



Phytochemical and Antimicrobial Activity of Methanolic Leaf Extract of *Eclipta prostrata L.*

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Abstract: *Eclipta prostrata L.* is a widely distributed medicinal plant belonging to the Asteraceae family. The plant is rich in many volatile compounds and several bioactive components that aid the potential pharmacological activity of the plant. The knowledge about the presence of such bioactive compounds in plants is important to derive the application and economic importance of such plants. The present study aims to evaluate the phytochemical profile of *E. prostrata L.* by the qualitative and quantitative methods. The phytochemical profiling was done by standard screening procedure, while the antimicrobial potential in the methanolic leaf extract was determined by the agar well diffusion method. The phytochemical profiling revealed that the methanolic leaf extract contains phenols, alkaloids, flavonoids, glycosides, and saponins. The leaf extract is also a rich source of alkaloids, phenols, carbohydrates, and reducing sugars as suggested by the quantitative analysis. The methanolic extract at its low concentration of 50mg/ml was found to be effective against the selected bacterial pathogens and among them it was highly effective against *Staphylococcus aureus*. But the extract lacked any antifungal potential against selected isolates, *Candida albicans*, and *Aspergillus flavus*. The study emphasizes the pharmacological importance of the selected plant by revealing its potential bioactive compound profile and its antimicrobial activity. In future, the investigation can focus on determining the phytochemical profile of the plant in other solvent systems. Isolation, purification, and application of the active constituent can be studied further. The compound profile of extracts could reveal the exact information of the metabolites present in them, which will help derive its applications. This information could help deduce the methodology to purify or concentrate the respective bioactive compound in the extract of *E. prostrata L.*

Keywords: *E. prostrata*, Methanolic extract, Phytochemical profiling, Quantification, Antimicrobial activity.

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I. INTRODUCTION

Medicinal plants are the richest bio-resource of drugs from the traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs. The plants have great potential uses, especially as traditional medicine and pharmacological drugs. Dependency on traditional medicines has been shown by a large proportion of the world population¹. Medicinal plants are useful for healing as well as for the curing of human diseases because of the presence of phytochemical constituents. The knowledge of the chemical constituents of plants is desirable because such information will provide insight into the synthesis of complex chemical substances. Therefore, there is the need to look inwards to search for herbal medicinal plants to validate the ethnomedicinal use and subsequently the isolation and characterization of compounds that will be added to the potential list of drugs². *Eclipta prostrata* L, is a medicinal plant that belongs to the Asteraceae family and is widely distributed throughout Asia and commonly found in the marshy lands, hedges, and roadsides³. It is an aromatic plant containing a rich volatile component that might be responsible for its pharmacological potential⁴. The traditional use of this plant includes blackening and growth promotion of hair, strengthening of hair, anti-venom against snake bites, and as a cholagogue and deobstruent in hepatic enlargement⁵. The herb is also known for its medicinal value as an analgesic, antiseptic, antiviral, antibacterial, antioxidant, anti-hemorrhage, and anti-hyperglycemic agent. The juice of the plant with honey is given to infants for catarrh⁶. The chloroform extracts also exhibits a significant anti-diabetic activity. The plant also shows immunomodulatory actions and is, therefore, used as a potential memory modulator⁷. The main class of bioactive compounds identified in *E. alba/prostrata* includes coumestans; these phytoestrogens are phenolic substances with structures similar to naturally occurring human steroid hormones⁸. This plant also contains other constituents, such as polypeptides, polyacetylenes, thiophenes derivatives, steroids, and terpenoids⁹. Coumestans are found in various plants, and this class is present in several naturally occurring products with diverse biological activities, including antibacterial, antifungal, and mycotoxic activities¹⁰. The herb is commonly known as a false daisy and bhringraj and is well known for its hair growth-promoting capacity. This herb is also reported to have antihepatotoxic, antihyperglycemic, immunomodulatory antipyretic, antioxidant, antibacterial, anthelmintic, antihepatotoxic, anti-inflammatory, antihemorrhagic properties¹¹. The constituents are playing a significant role in the identification of crude drugs. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such a medicinally important plant.

2. MATERIAL AND METHODS

Extract Preparation

Fresh young and green leaves of *E. prostrata* were collected and then washed for 5 minutes with 5% mercuric chloride solution followed by four times washing under distilled water. The leaves were identified with the help of authentic taxonomic literatures like Botany of Bihar and Orissa by H.H. Hains¹² and also confirmation was done by botanist Md. Sarfaraz Ahmad, Associate Professor and Head, Department of Botany, Jai Prakash University, Chapra, Bihar. The leaves were dried under shade and finally grounded into a fine

powder. The powder was used for the preparation of methanol extract by the soxhlet method, where 5 cycles were run for the extraction. The resultant concentrated extract was used for further analysis and study.

Qualitative Analysis

1. Alkaloids

Dragendorff's test: In 2 ml solution of extract in a test tube 0.1ml of dil. HCl and 0.1ml of Dragendorff's reagent was added. The development of orange-brown colored precipitate suggested the presence of alkaloids¹³.

2. Flavonoids

Shinoda test: About 0.5 ml of extract was dissolved in methanol, warmed, and then filtered. Three pieces of magnesium chips were then added to the filtrate followed by a few drops of conc. HCl. A red to purple coloration indicates the presence of flavonoids¹⁴.

3. Steroids

Liebermann Burchard Test: For this test 10 ml of chloroform was added to 1ml of the plant extract. To this mixture, an equal volume of concentrated sulfuric acid was added by sides of the test tube. The tubes were observed for the upper layer with red color while the lower layer of sulfuric acid turns yellow with green fluorescence indicating the presence of steroids¹⁵.

4. Phenolic Compounds

Ferric chloride test: 0.5 ml of the extract was treated with a few drops of 5% aqueous ferric chloride solution. The formation of deep blue-black color indicates the presence of phenolic compounds¹⁶.

5. Saponins

Froth test: 10ml of the extract was made using 2gm of the powdered extract and water. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with a few drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion¹⁷ which indicates the presence of saponins..

6. Terpenoids

Salkowski test: 2 ml of extract was treated with an equal volume of acetic anhydride. This followed the addition of a few drops of concentrated sulfuric acid and observed for the formation of blue-green rings that indicates the presence of terpenoids¹⁵.

7. Glycosides

To the methanol extract a few drops of glacial acetic acid, ferric chloride, and conc. H_2SO_4 acid was added. The mixture was then observed for a reddish-brown color at the junction of two layers and bluish-green color in the upper layer shows the presence of glycosides¹⁴.

Quantitative Analysis

• Determination of Alkaloids

Determination of total alkaloid content in the extract was done by the method described by Shamsa et al.¹⁸. The plant extract (1 mg/ml) was dissolved in 2 N HCl and then filtered. The pH of the phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. One ml of the extract solution was transferred to a separating funnel and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. This mixture was shaken properly and the complex so formed was extracted with chloroform by vigorous shaking. The chloroform layer was collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm.

• Determination of Phenols

Total phenolic content was determined using the Folin-Ciocalteu reagent¹⁹. Folin-Ciocalteu colorimetry is based on a chemical reduction of the reagent, a mixture of tungsten and molybdenum oxides. The products of the metal oxide reduction have a blue color with absorption maxima at 765 nm. The intensity of light absorption at that wavelength is proportional to the concentration of phenols. By using the standard Gallic acid calibration curve, the concentration of phenolic content in extract was measured as Gallic acid total equivalents using the units mg/gm (GAE).

• Determination of Cyanide

Five grams of each sample was dissolved in 50ml distilled water in a corked conical flask to extract cyanide. The cyanide extraction was allowed to stay overnight. The extract was filtered through filter paper. Alkaline picrate solution was prepared by dissolving 1g of picric acid and 5g of sodium carbonate in warm distilled water in a 200ml volumetric flask. To 1 ml of the sample extract 4ml alkaline picrate solution was added and this was incubated in a water bath for 5min for color development. After the development of the reddish-brown color, the absorbance of the solution was read at 490 nm in a spectrophotometer. The standard cyanide solution was prepared from different concentrations of potassium cyanide solution. The concentration of the Cyanide was expressed in mg/ml.

• Determination of Total Soluble Carbohydrates

The total sugars were estimated following the Phenol-sulphuric acid method described by Dey²⁰. The plant material (0.250mg of oven-dried plant powder) was suspended in 20ml of 90% methanol in a 50 mL conical flask. The test tubes were sealed with a cork and the suspension was incubated in a hot water bath and maintained at 60°C for one hr. The extract was decanted and collected in a 25ml capacity volumetric flask and re-extracted with another 10ml volume of 90% methanol. Both the extracts were collected and the final volume was made 25ml with 90% methanol. For the estimation purpose, 0.2ml plant extract was taken in a test tube and to this 1ml 5% phenol was carefully added and mixed thoroughly. 5ml of concentrated sulphuric acid (analytical grade) was added very carefully to the above test tube. This was mixed thoroughly

by vertical agitation with a glass rod with a broadened end. The mixture was cooled at room temperature in air and the absorbance was read at 485 nm against the blank containing distilled water instead of extract. The number of soluble sugars was estimated with the help of standard curve of glucose and expressed in mg/ml.

• Determination of Reducing Sugars

The reducing sugars were estimated by employing arseno-molybdate reagent introduced by Nelson²¹ for colorimetric determination of the cuprous oxide, formed in the oxidation of the sugars by alkaline copper tartrate reagent. The soluble carbohydrates were extracted from 0.250 mg of powdered leaf sample with 80% neutral ethanol. The extract was filtered through Buckner's funnel using Whatman No. 1 filter paper. The filtrate thus obtained was condensed to 5 ml in a water bath and to this 2gm lead acetate and potassium oxalate (1:1) were added for decolorization, then 40 ml distilled water was added and the solution was filtered. The volume of the filtrate was measured and it served as an extract for the determination of reducing sugars. To the 0.4mL this extract requisite amount of distilled water was added to make the final volume to 1 ml. Then 1 ml of Somogyi's alkaline copper tartrate reagent (4g CuSO₄, 5H₂O, 24g anhydrous Na₂CO₃, 16g Na-K-tartarate, and 180g anhydrous Na₂SO₄ dissolved in 1 liter distilled water) was added to each test tube and all these test tubes were transferred to boiling water bath for 10 minutes. Tubes were removed and cooled to room temperature and to them 1 ml of Arseno-molybdate reagent (25g Ammonium molybdate dissolved in 450 ml distilled water, 3g sodium arsenate dissolved in 25ml distilled water, 21 ml concentrated HCl. These ingredients were mixed well and digested for 48 hours at 37°C) was added to each test tube. The contents were diluted to 10mL with distilled water and after 10 minutes, the absorbance of the reaction mixture was measured at 660nm on a UV-visible double beam spectrophotometer. The amount of reducing sugar was calculated with the help of a standard curve obtained by using different concentrations of glucose solution.

Determination of the Antimicrobial Activity

The extract was used for determining its antimicrobial potential against various bacterial and fungal species using the Agar well diffusion method. For this process extract solution of 50 mg/ml in methanol was prepared to be used as a sample. The organism selected for this study includes *Staphylococcus aureus* (MTCC96), *Pseudomonas aeruginosa* (MTCC424), *Klebsiella pneumoniae* (MTCC530), *Candida albicans* (MTCC183), and *Aspergillus flavus* (MTCC277). For this, 0.1ml of an overnight grown culture of the selected organism was spread on the fresh culture media plates and then wells were punctured using a well borer. The wells were filled with extract, positive control and, negative control in respective wells where positive control was filled with antibiotic ciprofloxacin (50ppm) for bacteria and antifungal agent fluconazole (50ppm) for fungus. After this, the plates were incubated overnight and the next day were checked for the appearance of a clear zone around the sample extract well called the zone of inhibition. The diameter of this zone was measured in mm depicting the strength of antimicrobial potential carried by the extract.

RESULTS

In the present study, the medicinal plant *Eclipta prostrata* was considered to be analyzed for its phytoconstituents as they are the ones responsible for the pharmacological activity of the plants. The extract obtained by the soxhlet method was firstly undertaken for the qualitative analysis by standard procedures. The analysis revealed that the methanolic extract contains bioactive compounds like phenols, alkaloids, flavonoids, glycosides, saponins, and steroids (Table 1). Then the extract was used for the quantitative determination of some of the potential phytochemical compounds. The quantification result

(Table 2) depicts that the methanolic extract has a rich content of Alkaloids, phenols, carbohydrates, reducing sugar, and cyanide. The presence of such potential compounds indicates the pharmacological importance of the plant. The antimicrobial potential of the methanolic extract was assessed against active pathogens by the agar well diffusion method (Figure 1). The methanolic extract was found to be effective against bacterial isolates of the selected pathogens but did not show any significant results against fungal species. The results were in quite a good accordance with the positive control (Table no 3) and the extract was highly effective against *S. aureus*.

Table no. 1 Qualitative analysis of phytochemical in the methanolic leaf extract of *Ecliptaprostrata*

S. No	Phytochemical	Result
1	Alkaloids	+
2	Flavonoids	+
3	Glycosides	+
4	Saponins	+
5	Steroids	+
6	Terpenoids	-
7	Phenols	+

***(+)** Presence **(-)** Absence

Table no. 2 Quantification of phytochemical in methanolic leaf extract of *Ecliptaprostrata*

S. No.	Phytoconstituents	Concentration/amount
1	Alkaloids	30 mg/gm
2	Phenols	24.4 mg/gm
3	Cyanide	5.94 mg/ml
4	Soluble Carbohydrates	4.91 mg/ml
5	Reducing sugar	1.72mg/ml

Table no. 3 Antimicrobial potential of methanolic leaf extract of *E. prostrata* against selected pathogen

Sample name	Zone of inhibition in mm				
	<i>K. pneumonia</i> (T1)	<i>P. aeruginosa</i> (T2)	<i>S. aureus</i> (T3)	<i>C. albicans</i> (T4)	<i>A. flavus</i> (T5)
Methanol extract 50mg/ml	25	15	20	Nil	Nil
Positive control	35	35	35	18	15

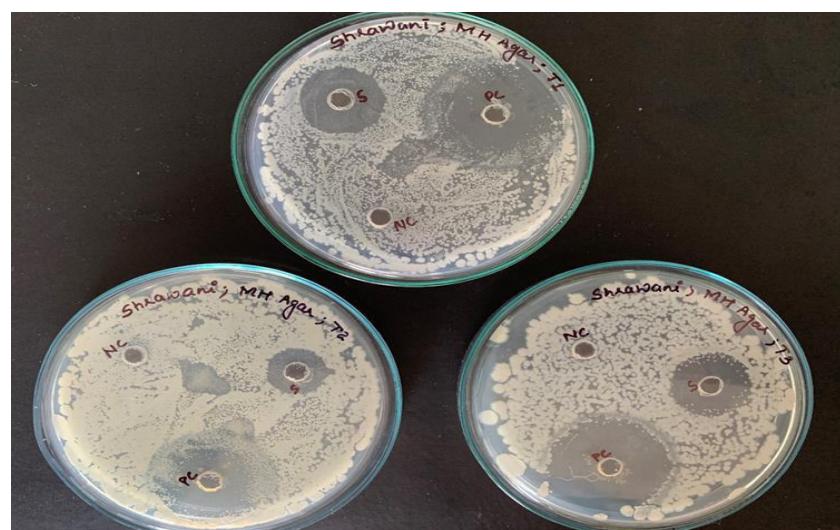


Fig 1: Antibacterial effect of methanolic extract of *E. prostrata* using Agar Well Diffusion method against test organisms T1 (*K. pneumonia*), T2(*P. aeruginosa*) and T3 (*S. aureus*); NC- Negative control (methanol); PC- positive control (50 ppm ciprofloxacin); S – sample (Methanolic extract 50mg/ml)

Traditional medicine is also known as indigenous or folk medicine that comprises medical knowledge systems developed over generations within various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or a combination of more than one plant. Indian contribution to the herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for the medicinal activity of plant species²². Hence, in the present study phytochemical screening of *E. prostrata* L. was carried out, the qualitative and quantitative phytochemical analysis of this plant confirms the presence of different potential bio-active compounds. On phytochemical screening of *E. prostrata* leaves, the presence of steroids, glycosides, saponins, flavonoids, alkaloids, and phenols was detected. This result was in accordance with the findings of Arunachalam et al.²³ and also with the findings of Dhandapani et al.²⁴ who reported the presence of steroids, phenols, reducing sugars and carbohydrates in the methanolic and aqueous extract. The differences may be due to the solvent capacity to extract the active principles, the difference in the extraction methods, and collection time. *Eclipta prostrata* has significant antimicrobial activity against common pathogens due to the wedelolactone components²⁴. Similar studies elsewhere also recorded that the ethanol aerial parts extract of *Eclipta alba* revealed high antibacterial activity against *S. aureus*, *E. coli*, and *S. typhi*^{26, 27}. The present findings also reported the antimicrobial potential of the extract against some of the active bacterial and fungal pathogens. From the investigation carried out by Pandey et al.,²⁸ it was clear that the solvent of extraction and method of extraction affected the degree of antimicrobial activity.

CONCLUSION

The present findings clearly showed that the selected plant *Eclipta prostrata* extract is a rich source of various effective

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