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Phytochemical Screening and Antimicrobial Activity Study of *Eclipta Alba* Against Bacteria

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Abstract

Plants are important due to their nutritive value and as a major source of medicines. *Ecliptaalba* is an herbal products and modern drugs. It is widely used in traditional cuisine and in folk medicine for the treatment of various ailments. The present study was carried out on the preliminary phytochemical screening of *Eclipta alba. The* plant leaf samples were collected powdered and bioactive compound were extracted by chloroform, acetone and water Soxhlet extractor. Phytochemical analysis should that the antibacterial activity of *Eclipta alba* is due to the presence of phytochemical compounds like alkaloids, flavonoids, steroids, triterpenes, phenolic compounds, saponins, carbohydrates, tannins and amino acid. The antimicrobial activity was determined by using agar well and disc diffusion method extracts with 1ml of dimethyl sulfoxide (DMSO) and added into the well. Chloroform, acetone and water extracted samples were tested against *Salmonella typhi* the most resistant microbial strain. The most susceptible gram-positive bacterium was *bacillus subtilis* which was inhibited by all extracts from *Eclipita alba* while the most resistance gram positive bacterium. While *Salmonella typhi* and *E. coli* were highly resistant among the gram-negative bacteria. The plant leaves of *Eclipta alba* are used various biological activities are possessed by *Eclipta alba* such as memory disorders, treatment, general tonic, edema, fever and rheumatic joint pains treatment digestion, hepatitis, enlarged spleen, and skin disorders

Keywords

Eclipta *Alba*, Phytochemical, Antimicrobial activity.

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INTRODUCTION:

Ecliptaalba (L.) is an annual herbaceous plant, commonly known as false daisy. It is a prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate belonging to family Asteraceae. The plant is commonly

used in hair oil all over India for healthy black and long hair[19]. The fresh juice of leaves is used for increasing appetite, improving digestion[12] and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma[24] and popularly used to enhance memory and learning[13]. The plant has a reputation as an ant ageing agent in Ayurveda[24]. It is used as a general tonic for debility. Externally it is used for inflammation[28], minor cuts and burns and the fresh leaf-juice is considered very effective in stopping bleeding[14]. Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections. The present study was carried out to test the antibacterial efficacy of the aerial parts extracts of *Ecliptaalba* with reference to bacterial spp. The plant used for the present study was *Ecliptaalba*, used for memory disorder treatment, edema, fevers and rheumatic joint pains treatment, digestion, hepatitis, enlarged spleen, antioxidant activity and skin disorders and as general tonic.

MATERIALS AND METHODS:

Plant material:

The plant of *Eclipta alba (*Family) Asteraceae were collected around Namakkal, Namakkal District, Tamilnadu. The plant materials were cleaned with distilled water and shade dried at room temperature. The shade dried plant materials were powdered by using electric blender.

Preparation of plant extracts:

The plant leaf powder (500g) of *Ecliptaalba* were extracted separately to exhaustion in a Soxhlet apparatus using chloroform, acetone and distilled water solvent systems. All the extracts were filtered through a cotton plug followed by What man filter paper (No.1) and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to get yield from chloroform, acetone and distilled water fractions respectively. The extracts were preserved in airtight containers and kept at 4°C until further use. All the extracts were tested for antimicrobial activity against the *Escherichia coli, Bacillus subtilis, and Salmonella typhi.*

Test Organisms:

Bacterial isolates used in this study were *Bacillus subtilis, Escherichia coli* and *Salmonella typhi.* All the strains were obtained from the Department of Micro Biology, Selvamm Arts and Science College Namakkal, Namakkal District, TamilNadu. Cultivated nutrient agar medium (NA) and incubated at 37°c for 24 hours. Stock cultures were maintained at 4°C.The pure cultures of bacteria maintained in the biotechnology lab were used for the project work. The test organisms were maintained on Nutrient agar medium. The following gram positive and gram-negative bacterial species were used ininvitro antimicrobial studies.

Culture media and inoculums preparation:

Nutrient broth (NB) (Himedia,1993) were used as media for culturing of bacterial strains. A loop full of microbial cultures was inoculated in the nutrient broth at 37^{o}C for 24 hrs.

PHYTOCHEMICAL SCREENING ANALYSIS:

Phytochemicals tests were done to find the presence of the bioactive chemical constituents such as alkaloids, flavonoids, terpenoids, steroids, triterpenes, phenolic compounds, saponins, carbohydrates, amino acid and tannins compounds and by the following procedure.

Test for Alkaloids (Meyer's Test):

The extract of *Ecliptaalba* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent[1]. The samples were then observed for the presence of turbidity or yellow precipitation[5].

Test for Flavonoids:

4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed, and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavones[1].

Test for Terpenoids:

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid.

Test for Steroids:

4 mg of extract was treated with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid and green bluish colour for steroids.

Test for Triterpenes:

300 mg of extract was mixed with 5 ml of chloroform and warmed at 80°C for 30 minutes. Few drops of concentrated sulphuric acid was added and mixed well and observed for red colour formation[11].

Test for Phenolic Compounds:

300 mg of extract was diluted in 5 ml of distilled water and filtered. To the filtrate, 5% Ferric chloride was added and observed for dark green colour formation.

Test for Saponins:

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously. Persistent froth indicated the presence of saponins[11].

Test for Carbohydrates:

Treat the test solution with few drops of alcoholic alpha-napthol. Add 0.2ml of Conc. Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction[11].

Test for Amino acids:

To the 3ml of crude sample, 3 drops 5% ninhydrin was mixed and heated for 10min in boiling water bath. Purple or bluish color indicated presence of amino acids.

Test for Tannins:

To 0.5 ml of extract solution, 1 ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins.

ANTI MICROBIAL ACTIVITY

Agar Well Diffusion Method:

The antimicrobial activity was determined by agar well disc diffusion method. Plates for antibacterial microbial activity was prepared using nutrient agar medium and sterilized, the wells were cut by using gel puncture and previously prepared cultured organisms were swabbed on the culture plates. A volume of 20 µl from 5 µg, 10 µg and 15 µg of the plant extracts were added into the well. The assessment of antimicrobial activity was based on the measurement of the concentration diameter of the inhibition zone formed around the well.

Agar Disc Diffusion Method:

The antimicrobial activity was studied using as test agent a range of different strains of human pathogenic bacteria of which there were one antibiotic agent (streptomycin). Invitro antimicrobial was carried out by disc diffusion technique in Whatman No.1 filter paper discs with 4mm diameter were impregnated with known amount test samples of the *Ecliptaalba*. The discs were loaded each with 10 µl of the extract by first applying 5 µl with the pipette, allowed to evaporate, then applying another 5 µl, then drying again. The positive control contained a standard antibiotic disc. (Bauer, Kirby & Turck 1966) Sterile discs used as negative control. The impregnated discs along with control (streptomycin) were kept at the center of agar plates, seeded with test bacterial cultures. The discs were then placed individually using sterile forceps in appropriate grids which were marked on the undersurface of the plated Petri plates and kept for incubation at room temperature (27°C±2) for 24 hrs. After incubation, plates were observed for zones of inhibition and recorded in millimeters.

RESULTS:

Phytochemical screening:

Preliminary phytochemical screening was performed for the plant material and the study revealed that alkaloids, flavonoids, steroids, saponins, carbohydrates, Amino acid and Tannins were present in the *Eclipta alba* (Table 1) whereas Terpenoids, Triterpenes and Phenolic Compounds were absent in acetone extract in the *Eclipta alba.*[02], (Poonam and Singh, 2002).Alkaloids, Flavonoids, Carbohydrates and Amino acid were absent in chloroform extract.*(Mohd Yousuf Malla et al 2013) and* whereas Steroids, saponins, carbohydrates, Amino acids and Tannins were absent in distilled water extract in the *Eclipta alba.(Jehan Bakht 2011).*

The antimicrobial activities of different solvents extracted (Acetone, Chloroform & Distilled water) samples from different microorganisms *(Escherichia coli, Bacillus subtilis, Salmonella typhi*) and different method (agar well diffusion and agar disc diffusion) leaf extracts of *Eclipta alba* is more sensitive to than *Salmonella typhi* to other two bacterial strains. These results confirm the findings of Rahman and Rashid (2008) (Table 2 and 3)

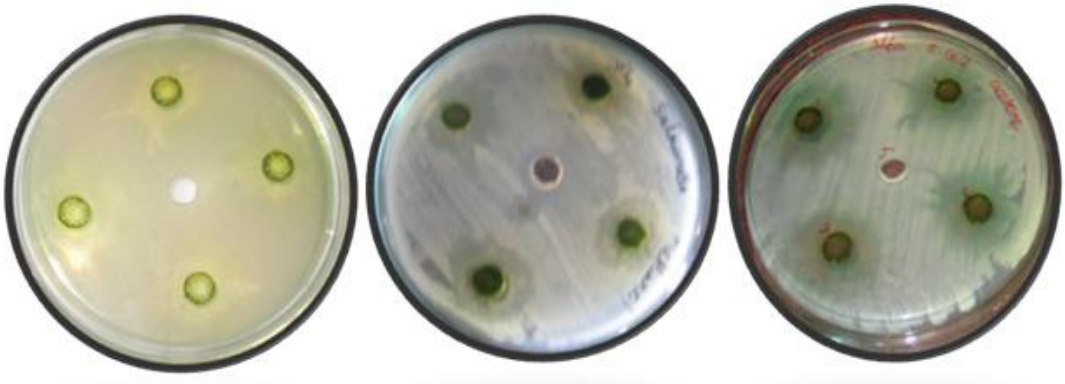
Preliminary phytochemical screening of *Ecliptaalba* (Table 1)

Phytochemical Compounds	Acetone	Chloroform	Distilled water
Alkaloids	+	-	+
Flavanoids	+	-	+
Terpenoids	-	+	+
Steriods	+	+	-
Triterpenes	-	+	-
Phenolic Compounds	-	+	+
Saponins	+	+	-
Carbohydrates	+	-	+
Amino acids	+	-	-
Tannins	+	+	-

Agar Well Diffusion Method (Table 2)

Microorganisms	mg/10ml	Chloroform (mm)	Acetone (mm)	Distilled water (mm)
Escherichia coli	70	11	9	8
	80	15	13	10
	90	19	17	14
	100	22	24	22
Bacillus subtilis	70	8	7	7
	80	12	9	9
	90	14	12	13
	100	15	17	15
Salmonella typhi	70	12	11	8
	80	13	15	12
	90	15	19	15
	100	23	24	22

Agar Well Diffusion Method

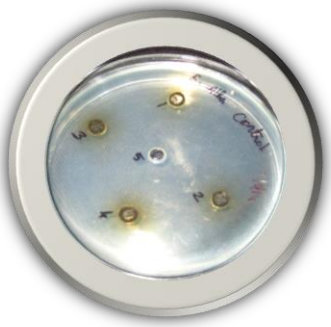


Escherichia coli ***Bacillus subtilis*** ***Salmonella typhi***

Agar Disc Diffusion Method (Table 3)

Microorganisms	mg/10ml	Chloroform (mm)	Acetone (mm)	Distilled water (mm)
Escherichia coli	70	7	8	8
	80	10	13	8
	90	12	14	10
	100	18	20	22
Bacillus subtilis	70	8	6	6
	80	9	9	8
	90	10	10	9
	100	12	12	11
Salmonella typhi	70	9	10	12
	80	10	11	12
	90	14	12	13
	100	19	23	23

Escherichia coli



Bacillus subtilis

Salmonella typhi

DISCUSSION:

The present study discusses on the phytochemical and antimicrobial activities of the plant *Ecliptaalba.* The plant *Eclipta alba* contain phytoconstituents like alkaloids, flavonoids, terpenoids, carbohydrates, saponins and tannins and this study could serve as a constructive reference to allow further in-vivo analysis which can be conducted to evaluate the extent of protective effects of *Eclipta alba* against chemically induced cellular damage. The present antimicrobial activity of different extracts of *Eclipta*alba leaves showed that the highest zone formation in acetone followed by water and chloroform extracts against the gram positive and negative bacteria. The most susceptible bacillus subtilis was inhibited all extracts while Salmonella typhi and Escherichia coli were highly resistant in acetone followed by distilled water extracts.

CONCLUSION:

The present investigations revealed that the chloroform, acetone, distilled water extracts of *Ecliptaalba* contain significant amount of phenols and flavonoids. It is noticed that the highest concentration of phenolic compounds in the extracts were obtained using solvents of high polarity. Further intention of this study was to correlate relationship of these secondary metabolites to possible biological activities and evaluate *Eclipta alba* as a potential source of natural bioactive chemicals. The result of the present study showed that the selected plant *Ecliptaalba* extracts was effective against the bacterial spp. tested. This can be used to treat various diseases like pimples, typhoid, food borne 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 antimicrobial activity. The results of the present study also support the medicinal usage of the studied extracts can be used as antimicrobial agents in new drugs for therapy infectious diseases caused by pathogens.

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