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Pharmacological evaluation of *Eclipta prostrata:* Antioxidant, antimicrobial, and cytotoxic potential of aqueous leaf extracts

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Abstract
Eclipta prostrata (L.) L., commonly known as *Eclipta alba* or False daisy, is a traditional medicinal herb from the Asteraceae family, widely distributed across tropical and subtropical regions. For generations, it has been used in Indian, Nepalese, and Bangladeshi medicine to treat skin ailments, liver disorders, and digestive issues. This study explores the plant's antioxidant, antimicrobial, and cytotoxic potential using aqueous leaf extracts. Free radical scavenging activity was analysed through various *in vitro* procedures, including DPPH, ABTS, FRAP and radical scavenging tests, all of which demonstrated strong free radical neutralization and reducing power, indicating the extract's ability to combat oxidative stress - an underlying factor in many chronic diseases. The antimicrobial effects were evaluated against bacterial strains & fungal strains, with significant zones of inhibition observed, supporting its traditional use for treating infections. Cytotoxicity assay was tested using the Brine shrimp lethality assay (Artemia bioassay), revealing a dose-responsive increase in mortality, suggesting potential anticancer properties. Altogether, these findings validate the ethno medicinal uses of *Eclipta prostrata* and emphasize its promise as a natural source of free radical scavenger, antimicrobial, and cytotoxic agents for therapeutic development.

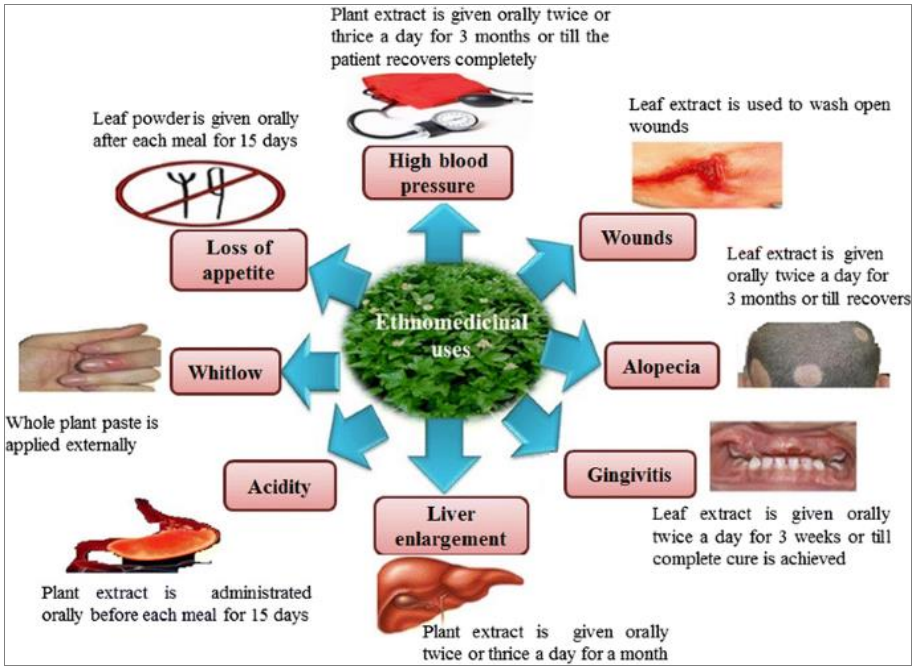
Keywords: *Eclipta prostrata*, medicinal herb, antimicrobial activity, antioxidant, brine shrimp, ferric reducing power, DPPH

Introduction

India stands as a global leader in medicinal herb production - rightfully known as the "botanical garden of the world"- thanks to its rich biodiversity and deeply rooted traditional herbal knowledge (Vidyashree and Kumar, 2022) [24]. Out of approximately 17,000 plant species in India, around 7,500 higher plants have medicinal properties, accounting for 7–13 % of the world's pharmacologically relevant flora (Vidyashree and Kumar, 2022) [24]. This massive botanical wealth, alongside well-established indigenous healthcare systems, gives India a unique global standing in herbal medicine. Demand for plant-based remedies - effective, culturally accepted, and affordable - is surging in both rural and urban areas, fueling ongoing efforts to discover new therapeutic agents from native plants (Vidyashree & Kumar, 2022) [24]. Bioactive compounds derived from such plants continue to play a vital role in modern drug development (Timalsina and Devkota, 2021) [23].

One remarkable example is *Eclipta prostrata* (L.) L., also known as Eclipta alba, or False daisy/Ink plant in English, and Bhringraj in many Indian languages (Timalsina and Devkota, 2021; Yang *et al*., 2023) [2, 23, 27]. This medium-sized annual herb with white flowers thrives in moist habitats like rice fields and riverbanks, spanning tropical and subtropical regions of Asia, Africa, and South America (Timalsina and Devkota, 2021; Silalahi, 2022) [21, 23]. Long valued in ethno medicine, it is used for skin disorders, hair loss, wounds, snakebites, catarrh in infants, and to strengthen liver and digestive health; leaf juice is also administered to stimulate and enhance the hair growth (Timalsina and Devkota, 2021; Yang *et al*., 2023; Silalahi, 2022) [21, 23, 27].

Several recent studies have explored the rich chemical composition of *Eclipta prostrata,* revealing the existence of biologically effective compounds such as flavonoids, phenolics, alkaloids, proteins, lipids, and phytosterols (Chung *et al*., 2017) [8]. Among these, flavonoids - natural plant pigments typically found in stems, leaves, flowers, and fruits - are especially significant due to their antioxidant potential and diverse biological activities (Wang *et al*., 2021) [25]. These compounds not only contribute to plant growth and coloration but also exhibit diverse health promoting properties, including anti-inflammatory, anticancer, and anti-amoebic effects (Gao *et al*., 2018; Duan *et al*., 2017; Albuquerque *et al*., 2019) [1, 10, 11]. This has made flavonoids a key focus in fields like pharmacology, food science, and medical research. In fact, Kim *et al.* (2015) [14] identified that E. prostrata contains high levels of flavonoids with ortho-diphenolic structures, enhancing their ability to neutralize free radicals.



Ethnopharmacological uses, Phytochemistry, Biological activities, and Biotechnological applications of Eclipta prostrata

Redox imbalance arises from the disruption in the balance between the production of excessive generation of ROS and the efficacy of body's antioxidant defense systems which can lead to cellular damage (Ardestani and Yazdanparast, 2007; Singh *et al*., 2020) [3, 22]. These ROS can impair vital cellular macromolecules such as DNA, lipids, and proteins, contributing to the onset of disorders like cancer, Alzheimer's disease, and multiple sclerosis (Bakoyiannis *et al*., 2019; Wang *et al*., 2021) [4, 25]. Although industrial antioxidants like BHA and BHT are commonly used in the food sector, concerns about their potential adverse effects - such as hepatotoxicity and carcinogenicity have encouraged a shift toward natural, plant-derived alternatives (Lei *et al*., 2008) [15]. Medicinal plants like *Eclipta prostrata* have shown promise as safer and more cost-effective options with broad therapeutic applications (Jin & Yin, 2012) [13].

The present study examines on evaluating the antioxidant, antimicrobial and cytotoxic properties of *Eclipta prostrata* aqueous extract, aiming to provide meaningful insights into their medicinal value and their potential in combating antibiotic resistance.

Materials and Methods

Procurement of medicinal herb *Eclipta prostrata*

Fresh leaves of *Eclipta prostrata* (L.) L. was collected from the Vellore region in Tamil Nadu, India. Once collected, the fresh leaves of Eclipta were rinsed with running tap water to eliminate dust and debris, subsequently rinsed with deionised water to ensure cleanliness. The leaves were then air dried in the shade at ambient temperature for 7–10 days, avoiding direct sunlight to preserve their phytochemical integrity, as recommended by Bimakr *et al*. (2011) [7]. After complete drying, the leaves were coarsely powdered using a mechanical grinder and stored in airtight containers until further extraction.

Preparation of aqueous extract of *Eclipta prostrata* medicinal herb

For aqueous extraction, 100 g of powdered leaf material of *Eclipta prostrata* was macerated in 300 mL of deionized and sustained at standard ambient temperature for 24-48 hours subjected intermittent agitation. The extract was subjected to filtration and lyophilized or evaporated under reduced pressure to obtain a dry aqueous extract, as described in the methods by Do *et al*. (2014) [9] and Panda and Padhi (2020) [19]. The dried extract was stored at 4°C in amber-colored bottles to protect it from light and degradation.

Antioxidant potential of the aqueous extract of *Eclipta prostrata* medicinal herb

1. DPPH Free Radical Scavenging Assay

To evaluate the antioxidant potential of Eclipta prostrata, the DPPH scavenging assay was conducted following the protocol of Williams *et al*. (1995) [26]. A 0.1 mM DPPH solution in methanol was freshly formulated, and different concentrations of the plant extract were mixed (an aliquot of 1 mL of extract was mixed with 1 mL of DPPH solution) and incubated in the dark at ambient temperature for 30 minutes. Absorbance was measured at 517 nm, and the percentage was determined using the standard formula;

DPPH Scavenging (%) = $[(A_0 – A_s) / A_0] \times 100$,

Where A_0 = absorbance of the control and A_s = sample.

2. Ferric Reducing Antioxidant Power (FRAP) Assay

According to the method of Benzie and Strain (1996) [6], the FRAP reagent was formulated by combining 300 mM acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a volumetric ratio of 10:1:1. A 100 µL aliquot of the plant water-based extract was combined with 900 µL of freshly formulated FRAP reagent and incubated for 30 minutes at 37°C. The absorbance, corresponding to the reducing capacity, was read at 593 nm.

3. ABTS Radical Cation Decolorization Assay

The ABTS was performed based on the method described by Re *et al*. (1999) [20]. The $ABTS^+$ radical cation was produced by reacting 7 mM ABTS with 2.45 mM potassium persulfate, followed by incubation for 12 to 16 hours in the

dark. A 1 mL aliquot of the resulting solution was combined with 10 µL of the plant extract. After 6 minutes, the absorbance was read at 734 nm to evaluate the antioxidant capacity.

4. Superoxide Radical Scavenging Assay

This assay followed the protocol of Nishikimi *et al*. (1972) [18]. The reaction mixture contained 0.1 mM EDTA, 0.1 mM xanthine, 0.1 mM NBT, and xanthine oxidase (0.1 units/mL) in phosphate buffer (pH 7.4). Xanthine oxidase initiated the reaction, which was allowed to proceed for 30 minutes. The absorbance was read at 560 nm to estimate superoxide scavenging.

5. Nitric Oxide Scavenging Assay

NO activity was evaluated following the protocol of Marcocci *et al*. (1994) [17]. A reaction mixture containing 10 mM sodium nitroprusside in phosphate buffer (pH 7.4) and the plant extract was maintained at ambient temperature for 15 minutes. Subsequently, 1 mL of Griess reagent was dropped to the mixture, and the absorbance was read at 546 nm. The nitric oxide inhibition % was calculated in a manner analogous to the DPPH assay.

6. Hydroxyl Radical Scavenging Assay

OH Scavenging potential was evaluated according to the method described by Halliwell *et al*. (1987) [12]. The reaction mixture consisted of EDTA, Fe Cl_3, H_2O_2, deoxyribose, ascorbic acid, and phosphate buffer. The plant extract was combined and the mixture was maintained at 37°C for 1 hour. After incubation, trichloroacetic acid (TCA) and Thiobarbituric acid (TBA) were added, followed by boiling the mixture for 10 minutes. The absorbance of the resulting chromogen was read at 532 nm to determine the extent of hydroxyl radical quenching ability.

7. Total Phenolic Content (Folin-Ciocalteu Method)

Phenol class compound concentration was calculated using the method described by Makkar *et al*. (1993) [16]. An aliquot of 0.1 mL of the plant extract was combined with 0.1 mL of 2N Folin–Ciocalteu reagent and 2.8 mL of 10% sodium carbonate solution. The reaction mixture was allowed to stand at ambient temperature for 40 minutes. Absorbance was then recorded at 725 nm. Gallic acid served as the standard, and the phenolic content was quantified in terms of mg GAE per gram of extract.

Antimicrobial activity of the aqueous extract of *Eclipta prostrata* medicinal herb

The antimicrobial potential of the water extract of *Eclipta prostrata* was evaluated using the agar well diffusion assay against selected bacterial strains such as *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* and fungal strains such as *Candida albicans, Fusarium oxysporum Aspergillus niger*, following the methodology described by Balouiri *et al*. (2016) [5]. Fresh plant extract was prepared in sterile distilled water at varying concentrations (100, 250, and 500 mg/mL). Bacterial and fungal cultures were cultured in NB and SDB, respectively, and incubated at 37°C (bacteria) and 28°C (fungi) until they reached the logarithmic growth phase. The microbial suspension was calibrated to match 0.5 McFarland turbidity standards to maintain consistent inoculum density.

Mueller Hinton Agar (MHA) was utilized for bacterial Strains, while Sabouraud Dextrose Agar (SDA) was utilized for fungal Strains. The test organisms were uniformly inoculated onto the agar plates using sterile cotton swabs. Wells of 6 mm diameter were aseptically punched using a sterile cork borer, and each well was loaded with 100 µL of the plant extract. Plates were pre-diffused at room temperature for 30 minutes and then incubated for 24 hours at 37°C for bacteria and 48 hours at 28°C for fungi. After incubation, the antimicrobial activity was determined by measuring the diameter of the inhibition zones in millimetres. Ciprofloxacin (10 µg/mL) and fluconazole (10 µg/mL) were used as standard controls for bacterial and fungal strains, correspondingly. Sterile deionized water functioned as the negative control.

Toxicity analysis of the aqueous Extract of *Eclipta prostrata* medicinal herb

Brine Shrimp (*Artemia salina*) Lethality Assay

Artemia salina bioassay was utilized to screen for cytotoxic activity in the water-based extract of *Eclipta prostrata* by the methodology described by Ameen *et al*. (2011) [2]. Test solutions were prepared in seawater containing 1 % DMSO. Thirty brine shrimp nauplii were inoculated to each well of a 24-well microplate along with 1 mL of the test solution. A negative control (seawater containing 1 % DMSO) and a positive control (100 µg/mL Potassium dichromate) were used in the experiment. The plates were maintained at a temperature of 25–30°C for 24 hours under continuous illumination. To determine mortality, immobile nauplii were counted under a microscope. The mortality percentage was estimated using the formula:

Mortality (%) = (Number of Dead Nauplii / Total Nauplii) × 100

Brine Shrimp Hatching Assay

Brine shrimp (*Artemia salina*) eggs were incubated in a prepared seawater solution for hatching (35 g NaCl/L) and kept under continuous light at 25–30°C for 24 to 48 hours (Ameen *et al*., 2011) [2]. The hatching success rate was evaluated using the following equation:

Hatching (%) = [Number of Nauplii / (Nauplii + Unhatched Eggs)] × 100

Results

Preparation of aqueous extract of *Eclipta prostrata* medicinal herb

Water – based extraction of *Eclipta prostrata* yielded a dark brown, semi-solid extract after soaking 100 g of powdered leaf material in 300 mL of deionized water at ambient temperature for 24–48 hours with intermittent agitation. Following filtration, the extract was lyophilized (or evaporated under reduced pressure), resulting in a dry aqueous extract. The final yield was stored at 4 °C in amber-coloured bottles to prevent photodegradation and maintain extract stability. The extraction process followed the methods outlined by Do *et al*. (2014) [9] and Panda & Padhi (2020) [19]. (Figure 1a & 1b)

Fig 1a: Fresh leaves of Medicinal herb *Eclipta prostrata*

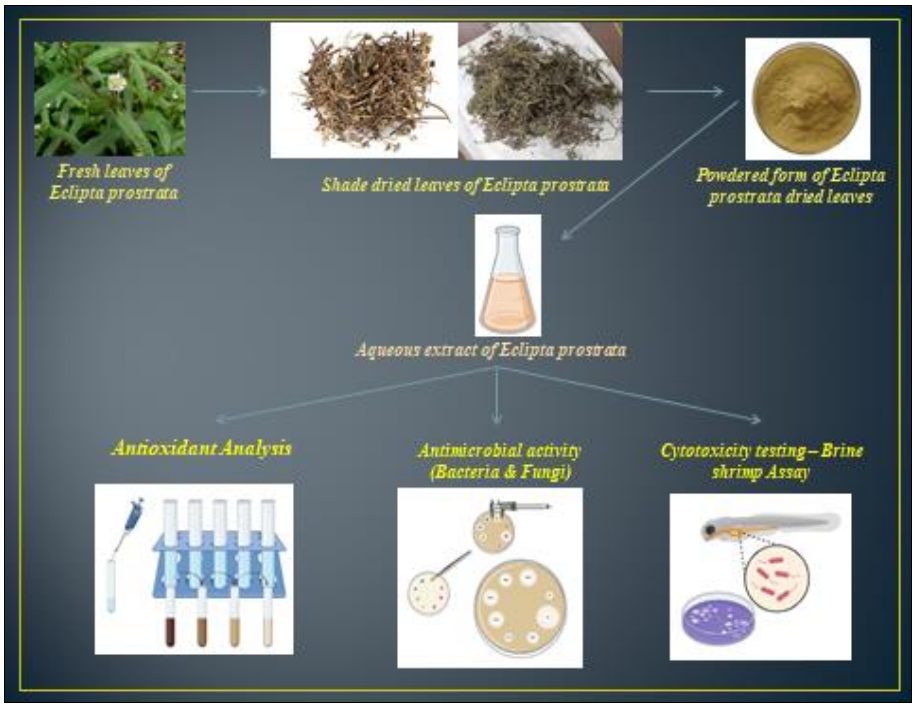


Fig 1b: Diagrammatic representation of the Antioxidant, Antimicrobial & Brine shrimp assay of *Eclipta prostrata*

Antioxidant potential of the water-based extract of *Eclipta prostrata* medicinal herb

The water-based extract of *Eclipta prostrata* leaves demonstrated notable antioxidant potential across several *in vitro* assays, with activity increasing consistently with concentration. In the DPPH radical scavenging assay, a steady enhancement in free radical neutralization was observed, starting at 20 % inhibition at 100µg/mL and reaching up to 80 % at 500µg/mL. Similarly, the FRAP assay showed a proportional rise in reducing power, with values increasing from 150 µmol Fe (II)/g extract at the lowest concentration to 750 µmol Fe (II)/g extract at the highest. The ABTS assay supported these findings, indicating scavenging activity that escalated from 25 % to 90 % across the same concentration range. Superoxide and nitric oxide radical scavenging assays also followed this trend, with inhibition rates rising from 15 % and 10 % at 100µg/mL to 75 % and 70 % at 500µg/mL, respectively. Although hydroxyl radical quenching activity was generally reduced compare to the other assays, it still showed a significant increase from 5% to 65% as the concentration increased. Total phenolic content also reflected the extract's antioxidant capability, ranging from 50 µg GAE/g extract at 100µg/mL to 250 µg GAE/g at 500µg/mL, confirming the phenolic compound's role in contributing to the observed antioxidant effects. (Table 1 & Figure 2)

Table 1: Antioxidant Activities of Aqueous *Eclipta prostrata* Extract

Conc. (µg/mL)	DPPH (%)	FRAP (µmol Fe/g)	ABTS (%)	Superoxide (%)	Nitric Oxide (%)	Hydroxyl (%)	Total Phenolic (µg GAE/g)
100	20	150	25	15	10	5	50
200	40	300	50	35	30	20	100
300	55	450	70	55	50	35	150
400	68	600	85	65	60	50	200
500	80	750	90	75	70	65	250

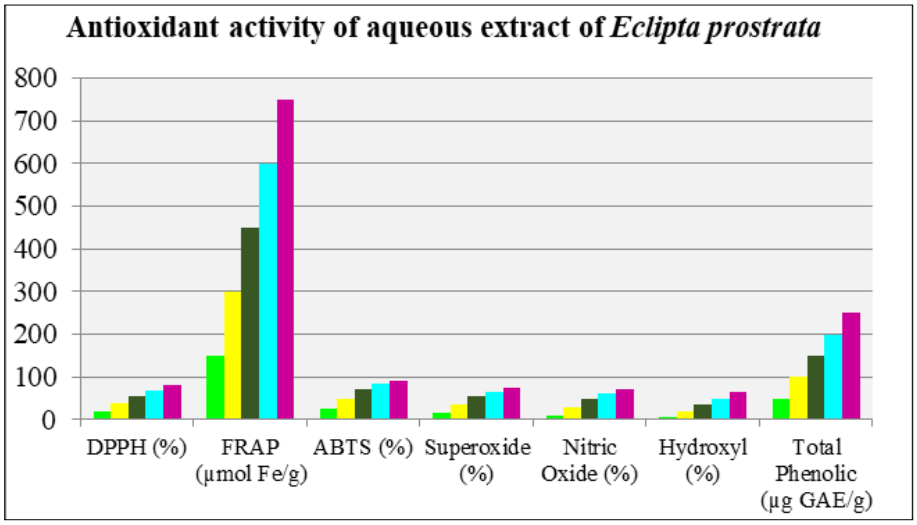


Fig 2: Antioxidant activity of aqueous extract of *Eclipta prostrata*

Antimicrobial efficacy of the water - based extract of *Eclipta prostrata* medicinal herb

The aqueous extract of *Eclipta prostrata* displayed strong antimicrobial activity, effectively acting against both Gram-positive and negative bacteria, along with several fungal species. Through the agar well diffusion assay, the extract produced zones of inhibition measuring 18 mm for *Staphylococcus aureus*, 16 mm for *Escherichia coli*, and 14 mm for *Pseudomonas aeruginosa* at a concentration of 500 mg/mL. These outcomes reflect the extract's potential as a broad-spectrum antibacterial agent. Antifungal efficacy was also noted against *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum*, with inhibition zones of 16 mm, 14 mm, and 12 mm, respectively. Overall, the findings substantiate the traditional medicinal application of *Eclipta prostrata* in managing microbial infections and underscore its promise as a natural antimicrobial source. (Table 2 & Figure 3)

Table 2: Antimicrobial Activity of aqueous extract of *Eclipta prostrata*

Microorganism Tested	Zone of Inhibition (mm)
Staphylococcus aureus	18
Escherichia coli	16
Pseudomonas aeruginosa	14
Candida albicans	16
Aspergillus niger	14

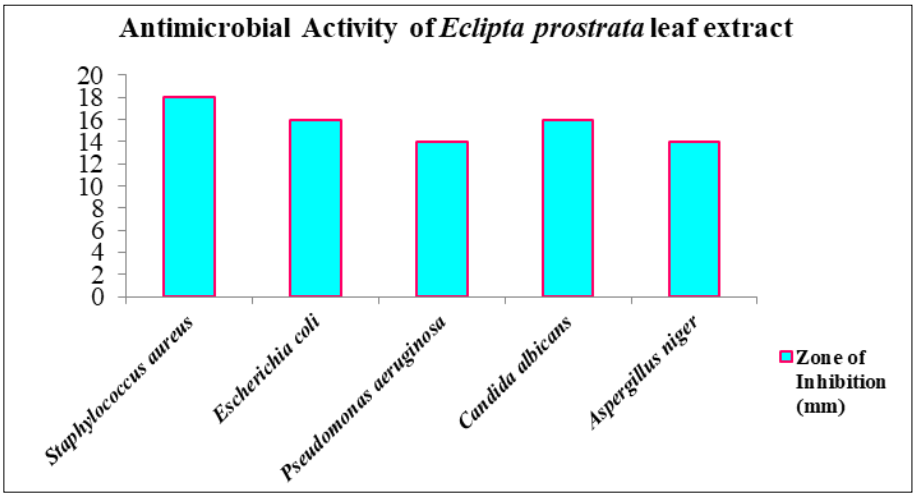


Fig 3: Antimicrobial Activity of *Eclipta prostrata* leaf extract

Artemia salina assay of the water-based extract of *Eclipta prostrata* medicinal herb

Toxic properties of the *Eclipta prostrata* aqueous extract were evaluated using the Brine Shrimp Lethality Assay, which revealed a concentration-responsive increase in lethality. At 20µg/mL, the extract caused a mortality rate of 16%, which gradually increased to 34 %, 37.6 %, and 44 % at 40, 60, and 80µg/mL, respectively. The highest test concentrations of 100µg/mL and 120 µg/mL demonstrated the most notable toxic effects, with mortality rates of 53.3 % and 66.6 %. These findings suggest that the extract contains bioactive compounds with potential cytotoxic or antitumor activity. Furthermore, the hatching percentage of Artemia salina nauplii was inversely proportional to extract concentration. The control group showed over 70 % hatching, while at 20µg/mL, the hatching percentage

dropped to 57 %. This further declined to 32.6 % and 22.6 % at 100µg/mL and 120µg/mL, respectively. The decrease in hatching success indicates the extract's possible toxicity on embryonic development at higher doses. (Table 3 & Figure 4, Figure 5)

Table 3: Cytotoxicity Assay (Brine Shrimp Lethality) of *Eclipta prostrata* aqueous leaf extract

Concentration (µg/mL)	Mortality (%)	Hatching (%)
20	16.0	57.0
40	34.0	51.0
60	37.6	42.6
80	44.0	37.0
100	53.3	32.6
120	66.6	22.6

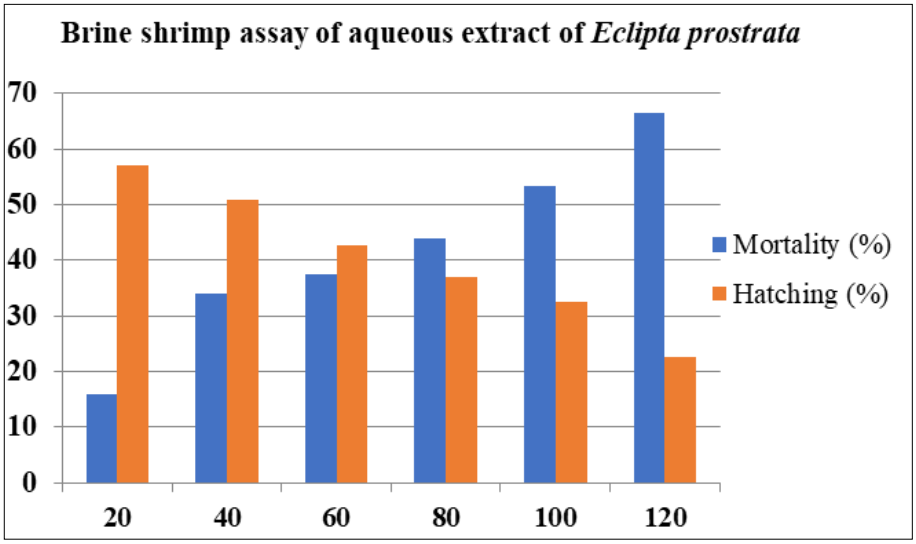


Fig 4: Brine Shrimp assay of aqueous leaf extract of *Eclipta prostrata*

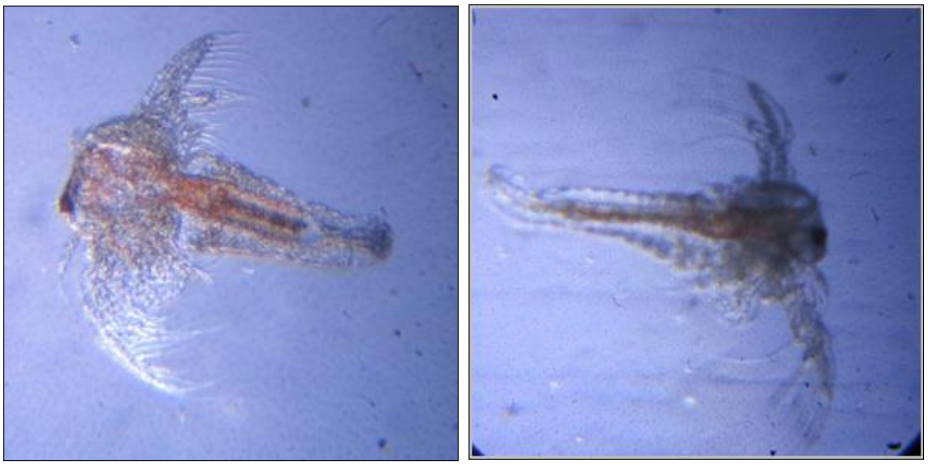


Fig 5: Brine shrimp larvae after exposure to concentrations of *Eclipta prostrata* medicinal herb aqueous extract

Discussion

The current study demonstrates that the aqueous extract of Eclipta prostrata possesses notable antimicrobial properties, showing effectiveness against a variety of bacterial and fungal species. This supports its traditional role in ethnomedicine. Zones of inhibition recorded were 18 mm for *Staphylococcus aureus*, 16 mm for *Escherichia coli*, and 14 mm for *Pseudomonas aeruginosa*, suggesting a broad-spectrum antibacterial potential. The antimicrobial effect is likely due to the existence of functional secondary metabolites such as flavonoids, tannins, alkaloids, and phenolic compounds. These compounds are recognized for their ability to damage microbial cell walls, disrupt enzymatic activity, or interfere with nucleic acid synthesis (Chung *et al*., 2017; Kim *et al*., 2015) [8, 14].

The aqueous extract of *Eclipta prostrata* also displayed promising antifungal activity, showing clear inhibition against *Candida albicans*, *Aspergillus niger*, and *Fusarium oxysporum*. These results align with previous studies reporting the antifungal efficacy of Eclipta prostrata and other medicinal plants rich in phytoconstituents (Albuquerque *et al*., 2019) [1]. Notably, *C. albicans*, a common opportunistic fungal pathogen, was found to be particularly susceptible, which suggests potential applications in treating fungal infections of mucosal and dermal origin.

The inhibitory or suppressive effect against a broad spectrum of both Gram-positive and negative bacteria suggests that the active compounds in the extract can penetrate a variety of bacterial cell envelopes. Gram-negative bacteria are often more tolerant to owing their outer lipopolysaccharide membrane; hence, inhibition of their growth demonstrates the potency of the extract (Duan *et al*., 2017) [10]. The antimicrobial action pathway may involve membrane disruption, enzyme inhibition, or ROS generation, as observed in studies involving flavonoid-rich plant extracts (Gao *et al*., 2018) [11]. Furthermore, the extract's antioxidant capacity demonstrated in this study may synergistically enhance antimicrobial action by reducing oxidative stress within microbial cells (Wang *et al*., 2021) [25]. In summary, these results validate the ethno medicinal application of *Eclipta prostrata* for treating infections and underscore its potential to serve as a source of natural antimicrobial agents.

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