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Antibacterial activity of *Eclipta Alba* Linn. (Asteraceae)

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Abstract - Eclipta alba Linn. (ASTERACEAE) is an annual herb, with curative properties and severely used as analgesic, antibacterial, antioxidant and antihyperglycemic among folklore to cure various ailment. The antibacterial studies were done by agar well diffusion methods. The MIC and MBC methods were also used. Hexane extract of Eclipta alba showed high antibacterial activity against S. aureus, B. cereus, E. coli, S. typhi whereas acetone, ethanol, methanol and aqueous extracts showed intermediate activity against S. aureus, B. cereus, E. coli and S. typhi.

Keywords - Eclipta alba Linn., analgesic, antibacterial, antioxidant and antihyperglycemic activity.

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INTRODUCTION

Eclipta alba Linn. (ASTERACEAE) is and annual herb, with tap-root stock.

Stem: terete, violet-tinged with variable length of internodes.

Leaves: lanceolate, opposite-decussate, entire, acute, cuneate and 1-nerved at base.

Capitula: white on unequal length peduncle. Main active principles consist of coumestans like wedelolactone, dimethyl wedelolactone (Wanger *et al.,* 1986), eclabatin (Upadhyay *et al.,* 2001), oleanane and teraxastane glycosides (Jadhar *et al.,* 2009; Khare, 2004)

Eclipta alba is and active ingredient of many herbal formulation prescribed for liver related ailments and shows effect on liver cell generation. It is also applied externally to cure cutaneous affections (Dalal *et al.,* 2010). The pure extract of leaves is applied externally on head as hair tonic (Roy *et al.,* 2008). The plant extract has an antiageing properly in Ayurveda (Thakur and Mengi, 2005). The present study was carried out to test the antibacterial efficacy of the leaf extract of *Eclipta alba* Linn. with reference to bacterial spp.

MATERIALS AND METHODS

Materials:

- Leaves of *Eclipta alba*
- Soxhlet apparatus
- Acetone, ethanol, methanol, hexane, water
- Filter paper (Whatman No. 1)
- Rotary evaporator
- Incubator
- Test tube
- Petri dish
- Funnel
- Pure culture of bacteria at Microbiology Lab, Patna University.
- Muller Hinton Agar (MHA)
- Nutrient broth (NB)

Methods:

1. **Preparation of leaf extract:**

The leaves of *Eclipta alba* were cleaned in water and shade dried at room temperature. The infusion weighing 500gm were extracted separately to exhaustion in a Soxhlet apparatus using acetone, ethanol, methanol, aqueous and hexane solvent systems. All the extracts were filtered through a cotton pheg followed by whatman filter paper no. 1 and then concentrated by using rotary evaporator at 40°C and reduced pressure to get 2.85 gm, 2.37 gm, 3.2 gm, 4.52 gm and 4.69 gm yield from acetone, ethanol, methanol, aqueous and hexane fractions respectively. The resultant extracts were preserved in airtight containers and kept at 4°C till further use. All the extracts were tested for antibacterial activity against Gram +ve and Gram -ve bacterial strains of different species by *in vitro* methods.

2. Test organism:

- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Bacillus cereus*
- *Escherichia coli*
- *Salmonella typhi*
- *Pseudomonas aeruginosa*
- *Proteus mirabilis*

3. Culture media:

Muller Hinton Agar (MHA)/nutrient broth (NB). A loop full of bacterial cultures was inoculated in the nutrient broth at 37°C for 24 hours.

4. Antibacterial activity:

The extracts prepared in different solvents were studied for antimicrobial activity. A loopful of Gram +ve and Gram -ve bacterial strains were inoculated for 24 hours in 30 ml of nutrient broth placed in a conical flask. In agar well diffusion method, the media and the test bacterial cultures were inoculated into petri dishes. The test strains 0.25 ml was inoculated into the media. After the medium solidified, a well was made in the plates with sterile borer. The extract compound (50 µl) was introduced into the well and the plates were inoculated at 37°C for 24 hours. The microbial growth was determined by measuring the across of the zone of inhibition. Ciprofloxacin (25 µg) was the reference drug used as a control for test organism.

5. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC):

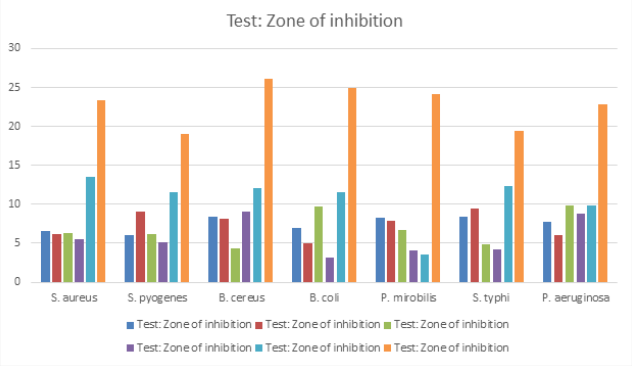
The antibacterial activities were measured using a dilution technique (Topley, 1998). The plant extract (100 ml) was solubilized in 1ml dimethyl sulfoxide (DMSO) and serially two-fold diluted in Muller Hinton broth to obtain a concentration range of 15.6 – 1000 mg/ml. The broth containing only DMSO diluted in the sae way, which did not influence bacterial growth was included as control. The bacterial strains were suspended in sterile physiological Tris buffer (pts: 7.4, 0.05 M), homogenized and adjusted to an optical density of 0.05 at 530 nm. This suspension was used as the inoculum for the test in the agar plates. Bacterial suspensions (100 µl) were inoculated using a micropipette. The MIC was defined as the minimal concentration of the plant extract which completely inhibited the visible growth (= turbidity) of the bacteria in tubes. The MBC was defined as the minimal concentration of the extract which completely inhibited the visible growth of the bacteria on solid media in Petri dishes that were inoculated at 37°C for 24 hours.

RESULTS AND DISCUSSIONS

In the present study, the antibacterial activity of *Eclipta alba* Linn. (ASTERACEAE) leaf extracts were evaluated using different solvents against Gram +ve and Gram -ve bacterial strains of varied bacterial types. The hexane extract showed best activity against *S. aureus* and *E. coli* (MIC 90.0 µg/ml) and also against

Table 1: Antibacterial activity by agar well diffusion method

Test: Zone of inhibition						
Organism	AE	ET	MT	AQ	HE	Ref. drug Ciprofloxacin 25 µg
S. aureus	6.6	6.2	6.3	5.5	13.5	23.4
S. pyogenes	6.0	9.0	6.2	5.1	11.5	19.1
B. cereus	8.4	8.1	4.3	9.0	12.1	26.1
B. coli	6.9	5.0	9.7	3.2	11.5	25.0
P. mirobilis	8.3	7.9	6.7	4.1	3.5	24.1
S. typhi	8.4	9.5	4.9	4.2	12.3	19.4
P. aeruginosa	7.8	6.1	9.9	8.8	9.8	22.9



Solvent:

AE (= Acetone)

ET (= Ethanol)

MT (= Methanol)

AQ (= Aqueous)

HE (= Hexane)

Table 2: Determination of MIC

Sa = *S. aureus*

Sp = *S. pyrogenus*

Bc = *B. cereus*

Ec = *E. coli*

Pm = *P. mirabilis*

St = *S. typhi*

P. mirabilis and *S. typhi* (MIC 125.0 µg/ml). Acetone, ethanol and methanol extracts showed better activity on test bacterial spp., with MIC of 100-500 µg/ml. Aqueous extract showed good activity against test bacterial spp, with MIC 500-1000 µg/ml.

The results for MBC (Table-2) were similar to MIC results, but in the case of MBC, the confirmation was made by absence of growth in culture plates.

The present study was concluded to investigate the *in vitro* antibacterial activity of some folklore medicinal plants used by people of India to evaluate the scientific basis of their applications from the present investigation, it was clear that the solvent of extraction and method of extraction affected the degree of antimicrobial activity.

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