

In vitro Antibacterial Activity of Alkaloids Isolated from Leaves of *Eclipta alba* Against Human Pathogenic Bacteria

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ABSTRACT

The susceptibility of five human pathogenic bacterial species to an alkaloids isolated from the leaves of this plant was screened using the agar well diffusion and broth micro-dilution assay. The purity of isolated alkaloids was checked by TLC and qualitative phytochemical analysis and total alkaloids were quantified. In the present study, the inhibitory action of the alkaloid was found to increase with an increase in concentration against all bacterial strains. The maximum zone of inhibition was observed at the concentration of 500 µg/ml against all the bacteria. In this study, the *S. aureus* and *E. coli* are the more susceptible than the other selected human pathogenic bacteria. Based on the observations, *E. alba* appears to be a valuable source for antimicrobial properties and helps to produce antimicrobial agents to treat human pathogenic infections. **Context:** Different parts of *Eclipta alba* crude extracts are used traditionally for the treatment of several diseases of liver, skin and stomach. **Aims:** To study the *in vitro* antimicrobial activity of alkaloids isolated from *E. alba* against human pathogenic bacteria. **Settings and Design:** The susceptibility of five human pathogenic bacterial species to an alkaloids isolated from the leaves of this plant was screened using the agar well diffusion and broth micro-dilution assay. **Methods and Material:** The purity of isolated alkaloids was checked by TLC and qualitative phytochemical analysis and total alkaloids were quantified. Agar-well diffusion and Broth micro-dilution methods were selected to assess the antimicrobial activity. **Statistical analysis used:** One-way analysis of variance (ANOVA) was used to determine statistical significance. **Results:** In the present study, the inhibitory action of the alkaloid was found to increase with an increase in concentration against all bacterial strains. The maximum zone of inhibition was observed at the concentration of 500 µg/ml against all the bacteria. In this study, the *S. aureus* and *E. coli* are the more susceptible than the other selected human pathogenic bacteria. **Conclusion:** Based on the observations, *E. alba* appears to be a valuable source for antimicrobial properties and helps to produce antimicrobial agents to treat human pathogenic infections.

Key words: *Eclipta alba*, Alkaloid, Human pathogenic bacteria, Antimicrobial activity, Agar-well diffusion, Broth micro-dilution assay.

Key Messages: The results of the present study support the medicinal usage of the alkaloids isolated from the leaves of *E. alba* can be subjected to identification and isolation of the therapeutic antimicrobials and undergo further pharmacological screening that can be used as sources for new drugs.

INTRODUCTION

In the modern medicinal system, people have started looking at the ancient healing systems like Ayurveda, Siddha and Unnani due to the adverse effects associated with synthetic drugs. Especially in developing countries, herbal drugs play an important role in health care programs. The remarkable broad definition for medicinal plants was incorporated by the ancient Indian literature and it considered all plant parts to be potential sources of medicinal components.¹ A diverse array of chemicals are synthesized by medicinal plants. In hepatic and spleen enlargement *Eclipta alba* is used as a tonic and diuretic and it is also used for catarrhal jaundice and for skin diseases.² Plant-derived traditional medicines can be used to treat different diseases as they contain a vari-

ety of secondary metabolites to which the bacterial species may not be resistant.

The plant *Eclipta alba* (L.) is commonly known as false daisy which is annual herbaceous and belonging to Asteraceae family. The leaves of this plant are opposite, lanceolate and sessile. The plant is an erect, roughly hairy, annual, much branched and rooting at the nodes. It is also known as Bhringaraj and Karisalakanni, which is found a common weed throughout India ascending up to 6000 ft. *E. alba* has been used in various parts of tropical and sub-tropical regions like south America, Asia, Africa. The plant is commonly used in hair oil all over India for healthy black and long hair.³ This plant is well documented and several *in vitro* and *in vivo* studies describe its anti-ageing

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agent and anti-hepatotoxic properties.⁴ Coumestans like wedelolactone, desmethyl wedelolactone,⁵ furano-coumarins, ecalbatin,⁶ eleanane & taraxastane glycosides,^{7,8} are the consisting main active principles in *E. alba*. The safety, efficacy and quality of some of bioactive principles have not been scientifically validated and antibacterial activity was also not studied for isolated phytochemicals present in it. Therefore the objective of this present study is to study the antimicrobial activity of alkaloids isolated from the leaves of *E. alba* plant.

MATERIALS AND METHODS

Plant material and alkaloids extraction

Leaves of *E. alba* were collected in Kakatiya University Campus in July 2016. The plant was botanically authenticated by Prof. V.S. Raju, Senior Taxonomist at Department of Botany, Kakatiya University, India. The collected sample was grinded and this ground sample (500 g) was made alkaline with 30% ammonia and extracted with chloroform at room temperature for a total period of 24 h. The extract was partitioned between chloroform and 5% HCl. Finally chloroform was totally evaporated from the organic phase to form the alkaloid powder (5.2 gm).

Thin layer chromatography (TLC)

To determine the purity and relative to front (Rf) of isolated compound, a thin layer chromatography was carried out as per conventional one dimensional ascending method using silica gel 60F254, 7x6 cm (Merck) were cut with ordinary household scissors. Plate marking were made with soft pencil. Glass capillaries were used to spot the sample for TLC. Applied 1 µl of extract by using capillary at distance of 1cm. After pre-saturation with mobile phase (chloroform:methanol- 0.5:9.5) for 20 minutes the plates were dried and the spot which appeared were developed with iodine vapour. The movement of sample was expressed by its retention factor (Rf) and values were calculated as: Rf = Distance travelled by the solute/Distance travelled by solvent front TLC plates

Qualitative Determination of Phytochemicals

Qualitative phytochemical analyses of the isolated alkaloid were performed by following the protocol of J.B. Harborne (1973).⁹

Tannins: 200 mg of plant material was boiled in 10 mL distilled water and few drops of FeCl₃ were added to the filtrate; a blue-black precipitate indicated the presence of Tannins.

Alkaloids: 200 mg plant material was boiled in 10 mL methanol and filtered. 1% HCl was added than 6 drops of Dragendorff reagent was added, and brownish-red precipitate was the indication for the presence of alkaloids.

Saponins (Frothing test): 5 mL distilled water was added to 200 mg plant material. 0.5 mL filtrate was diluted to 5 mL with distilled water and shaken vigorously for 2 minutes. Formation of stable foam indicates the presence of saponins.

Cardiac Glycosides (Keller-Kiliani test): 2 mL filtrate was treated with 1 mL glacial acetic acid containing few drops of FeCl₃. Conc. H₂SO₄ was added to the above mixture giving green-blue colour depicting the positive results for presence of cardiac glycosides.

Steroids (Liebermann-Burchard reaction): To 10 mL chloroform 200 mg plant material was added. In the ratio of 1:1, Acetic anhydride was added which resulted into the formation of blue-green ring pointing towards the presence of steroids.

Terpenoids (Salkowski test): 2 mL of chloroform (CHCl₃) and 3 mL of concentrated sulphuric acid (H₂SO₄) were carefully added to 200 mg plant material. A reddish brown colouration signified the presence of terpenoids.

Flavonoids: To the aqueous filtrate 5 mL of dilute ammonia solution was added, followed by concentrated H₂SO₄. A yellow colouration indicated the presence of flavonoids.

Anthraquinones: 500 mg of dried plant leaves were boiled in 10% HCl for 5 mins and filtrate was allowed to cool. Few drops of 10% NH₃, equal volume of CHCl₃ was added to 2 mL filtrate. The formation of rose-pink colour implies the presence of Anthraquinones.

Reducing Sugars: To the 10 mL of aqueous extract a few drops of Fehling's solution A and B were added; an orange red precipitate suggests the presence of reducing sugars.

Quantitative Determination of alkaloids

The content of alkaloids was measured by following the protocol described by Harborne (1984)¹⁰ To prepare the suspension, dispersing 5 gm of the dried leaves in 10% acetic acid solution in ethanol and kept at 28°C for 4 hrs which was further filtered through Whatman Number 42. Alkaloid was precipitated by concentrating the filtrate to one quarter of its original volume and then drops of conc. aqueous NH₄OH were added. Finally the precipitate was washed with 1% ammonia solution and dried at 80°C in the oven. The alkaloid content alkaloid was calculated and expressed as mg/gm of sample.

Bacterial Cultures

Clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella boydii*, *Staphylococcus aureus* and *Streptococcus faecalis* were obtained from the Department of Microbiology, Kakatiya University, Telangana State, India. On nutrient agar slopes (Hi-media) all the test strains were maintained and were sub-cultured once in every two-week. These bacteria served as test pathogens for antibacterial activity assay.

Antibacterial assays

Agar-well diffusion

The assay was conducted as described by Perez *et al.* (1990). Briefly, on nutrient agar microorganisms from growth are incubated at 37°C for 18 h and were suspended in saline solution 0.85% NaCl. To inoculate the suspension 90 mm diameter petri plates with a sterile non-toxic cotton swab on a wooden applicator was used. In the agar six mm diameter wells were punched and filled with 50 µL of different concentration (125, 250 and 500 µg/ml) of alkaloid sample. According to our control experiments, 1% (v/v) DMSO dissolution of the alkaloids was aided which did not affect the growth of microorganisms. Ciproflaxacin antibiotic was used as positive reference standard to determine the sensitivity of the strains. Discs were directly placed onto the bacterial culture and then the plates were incubated in air at 37°C for 24 h. Antibacterial activities were evaluated by measuring inhibition zone diameters. The experiments were conducted twice.

Broth microdilution assay

To determine the minimal inhibitory concentrations (MIC) of isolated alkaloids against the selected test microorganisms broth microdilution method was used and this was recommended by the National Committee for Clinical Laboratory Standards.¹¹ The tests were performed in 96 well-plates. In 1% DMSO the alkaloids were dissolved and were transferred in plates to obtain a twofold serial dilutions ranging from 10 to 640 µg/ml. Then the plates were inoculated with microbial suspensions and diluted to have 10⁵ cfu/ml in each well. The final volumes in wells were 200 µl. MIC was recorded after 24 h incubation in air at 37°C, as a lowest extract concentration demonstrating no visible growth in the broth.

Statistical analysis

Values are expressed as mean \pm SE. One-way analysis of variance (ANOVA) was used to determine statistical significance and values with $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Phytochemical Profiling

The present study was carried to investigate the presence of medicinally important phytochemical (alkaloids) in *E. alba*. The qualitative test which describes the appearance of alkaloid is listed in the Table 1 and was isolated from the leaves of *E. alba*. In this study we checked whether other phytochemicals present or not and these results noted that, the absence of tannins, saponins, flavonoids, terpenoids, carotenoids, cardiac glycosides, and reducing sugars. The presence of different phytochemicals and the antimicrobial activity of ethanolic, petroleum ether and methanolic extracts of *E. alba* have been previously reported.¹² However, our study is first ever report to the best of our knowledge on antibacterial activity of alkaloid from *E. alba* in India. The purity of alkaloids were confirmed by performing TLC which shows the appearance of one spot using solvent system chloroform: methanol (0.5:9.5). The Rf value of the peak was 0.81.

The quantitative phytochemical estimation specifies that the leaves of *E. alba* contain a significant amount of alkaloid content. The alkaloids content was quantitatively estimated and was found in the range of 0.4 mg/gm. Earlier reports on quantitative yield also revealed that *E. alba* contained highest quantity of alkaloids as compared to other medicinal plants.¹²⁻¹⁴ Simple phenols and phenolic acids, quinones, flavones, flavonoids and flavonols, tannins, coumarins, alkaloids, terpenoids and essential oils, lectins and polypeptides are the major groups of antimicrobial compounds.¹⁵

Antibacterial Study

The antibacterial activity of isolated alkaloidal compound was determined by using agar well diffusing methods. The results in Table 2 show that the isolated compound has good antibacterial activity against selected human pathogenic bacteria. In the present study, the inhibitory action of the alkaloid was found to increase with an increase in concentration against all bacterial strains. The tested bacterial strains showed different patterns of inhibition. This was supported by an earlier study on an alcoholic extract that exhibited greater activity than the aqueous and hexane extracts against bacteria, with no cellular toxicity.¹⁶ The broad spectrum of antibacterial activity was reported for *E. alba*.^{17,18}

The isolated alkaloid compound at a concentration of 500 μ g/disc showed maximum inhibition against *S. aureus* (16.5 mm), followed by *E. coli* (14.7 mm), *S. boydii* (13.2 mm), *S. faecalis* (12.5 mm) and *P. aeruginosa* (9.8 mm) by broth dilution method. The maximum zone of inhibition was observed at the concentration of 500 μ g/ml against all the bacteria.

E. alba leaves methanolic extract showed maximum activity against gram-negative bacteria and showed the highest inhibition zones against *P. aeruginosa* and *E. coli*.¹⁹ This study confirms the presence of the alkaloid compounds may be responsible for the antibacterial activity against various bacterial strains.

The varying concentrations between 10 to 640 μ g/ml of the isolated alkaloid compound of *E. alba* were tested in order to determine their MICs. The MICs of the isolated alkaloid compound against the five tested bacterial is presented in Table 3. The lowest MICs were obtained in the alkaloid compound having 42 μ g/ml against *E. coli*, 57 μ g/ml against *S. aureus*, 61 μ g/ml against *S. boydii*, 82 μ g/ml against *P. aeruginosa* and 89 μ g/ml against *S. faecalis*. The MIC ranged from 10 to 640 μ g/ml for all studied microorganisms while for ciprofloxacin it ranged from 0.1 to 10 μ g/ml. These alkaloids known to be biologically active as well as showing antimicrobial activities. In this study, the *S. aureus* and *E. coli* are the more susceptible than the other selected human pathogenic bacteria. In this study, this antimicrobial activity may be due to the presence of (OH) group in the structure isolated alkaloids which increased the activity to inhibit the bacterial growth by changing the nature of cell protein (denaturation), thus increasing the permeability of cell membranes,²⁰

Table 1 : The qualitative chemical analysis for the isolated alkaloids of *E. alba*

Phytochemical test	Results
Tannins (Folin-Denis test)	-
Alkaloids (Dragendorff test)	+
Saponins (Frothing test)	-
Cardiac Glycosides (Keller-Kiliani test)	-
Steroids (Liebermann-Burchard reaction)	-
Terpenoids (Salkowski test)	-
Flavonoids (Pb acetate test)	-
Anthraquinones (Colour test)	-
Reducing Sugars (Fehling's test)	-

Table 2: The zone of inhibition produced by alkaloid compounds isolated from *E. alba* against human pathogenic bacteria

Bacteria Strains	Inhibition zone (mm)				
	125 μ g/ml	250 μ g/ml	500 μ g/ml	Standard (Ciprofloxacin, 25 μ g/disc)	Control (DMSO)
<i>Escherichia coli</i>	11.0 \pm 0.03	11.4 \pm 0.03	14.7 \pm 0.01*	20.3 \pm 0.07	0
<i>Pseudomonas aeruginosa</i>	0.00 \pm 0.00	9.0 \pm 0.01	9.8 \pm 0.05	10.5 \pm 0.06	0
<i>Shigella boydii</i>	10.2 \pm 0.06	10.5 \pm 0.00	13.2 \pm 0.02*	11.2 \pm 0.07	0
<i>Staphylococcus aureus</i>	12.5 \pm 0.02	13.3 \pm 0.01	16.5 \pm 0.02*	23.5 \pm 0.12	0
<i>Streptococcus faecalis</i>	9.7 \pm 0.07	10.0 \pm 0.02	12.5 \pm 0.06*	20.6 \pm 0.13	0

Values are expressed as mean \pm SEM and analyzed by one-way analysis of variance (ANOVA) followed by Dennett's *t* test; * $P < 0.05$;

Table 3: Antibacterial activity of alkaloid compounds isolated from *E. alba* against selected human pathogenic bacteria by minimum inhibitory concentration (MIC) method

Bacterial strains	MICs ($\mu\text{g/ml}$) of isolated alkaloid compound	Standard (Ciprofloxacin)	Control (DMSO)
<i>Escherichia coli</i>	42	1	0
<i>Pseudomonas aeruginosa</i>	82	0.8	0
<i>Shigella boydii</i>	61	1	0
<i>Staphylococcus aureus</i>	57	1	0
<i>Streptococcus faecalis</i>	89	1.3	0

either by increasing the permeability of the cell membrane of the bacteria. The cell membrane causes loss or leakage of the contents of a cell of bacteria to the outside or through a direct link membrane of cell bacteria, causing the demise of polar membrane of bacteria, which leads to the death of a cell bacteria gradually.^{21,22}

CONCLUSION

The results of the present study showed that the isolated alkaloids of the leaves of *E. alba* were effective against the bacterial species tested. This can be used to treat various diseases like pimples, food borne infections, typhoid, oral and throat sores and nosocomial infections. This investigation has opened up the possibility of the use of this plant for formulating a drug for human consumption possibly for the treatment of bacterial infections. These findings support the traditional knowledge of local users about their selection of this plant sample as antimicrobial agents and it is a preliminary scientific validation for the use of this plant for antibacterial activity. The results of the present study also support the medicinal usage of the alkaloids isolated from the leaves of *E. alba* can be used as antimicrobial agents in new drugs for therapy and can be subjected to identification and isolation of the therapeutic antimicrobials and undergo further pharmacological screening that can be used as sources for new drugs.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest, financial or otherwise regarding the publication of this paper.

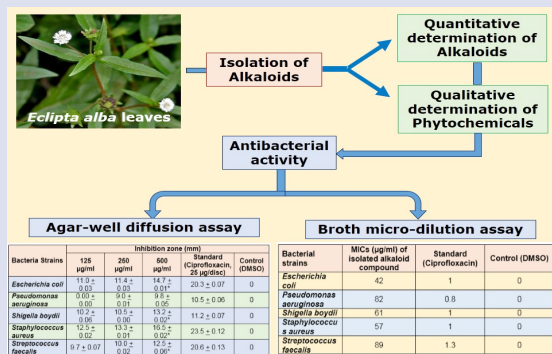
ABBREVIATION USED

TLC: Thin layer chromatography; Rf: Retention factor; MIC: Minimal inhibitory concentrations, NCCLS: National Committee for Clinical Laboratory Standards; DMSO: Dimethyl sulfoxide; ANOVA: One-way analysis of variance.

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GRAPHICAL ABSTRACT



SUMMARY

- The qualitative test describes the presence of alkaloid in *Eclipta alba*. The purity of alkaloids were confirmed by performing TLC.
- The alkaloids content was quantitatively estimated and was found in the range of 0.4 mg/gm.
- The isolated alkaloid compound at a concentration of 500 µg/disc showed maximum inhibition against *S. aureus* (16.5 mm).
- The lowest MICs were obtained in the alkaloid compound having 42 µg/ml against *E. coli*.

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