

Exploring the Potential of Eclipta Alba against S. Typhi: Harnessing Nature Power for Health

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ABSTRACT

The present study investigated the in vitro antibacterial potential of Eclipta alba whole-plant extracts against Salmonella Typhi and evaluated the antibacterial activity of fractions obtained from the most active extract. The whole plant was extracted using petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous solvents by Soxhlet extraction. The obtained extracts were subjected to preliminary phytochemical screening and antibacterial evaluation using the agar well diffusion method. The ethanolic extract showed the highest extractive value (13.00% w/w) and exhibited the strongest antibacterial activity, producing inhibition zones of 12.50, 13.40, and 16.50 mm at concentrations of 10, 20, and 40 mg/mL, respectively. Phytochemical screening revealed the presence of carbohydrates, amino acids, glycosides, flavonoids, fixed oils and fats, tannins and phenolic compounds, and proteins in the extracts, with variable occurrence of alkaloids and steroids. The ethanolic extract was further fractionated by column chromatography into ten fractions. Among these, the ethanol fraction (F10) demonstrated the highest antibacterial activity (3.45 ± 0.55 mm), followed by F4 and F9. Overall, the findings demonstrate the antibacterial potential of E. alba and support further investigation for isolation and characterization of its bioactive antibacterial constituents.

Keywords: Eclipta alba; Salmonella Typhi; Antibacterial activity; Phytochemical screening; Ethanolic extract; Agar well diffusion; Column chromatography; Bioactive compounds.

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INTRODUCTION

Medicinal plants are a wonderful gift of nature to the mankind and have been utilises since traditional and modern times. It providing man with health maintenance and disease prevention solutions [1]. These plants have bioactive compounds that play an important role in pharmaceutical development, while their effects on human and animal health continue to spark curiosity and scientific research [2].

Eclipta alba (Linn.) Hassk [Synonym = *Eclipta prostrata* (Linn.)] is an herbal plant, grows in tropical and sub-tropical countries. In India, this widely recognized Ayurvedic plant is referred to as

‘Bhringaraj’. *Eclipta alba* (L.) is commonly known as false daisy [3]. is an annual herbaceous, erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes. The leaves are opposite to each other, sessile and lanceolate belonging to family Asteraceae [4].

In Ayurveda which has been in use for treating different ailments especially related to the liver and hair [5]. The herb has been known as antimycotoxic, analgesic, antibacterial, antihepatotoxic, anti-hemorrhagic, anti-hyperglycemic, antioxidant and immunomodulatory and rejuvenating properties [6]. Coumestan, wedelolactone, desmethylwedelolactone

as well as β -sitosterol present in this plant are known for their antihepatotoxic, antioxidant and anticancer activities [7]. The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus. The plant is commonly used in hair oil all over India for healthy black and long hair [8]. Also, plant is well documented and several in vitro and in vivo studies describe its anti-ageing agent and anti-hepatotoxic properties [9].

The present study was carried out to test the potential of *Eclipta alba* whole plant extract against *S. Typhi* in Acetone, Ethanol, Hexane and Aqueous extract by using ciprofloxacin as a standard [10].

MATERIALS AND METHODS

Plant Material

The whole plant of *Eclipta alba* belonging to family Asteraceae were collected during month of October to December from botanical garden at Nagpur, Maharashtra, India. The plant was cleaned in running tap water and shade dried at room temperature. The plant was authenticated from Department of Botanical Science, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The shade dried plant material was powdered by using electric blender.

Preparation of Plant Extracts

The whole dried plant of *Eclipta alba* were powdered. Weighed about 30g and extracted in Soxhlet apparatus using Petroleum Ether, Ethyl acetate, Chloroform, Ethanol and Aqueous solvent (Merk Chemical India) system. All the extract were obtained by evaporation at low temperature (50-60°C) to get percent practical yield 8.01%, 12.60%, 7.85%, 13.00 and 10.50% from Petroleum Ether, Ethyl acetate, Chloroform, Ethanol and Aqueous solvent respectively. The extract preserved in air tight container and kept below 4°C for further use. All the extracts were tested for antibacterial activity against *S. Typhi* by In-Vitro techniques.



Figure 1: Extraction of *Eclipta alba* plant by Soxhlet process.

Phytochemical Screening

Qualitative phytochemical screening was performed for the detection of reducing sugars, alkaloids, saponins, flavonoids, terpenoids, tannins, cardiac glycosides and anthraquinone with the use of the standard methods.

Test Organism

The pure culture of *S. Typhi* (Non-pathogenic strain) were obtained from Rajiv Gandhi Institute of Biotechnology, Nagpur, Maharashtra. The test organism was maintained on nutrient agar medium in department of microbiology at Kamla Nehru College of Pharmacy, Butibori, Nagpur, Maharashtra.

Culture Media and Inoculum Preparation

Nutrient Broth (Himedia, India) were used as the media for culturing the bacterial strain. A one loop full of bacterial culture was inoculated in the nutrient broth at 37°C for 24 hours [11].

Antibacterial Activity: Agar Well Diffusion Method

The extract obtained from plant (whole plant) were studied for antityphoid activity. A loopful of bacterial strain i.e. *S. Typhi* were inoculated in 50 ml of nutrient broth in conical flask and incubated for 24hrs to activate the strain. In agar well diffusion method, the media and the test organism were incubated in to petri plates. The test strain 0.25 ml was inoculated into the media. The experiment was conducted under strict aseptic conditions. After solidification of media, a well was made in media by using sterile borer (5 mm). The extracts (50ul) were introduced into the well and the plates were incubated at 37°C for 24hrs. The microbial growth was determined by measuring the diameter of zone of inhibition. Ciprofloxacin (25ug) was the reference drug used as control for test organism [12].

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The standard broth dilution method (CLSI M07-A8) was used to study the antimicrobial efficacy of *Eclipta alba* extract by evaluating the visible growth of *S. Typhi* in the agar broth. Serial two-fold dilutions of *Eclipta alba* extract in concentrations ranging from 1000 ug/ml to 31.25 ug/ml with adjusted bacterial concentration (10^8 CFU/ml, 0.5 McFarland's standard) were used to determine MIC in BHI broth. The control contained only inoculated broth and incubated for 24 h at 37°C. The MIC endpoint is the lowest concentration of *Eclipta alba* extract where no visible growth is seen in the tubes. The visual turbidity of the tubes was noted, both before and after incubation to confirm the MIC value [13].

After the MIC determination of the *Eclipta alba* extract, aliquots of 50 μ l from all the tubes which showed no visible bacterial growth were seeded on nutrient agar plates and incubated for 24h at 37°C. When 99.9% of the bacterial population is killed at the lowest concentration of an antimicrobial agent, it is termed as MBC endpoint. This was done by observing pre and post-incubated agar plates for the presence or absence of bacteria [14-15].

Fractionation of the Ethanolic Extract of *Eclipta alba* L.

Column Chromatography

Column chromatography was used to achieve the fractionation of the ethanolic extract of *Eclipta alba* plant. 5g of the extract was subjected to column chromatography to separate the extract into various component fractions. Silica gel of 120-150 mesh was used as the stationary phase, and the mobile phase was subjected into the column by increasing polarity i.e. petroleum ether, ethyl acetate, chloroform, ethanol.

In setting up of the column chromatography, the lower part of the glass column was stocked with a glass wool with the aid of glass rod. The slurry was prepared by mixing 25g of silica gel and 250ml of the solvent such as petroleum ether was poured down carefully into the column, the tap of the glass column was left open to allow flow of the solvent system into conical flask below, the set-up was seen to be in order when the solvent system drain freely without carrying the silica gel or glass wool along, at the end of the packing, the tap was locked, the column was allowed to settle, after which clear solvent was allowed to drain down to the silica gel meniscus. The column was set up using wet packing method [16].

Thin Layer Chromatography (TLC)

Each fraction that was eluted was subjected to thin layer chromatography (TLC) as per conventional one-dimensional ascending method using pre-coated silica gel plates. Plate markings were made with soft pencil. A Glass capillary (1microlitre) was used to spot the samples at an interval of 1cm on the thin layer chromatographic plate and was allowed to dry. The chromatographic tank containing the solvent system of Toluene: Acetone: Formic Acid (11:6:1) was allowed for 20 min to pre-saturate. The thin layer chromatographic plate was then lowered down into the chromatographic tank. The tank was tightly covered to prevent escape of the solvents. After the solvent have travelled a distance from the origin, the plant components separated, the plates were removed and allowed to dry. Another chromatographic tank containing iodine crystal which was well covered was allowed for 30min to pre-saturate. The chromatographic plates were inserted into the pre-saturated iodine crystal chromatographic tank for

visibility (clear resolution) of bands. After the thin layer chromatography was done, the separated plant components were clearly seen and their retardation factor was calculated using the formula: [17]

$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent front}}$

Antibacterial Activity of Column Fractions of Ethanolic Extract of *Eclipta alba* L.

Ten (10) fractions were obtained and were screened for their antibacterial activity by agar well diffusion method (As previously described).

Results and Discussion

The result of the extractive value for different solvent of *Eclipta alba* L. leaves shown in Table 1 below. Also result of qualitative phytochemicals present in different solvents are presented in table 2. While the result for the antibacterial activity of leave extracts of *Eclipta alba* is presented on Table 3 and antibacterial activity of column fractions (F1-F10) of crude methanolic extract is shown on Table 4.

Table. 1: Extractive values for different solvents

Sr. No.	Extracts	Extractive Value (%W/W)
1	Petroleum Ether	8.01
2	Chloroform	12.60
3	Ethyl Acetate	7.85
4	Ethanol	13.00
5	Aqueous	10.50

The extractive values of the plant material were determined using solvents of increasing polarity, namely petroleum ether, chloroform, ethyl acetate, ethanol, and water. The percentage yield of the extracts ranged from 7.85% to 13.00% (w/w).

Among all the solvents tested, the ethanolic extract exhibited the highest extractive value (13.00% w/w), followed closely by the chloroform extract (12.60% w/w) and the aqueous extract (10.50% w/w). The petroleum ether extract showed a moderate extractive value of 8.01% w/w, whereas the ethyl acetate extract produced the lowest extractive value of 7.85% w/w. The higher extractive value observed in the ethanolic extract suggests that ethanol is more efficient in extracting a broad spectrum of phytoconstituents, including phenolics, flavonoids, alkaloids, glycosides, tannins, and other polar to moderately polar compounds. The comparatively lower yield obtained with ethyl acetate indicates the presence of fewer phytochemicals with intermediate polarity. These findings demonstrate that solvent polarity significantly influences the extraction efficiency and yield of bioactive constituents from the plant material.

Table. 2: Preliminary phytochemical screening of *Eclipta alba* Extract

Sr. No.	Parameters	Observation
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		PE	CH	EA	ET	AQ
1	Carbohydrates	+	+	+	+	+
2	Amino Acids	+	+	+	+	+
3	Glycosides	+	+	+	+	+
4	Flavonoids	+	+	+	+	+
5	Fixed oil & fats	+	+	+	+	+
6	Alkaloids	-	+	-	-	+
7	Tannins & Phenol compound	+	+	+	+	+
8	Steroids	+	-	+	-	+
9	Proteins	+	+	+	+	+
10	Terpenes	-	-	-	-	-

The results indicate that the plant contains a diverse range of phytochemical constituents, with ethanol and aqueous extracts containing most of the biologically important phytochemicals. The presence of flavonoids, phenolic compounds, glycosides, and alkaloids suggests that the plant may possess significant pharmacological activities, including antioxidant, antimicrobial, anti-inflammatory, and hepatoprotective properties.

In-Vitro anti-microbial study

Table 3: The zone of inhibition produced by extracts Eclipta alba against S. typhi

Zone of Inhibition (mm)				
Extracts	Activity against S. typhi			
	10mg/ml	20mg/ml	40mg/ml	Standard (Ciprofloxacin 25ug/ml)
Petroleum Ether	9.50	10.00	12.30	20.50
Chloroform	11.00	11.80	14.70	20.60
Ethyl Acetate	9.70	10.20	12.50	20.50
Ethanol	12.50	13.40	16.50	23.50
Aqueous	10.20	10.70	13.20	20.30

The antimicrobial activity of petroleum ether, chloroform, ethyl acetate, ethanol, and aqueous extracts was evaluated against Salmonella Typhi using the agar well diffusion method. The antibacterial activity increased with increasing extract concentration, indicating a dose-dependent response.

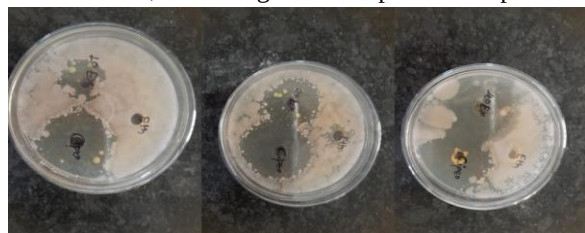


Figure 2: In-vitro antimicrobial study.

Among all the extracts tested, the ethanolic extract demonstrated the highest antibacterial activity, producing inhibition zones of 12.50 mm, 13.40 mm, and 16.50 mm at concentrations of 10, 20, and 40

mg/mL, respectively. The chloroform extract showed the second-highest activity, with inhibition zones ranging from 11.00 mm to 14.70 mm. The aqueous extract exhibited moderate antibacterial activity (10.20–13.20 mm), while petroleum ether and ethyl acetate extracts showed comparatively lower inhibitory effects.

The standard antibiotic Ciprofloxacin (25 µg/mL) produced inhibition zones ranging from 20.30 mm to 23.50 mm, confirming its superior antibacterial efficacy compared with the plant extracts.

Table 4: Antibacterial activity of column fractions (F) of Eclipta alba L.

Column Fraction	Solvents & Ratio	Organisms/Zone of Inhibition (in mm)
		<i>S. Typhi</i>
F1	Petroleum Ether (100%)	-
F2	Petroleum Ether:Ethyl Acetate (70:30)	-
F3	Petroleum Ether:Ethyl Acetate (30:70)	2.15±0.45
F4	Ethyl Acetate (100%)	2.85±0.25
F5	Ethyl Acetate:Chloroform (70:30)	2.35±0.55
F6	Ethyl Acetate:Chloroform (30:70)	-
F7	Chloroform (100%)	-
F8	Chloroform:Ethanol (70:30)	2.25±0.80
F9	Chloroform:Ethanol (30:70)	2.75±0.75
F10	Ethanol (100%)	3.45±0.55

Note: Values are presented as mean ± standard deviation of three replicates.

The results obtained from the antibacterial activity of the five extracts showed that ethanolic extract has the highest antibacterial activity against the tested organisms compared to other extracts (table 3). Hence, ethanolic extract was subjected to column and Thin Layer Chromatography, after which ten fractions were obtained.



Figure 3: Isolation of Ethanolic Extract of *Eclipta alba* by column chromatography.

The antibacterial activity of the ten column chromatographic fractions (F1–F10) obtained from the extract was evaluated against *Salmonella Typhi*. Among the ten fractions, six exhibited measurable antibacterial activity, whereas four fractions (F1, F2, F6, and F7) showed no detectable inhibitory effect.

The ethanol fraction (F10) exhibited the highest antibacterial activity with a zone of inhibition of 3.45 ± 0.55 mm, followed by the ethyl acetate fraction (F4) (2.85 ± 0.25 mm) and the chloroform:ethanol (30:70) fraction (F9) (2.75 ± 0.75 mm). Moderate antibacterial activity was observed in F5 (2.35 ± 0.55 mm), F8 (2.25 ± 0.80 mm), and F3 (2.15 ± 0.45 mm).

The absence of antibacterial activity in F1, F2, F6, and F7 suggests that these fractions either lacked active phytoconstituents or contained them at concentrations below the detectable level. Overall, the results indicate that the bioactive compounds responsible for antibacterial activity were predominantly eluted in the more polar solvent systems, particularly ethanol-containing fractions.

Column chromatographic separation successfully fractionated the extract into ten fractions with varying antibacterial activities against *Salmonella Typhi*. The observed differences in activity among the fractions indicate that the antibacterial phytochemicals were selectively distributed based on their polarity and affinity for the solvent system used during elution.

Among all fractions, F10 (100% ethanol) demonstrated the greatest antibacterial activity, suggesting that the active compounds possess relatively high polarity. This finding is consistent with the earlier observation that the crude ethanolic extract exhibited the strongest antibacterial effect, indicating that ethanol effectively concentrates the principal antibacterial constituents. These compounds

are likely to include flavonoids, phenolic compounds, tannins, and glycosides, which are well known for their ability to inhibit bacterial growth through disruption of cell membranes, inhibition of enzyme activity, interference with nucleic acid synthesis, and induction of oxidative stress.

Fractions F4 and F9 also exhibited appreciable antibacterial activity, indicating that moderately polar compounds may also contribute to the antibacterial effect. Conversely, the inactivity of petroleum ether-rich fractions (F1 and F2) and the chloroform fraction (F7) suggests that non-polar constituents have limited activity against *S. Typhi*. The absence of activity in F6 further indicates that not all intermediate-polarity fractions contain bioactive compounds in sufficient concentrations.

Conclusion

The antibacterial activity demonstrated against the tested bacterial strains indicates that the plant may serve as a promising source for the development of broad-spectrum antimicrobial agents. The findings of the present investigation further support the traditional medicinal use of the plant and suggest that its extracts contain bioactive compounds with significant antibacterial properties. These constituents may have potential applications in the development of novel antibacterial formulations for the management of gastroenteritis, typhoid fever, and wound infections. Furthermore, detailed characterization and isolation of the active constituents are required to identify the specific compounds responsible for the observed antibacterial activity.

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Conflict of Interest

The authors declared that they have no known conflict of interest.

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