here is consistent with this picture of broad but selective activity.

The activity is probably due to secondary metabolites such as phenolics, flavonoids and terpenoids. These are well-known antimicrobial plant constituents that can disrupt microbial membranes, inactivate enzymes and bind cell-wall components (Cowan, 1999; Chassagne *et al.*, 2021). Leite *et al.* (2019) linked the antibacterial effect of *S. trilobata* to flavonoids and terpenoids, and Sharma *et al.* (2022) identified several bioactive constituents in *E. alba* by GC-MS, some of which were predicted to bind the bacterial enzyme FabA. Solvent choice also matters. Karunambigai and Devi (2014) found that chloroform extracts of *E. alba* leaves and roots produced much larger zones than methanol, acetone or aqueous extracts, which mostly gave zones of 8-10 mm. The modest zones in the present study may therefore partly reflect the polarity of the alcoholic solvent, which extracts only part of the active compounds. This remains a hypothesis, because the compounds responsible for the activity were not identified here.

The two extracts showed broadly similar activity. *S. trilobata* gave the larger zone against *S. aureus* at 100 mg/mL (11.3 vs 9.3 mm), the two were almost equal against *E. coli* and *C. albicans*, and *E. alba* gave the larger zones against *M. furfur*. No extract was consistently superior. The zone of inhibition also depends on extract concentration, compound solubility, molecular size and diffusion through agar, so a larger zone does not necessarily mean greater potency, and the relationship with concentration need not be linear (Balouiri *et al.*, 2016). The fall in the *E. alba* zone against *M. furfur* between 75 and 100 mg/mL illustrates this. Agar diffusion is a qualitative screening method. It cannot separate bacteriostatic from bactericidal effects or give a quantitative measure of potency (Balouiri *et al.*, 2016). The results should therefore be read as preliminary evidence of antimicrobial potential. Determining the minimum inhibitory, bactericidal and fungicidal concentrations (MIC, MBC and MFC), with replicate testing, would put the activity on a quantitative footing, and phytochemical profiling with bioassay-guided fractionation would help identify the active constituents.

V. CONCLUSION

Alcoholic extracts of *Eclipta alba* and *Sphagneticola trilobata* inhibited *Escherichia coli*, *Staphylococcus aureus*, *Malassezia furfur* and *Candida albicans* in a concentration-dependent manner, with larger zones against the bacteria than against the fungi. Neither extract was clearly more active than the other. These results support further quantitative and phytochemical investigation of both plants as sources of antimicrobial agents.

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CONFLICTS OF INTEREST

The authors have declared that no conflicts of interest exist.

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