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PHYTO-CHEMICAL ANALYSIS AND ANTI-MICROBIAL ACTIVITY OF ECLIPTA ALBA.

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ABSTRACT

The need of natural antimicrobials anti cancer and antioxidents has increased now days as the microbes developing immunity of the synthetic drugs very fast. To overcome this researcher has started isolating compounds from natural sources and the best natural source and easily extracted is plant source. Eclipta alba is a weed plant which is very commonly found and has a very good anti microbial and antioxidant activity, so we have selected this as our test subject. It showed a very good activity than expected. The extracts were tested against bacterial and fungal organisms and the results were moderate.

KEYWORDS: antimicrobials anti cancer and antioxidents.

INTRODUCTION

Due to the heavy use of antibiotics now a days the microbes developed resistance against them. From the olden days Herbal drugs are been used for treating many disease causing microbes. [1,2] Development of synthetic drugs has caused a great damage by the side effects caused by them. The drug discovery has been changed from the day the researchers started searching the antibiotic compounds isolation from plant sources. [3] They showed the promising results as therapeutic agents. Eclipta alba is such a plant with high antimicrobial activity. It is a mesophytic herb commonly known as false daisy, belonging to Asteraceae family. [4] It spreads on land vary easily when its nodes touches the ground. [5,6] A common annual weed found throughout India. The Juice is used as remedy for hepatitis and is good for treating the urinary tract infection. This plant has anti-ageing and anti-hepatotoxic properties.

The study below is about the anti microbial activity shown by the extracts of plants from different parts of it.^[7]

Micro organisms

The test organisms Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Trichophyton rubrum, Pseudomonas aeruginosa, Salmonella typhi, Candida parapsilosis and Aspergillus niger were cultured onto nutrient agar in order to determine their viability. The identity of each test organism was confirmed using standard cultural, morphological and biochemical techniques. Stock cultures were maintained as Glycerol stocks at 4°C checking their viability time to time.

Evaluation of antimicrobial activity

The experiment was done with care and perfect handling in aseptic conditions. nutrient agar medium (25 ml) was taken in a sterile petridish and broth cultures of the test isolate (0.1 ml) containing 1.0×10^5 CFU/ml of organisms were added. The extracts were dissolved in Ethyl alcohol and used. The concentrations used for the test are 10, 20, 40 and 50 mg/ml. Ampicilin (10 μ g/ml) was used as standard antibacterial agent and Griseofulvin was used as standard antifungal agent.

MATERIALS AND METHODS

PHYTOCHEMICAL ANALYSIS

Plant material was collected from the college campus. Chemicals such as wagnes reagent, chloroform, 2% H2SO4, Concentrated sulphuric Acid, 10% Lead acetate, Benedict's reagent, 0.1% ferric chloride, Fehling's solution, dilute NaOH,2% HCL, 10% Ammonia, 10%HCL, distilled water, Ethyl Alcohol are provided by the management of the college.

Preparation of solutions

- **a) Fehling's solution: -** A mixture of equal volume of copper sulphate, sodium potassium tartar ate and sodium hydroxide is prepared in a beaker.
- b) Wagner's Reagent:- Mixing 2gm of Iodine, 6gm of potassium iodide in 100ml of water.

Collection of sample: Healthy leaves of Eclipta albawere taken and washed under running water to remove the dust and other external pollutants. The plant leaves, roots and flowers were air dried for few days. Normally it takes 15 to 21days for drying.

Grinding the sample: The dried leaves are grinded to a fine powder in a mixer and the powder is collected in clean polythene bags.

Preparation of plant extract with Ethyl Alcohol: Taken 10gms of leaf powder and added 50ml of ethyl alcohol stirred it constantly for 30 minutes and the solution was kept at room temperature for 24 hours (minimum) and then filtered. The filtered solution is again filtered with whatman filter paper No.3 and then it was stored at 4 degrees centigrade (in a freezer) until use.

Table showing results of Phyto Chemical Analysis Eclipta alba(Lin)

Sl. No	Phytochemicals	Distilled Water	Methanol	Acetone	Ethanol
1	Tanins	Positive	Positive	Positive	Positive
2	Anthraquinones	Positive	Positive	Negative	Positive
3	Flavanoides	Positive	Positive	Positive	Positive
4	Alkaloides	Positive	Positive	Positive	Positive
5	Terpenoids	Positive	Positive	Positive	Positive
6	Saponins	Positive	Positive	Positive	Positive
7	Cardiac glycosides	Positive	Positive	Positive	Positive
8	Glycosides	Negative	Positive	Positive	Positive
9	Reducing Sugars	Positive	Positive	Negative	Positive
10	Phlobatanins	Positive	Positive	Negative	Positive
11	Steroids	Positive	Positive	Positive	Positive
12	Phenolic	Positive	Positive	Positive	Positive
13	Aminoacids	Positive	Positive	Positive	Positive
14	Proteins	Positive	Negative	Positive	Negative

Antimicrobial activity

Leaves and Roots Collection

The leaves, flowers and roots for the present study from the plant *Eclipta alba* were collected from University College for Women, Koti, Hyderabad. And were allowed to dry under shade and made into a fine powder. The powder (100grams) was Soxhlet extracted with methanol and dried under rotavapor at 40-50°C for 3-4 hours. This measure was taken in order to evaluate the antimicrobial activity.

Equipment preparation

To conduct the experiment, the nutrient agar media was prepared by dissolving 28g of nutrient agar in 1000ml distilled water. It was sterilized in autoclave along with the petridishes, forceps, spreader, cotton balls and 25ml conical flasks. The sterilized agar was then

transferred into the petri-dishes and was allowed to solidify. Thereafter, the procedure was executed in laminar air flow to ensure proper aseptic conditions.

Preparation of Paper Discs

The mode of anti microbial activity of the above medicinal plant leaves were performed using the whatman no.1 paper. The fine round paper discs were obtained and were sterilized.

RESULTS AND DISCUSSION

Antimicrobial activity

The antimicrobial activity of all the three leaf extracts was examined against Gram positive and Gram-negative bacteria and fungal strains by measuring zone of inhibition.

The antimicrobial activity was performed by Agar disc diffusion method at concentration level of 2.5, 5.0, 7.0, 10µg/ml respectively.

Ampicillin (antibacterial), Itraconazole (antifungal) as the standard drug at a concentration of 200µg/ml. LB Agar was used as the culture media for antibacterial and potassium dextrose agar was used as culture media for the antifungal activity. The results of the antimicrobial activity are shown in figures and tables.

Eclipta albaleaves

Organism/conc ⁿ of extract	2.5μg/ml	5μg/ml	7.5μg/ml	10μg/ml
E.coli	1.0cm	1.1 cm	1.1 cm	1.2 cm
Staphylococcus aureus	1.2 cm	1.2cm	1.3 cm	1.5 cm
Bacillus subtilis	1.0 cm	1.2 cm	1.5 cm	1.5 cm
Salmonella typhi	0.9 cm	1.1 cm	1.3 cm	1.3 cm
Aspergillusniger	0.8cm	1.0 cm	0.8cm	1.1 cm
Candida parapsilosis	1.2 cm	1.3 cm	1.5 cm	1.5 cm
Trichophyton rubrum	1.0 cm	1.2 cm	1.2 cm	1.0 cm

The *Eclipta alba*leaf extract showed high activity against *Staphylococcus aureus* at very low concentration (2.5µg/ml) compared to *E.coli*, *Bacillus subtilis*, leaf extract showed high activity against *Candida parapsilosis* at a very low concentration (2.5µg/ml) compared to *Aspergillus niger*. The zone of inhibition is calculated in cm.

Eclipta alba roots

Organism/conc ⁿ of extract	$2.5\mu g/ml$	5μg/ml	7.5μg/ml	10μg/ml
E.coli	1.1cm	1.3cm	1.5cm	1.5 cm
Staphylococcus aureus	1.0 cm	1.3cm	1.5cm	1.8cm
Bacillus subtilis	1.0 cm	1.2 cm	1.3 cm	1.3 cm
Salmonella typhi	0.8 cm	0.9cm	1.0 cm	1.0 cm
Aspergillusniger	0.9cm	1.0 cm	1.2cm	1.1 cm
Candida parapsilosis	0.9cm	1.2cm	1.3cm	1.5cm
Trichophyton rubrum	0.7cm	0.9 cm	1.0 cm	1.1 cm

The *Eclipta alba*root extract showed high activity against all the bacteria at very low concentration (2.5µg/ml) when compared and root extract showed high activity against *Candida parapsilosis* and *Aspergillus niger* at a very low concentration (2.5µg/ml) compared to *Trichophyton rubrum*. The zone of inhibition is calculated in cm.

Eclipta alba Flowers

Organism/conc ⁿ of extract	2.5μg/ml	5μg/ml	7.5μg/ml	10μg/ml
E.coli	1.0cm	1.3cm	1.5cm	1.3 cm
Staphylococcus aureus	1.2 cm	1.3cm	1.5cm	1.5cm
Bacillus subtilis	1.1 cm	1.3 cm	1.5 cm	1.6 cm
Salmonella typhi	1.0 cm	0.9cm	1.0 cm	1.0 cm
Aspergillusniger	1.0cm	1.0 cm	1.3cm	1.2 cm
Candida parapsilosis	1.0cm	1.2cm	1.2cm	1.2cm
Trichophyton rubrum	0.8cm	0.9 cm	0.8 cm	1.0 cm

The *Eclipta alba* flowers extract showed an activity of 1.2cm with *Staphylococcus aureus* cultures at very low concentration ($2.5\mu g/ml$). Flower extract showed high activity against all three fungal cultures at a very low concentration ($2.5\mu g/ml$) when compared. The zone of inhibition is calculated in cm.

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