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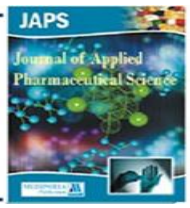

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Antibacterial activity of *Eclipta alba* (L.) Hassk

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ABSTRACT

Eclipta alba (L.) Hassk is small branched annual herbaceous plant with a long history of traditional medicines uses in many countries especially in tropical and subtropical regions. The herb has been known for its curative properties and has been utilized as antimytotoxic, analgesic, antibacterial, antihepatotoxic, antihaemorrhagic, antihyperglycemic, antioxidant, immunomodulatory properties and it is considered as a good rejuvenator too. A wide range of chemical compounds including coumestans, alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes and their glycosides have been isolated from this species. Extracts and metabolites from this plant have been known to possess pharmacological properties. The present study confirmed the antibacterial potential of aerial parts extracts of *Eclipta alba* in solvents like acetone, ethanol, methanol, aqueous and hexane against selected gram positive and gram negative bacterial species. The antibacterial studies were done by agar well diffusion methods. The MIC and MBC methods were also used. Hexane extract of showed *Eclipta alba* high antibacterial activity against *S.aureus, B.cereus, E.coli, S.typhi, K.pneumoniae,S.pyogenes* and *P.aeruginosa*. whereas acetone, ethanol, methanol and aqueous extracts showed intermediate activity against *S.aureus, B.cereus, E.coli, S.typhi, K.pneumoniae, P.aeruginosa, P.mirabilis and S.pyogenes.* The inhibitory activities of all the extracts reported were compared with standard antibiotics (Ciprofloxacin 25 µg/ml). An MIC of 90.0µg/ml shown by *E.coli* and *S.aureus* was considered to be the best (below 100µg/ml), an MIC of 125.0µg/ml shown by *E.coli, K.pneumoni, P.mirabilis* and *S.typhi* was considered to be better (100-500µg/ml) as such by the action of acetone, ethanol, methanol and hexane extracts on test bacterial spp respectively MIC between (500-1000µg/ml) was considered to be good. The aqueous extracts of *Eclipta alba* showed good activity against *S.pyogenes, B.cereus, E.coli* and *P.aeruginosa*. If the dilution was above 1000µg/ml the extract were considered inactive against *S.aureus, K.pneumoniae, P.mirabilis* and *S.typhi*. MBC results were similar to MIC results but in the case of MBC the confirmation was made by absence of growth in culture plates after 24 hrs of incubation at 37ºC. A potent antibacterial and hepatoprotective drug could probably be formulated from the plant extract of *Eclipta alba* to combat the effects of bacterial and hepatotoxic infections.

***Key words*:** *Salvia officinalis*, anti-inflammatory, fractionated extracts, peritonitis.

INTRODUCTION

Eclipta alba (L.) is an annual herbaceous plant, commonly known as false daisy. It is an erect or prostrate, much branched, roughly hairy, annual, rooting at the nodes; the leaves are opposite, sessile and lanceolate belonging to family Asteraceae. It is also known as Bhringaraj and Karisilakanni, which is found a common weed throughout India ascending up to 6000 ft. The genus name comes from the Greek word meaning "Deficient," with reference to the absence of the bristles and awns on the fruits. The specific Eclipta alba means white which refers to the color of the flowers. Main active principles consist of coumestans like wedelolactone, desmethylwedelolactone (Wanger et al, 1986) furanocoumarins,eclalbatin (Upadhyay et al, 2001) oleanane & taraxastane glycosides (Jadhav et al, 2009, Khare, 2004) *Eclipta alba* (L.) has been

used in various parts of tropical and sub-tropical regions like south America, Asia, Africa. It is an active ingredient of many herbal formulations prescribed for liver ailments and shows effect on liver cell generation .It is used as a tonic and diuretic in hepatic and spleen enlargement. It is also used in catarrhal jaundice and for skin diseases (Dalal et al, 2010). The alcoholic extract of the plant has shown antiviral activity against Ranikhet disease virus (Khare, 2004). The plant is commonly used in hair oil all over India for healthy black and long hair (Roy et al, 2008). The fresh juice of leaves is used for increasing appetite, improving digestion (Chery, 2007) and as a mild bowel regulator. It is commonly used in viral hepatitis to promote bile flow and protect the parenchyma (Thakur and Megni, 2005) and popularly used to enhance memory and learning (Jadav, 2009). The plant has a reputation as an ant ageing agent in Ayurveda (Thakur and Mengi, 2005). It is used as a general tonic for debility. Externally it is used for inflammation (Singh et al, 2005), minor cuts and burns and the fresh leaf-juice is considered very effective in stopping bleeding (Khan and Khan, 2008). Leaf juice mixed with honey is also used for children with upper respiratory infections and also used in eye and ear infections.

It is a source of coumestans-type compounds used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis (Wanger et al, 1986, Scott, 1998), Thakur and Mengi, 2005). It is widely used in India as a chologuague and deobstruent in hepatic enlargement, for jaundice and other ailments of the liver and gall bladder (Upadhyay et al, 2001, Lal et al, 2010). Vedic Guard, a polyherbal formulation is a synergistic combination of 16 medicinal plant extracts contains *Eclipta alba* as a major ingredient (Razdan et al, 2008). Charaka advises taking the juice of *Eclipta alba* with honey to prevent the onset of senility, and its oil as the best medicated massage oils for rejuvenation therapies. This plant is well documented and several *in vitro* and *in vivo* studies describe its antiageing agent and anti-hepatotoxic properties (Saxena et al, 1993). The present study was carried out to test the antibacterial efficacy of the aerial parts extracts of *Eclipta alba* with reference to bacterial spp.

MATERIALS AND METHODS

Plant material

The aerial parts of *Eclipta alba (*Family) Asteraceae were collected during the month of June-August 2010 from in and around Ghaziabad (U.P.), India. The plant materials were cleaned with distilled water and shade dried at room temperature. The plant materials were authenticated by the Department of Botany, M.M.H.College, Ghaziabad (U.P.) and the voucher specimens were kept at the Department of Botany. The shade dried plant materials were powdered by using electric blender.

Preparation of plant extracts.

The powdered aerial parts (500 g) of *Eclipta alba* were extracted separately to exhaustion in a soxhlet apparatus using acetone, ethanol, methanol, aqueous and hexane solvent (Merk Chemicals, India) 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 2.85g, 2.37g, 3.2g, 4.52g and 4.69 g yield from acetone, ethanol, methanol, aqueous and hexane fractions respectively. The extracts were preserved in airtight containers and kept at 4°C until further use. All the extracts were tested for antibacterial activity against the gram positive and gram negative bacterial spp. by *in vitro* methods

Test Organisms.

The pure cultures of bacteria maintained in the Microbiology Division of Indian Pharmacopoeia Commission, Ghaziabad,India were used for the microbiological work. The test organisms were maintained on Nutrient agar medium. The following gram positive and gram negative bacterial species were used in *in vitro* antibacterial studies; *Staphylococcus aureus* (MTCC 2940), *Streptococcus pyogenes* (MTCC 442) and *Bacillus cereus* (*MTCC 430), Escherchia coli* (MTCC 443), *Salmonella typhi* (MTCC 733), *Klebsiella pneumoniae* (MTCC 139), *Pseudomonas aeruginosa* (MTCC 741), and *Proteus mirabilis* (MTCC 1429).

Culture media and inoculum preparation

Muller Hinton Agar (MHA) / Nutrient broth (NB) (Himedia , India) were used as the media for culturing of bacterial strains. A loop full of bacterial cultures was inoculated in the nutrient broth at 37ºC for 24 hrs.

Preparation of Mc Farland Nephelometer standard

McFarland tube number 0.5 was prepared by mixing 9.95 ml of 1% Sulphuric acid in MHB and 0.05 ml 1% Barium chloride in distilled water in order to estimate bacterial density (NCCLS, 2004). The tube was sealed and used for comparison of bacterial suspension with standard whenever required.

Antibacterial activity: Agar well diffusion method (IP 2010)

The extracts obtained from the aerial parts were studied for antimicrobial activity. A loopful of gram positive and gram negative bacterial strains such as *S.aureus, S. pyogenes, B.cereus, E.coli, S. typhi, K.Pneumoniae, P. aeruginosa* and *P. mirabilis* were inoculated in 30 ml of nutrient broth in a conical flask and incubated for 24 hrs to activate the strain. In agar well diffusion method, the media and the test bacterial cultures were inoculated into petridishes. The test strain 0.25 ml was inoculated into the media. Adequate care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. After the medium solidified, a well was made in the plates with sterile borer (5mm). The extract compound (50 µl) was introduced into the well and the plates were incubated at 37 °C for 24 hrs. All samples were tested in triplicates. The microbial growth was determined by measuring the diameter of the zone of inhibition. Ciprofloxacin (25 µg) (Himedia, Mumbai, India) was the reference drug used as a control for test organisms.

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 mg) was solubilized in 1 ml of dimethyl sulfoxide (DMSO) and serially two fold diluted in Muller Hinton broth (Himedia, India) to obtain a concentration range of 15.6-1000 µg/ml. The broth containing only DMSO diluted in the same way, which did not influence bacterial growth, was included as control (Ciprofloxacin (25 µg). The bacterial strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenized and adjusted to an optical density of 0.05 at 530 nm (equivalent to 1 X 10^6 CFU/ml). This suspension was used as the inoculum for the test in the agar plates. Bacterial suspensions (100µl) were inoculated using a micropipette. The minimal inhibitory concentration (MIC) was defined as the minimal concentration of the plant extract which completely inhibited the visible growth (turbidity) of the bacteria in tubes. The minimum bactericidal concentration (MBC) was defined as the minimal concentration of the extract which completely inhibited the visible growth of the bacteria on solid media in petriplates that were incubated at 37^{o}C for 24 hrs.

RESULTS

In the present study the antibacterial activities of extracts of aerial parts of *Eclipta alba* using different solvents such as acetone, ethanol, methanol, aqueous and hexane extract were evaluated against eight bactrical spp. (table-1).Hexane extract showed high antibacterial activity against *S.aureus*, *B.cereus*, *E.coli*, *K.pneumoniae*, *S.typhi*, *S.pyogenes* and *P.aeruginosa*, whereas acetone, ethanol, methanol and aqueous extracts showed intermediate activity against *S.aureus*, *B.cereus*, *E.coli*, *K.pneumoniae*, *S.typhi*, *P.aeruginosa*, *P.mirabilis* and *S.pyogenes*. The inhibitory activity of all the extracts reported (table 1) were compared with the standard reference of the antibiotic ciprofloxacin (25 µg) activity. The results of the antibacterial screening tests for the extracts of *Eclipta alba* against both gram positive and gram negative bacteria by using the serial dilution techniques were given in the table 2. If the extracts displayed an MIC in well diluted form (e.g. below 100µg/ml), then the antimicrobial activity was considered to be the best, if in moderate dilution (e.g. from 100 to 500µg/ml) the antimicrobial activity was considered to be better, and if in more concentration (eg from 500 to 1000µg/ml) the antimicrobial activity was considered as good. If the dilution was above 1000µg/ml then the leaf extracts were considered inactive. The hexane extract showed best activity against *S.aureus* and *E.coli* (MIC 90.0µg/ml), and also against, *P.mirabilis*, *K.pneumoniae* 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 spp. with MIC of 500-1000 µg/ml. Extracts of *Eclipta alba* were found to be most active against test bacteria, such as *S.aureus*, *K.pneumoniae* and *E.coli*. This is shown in table 2. The results for minimum bactericidal concentration (MBC) were similar to minimum inhibitory concentration (MIC) results, but in the case of MBC the confirmation was made by absence of growth in culture plates.

Table 1.Antibacterial activity of *Eclipta alba* by Agar well diffusion method.

Test Oraganism	Zone of inhibition (in mm)					
	AE	ET	MT	AQ	HE	Reference 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	11.6	19.1
B. cereus	8.4	8.1	4.3	9.0	12.1	26.1
E.coli	6.9	5.0	9.7	3.2	11.5	25.0
K.Pneumonia	7.0	7.2	8.8	3.6	11.4	22.1
P.mirabilis	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

AE- Acetone, **ET** - Ethanol, **MT** - Methanol, AQ- Aqueous, **HE**- Hexane, **Reference drug** - Ciprofloxacin (25 µg).

Table 2.Determination of Minimum inhibitory concentration (MIC) for *Eclipta alba*

Plant extracts	MIC (µg/ml)								
	Sa	Sp	Bc	Ec	Kp	Pm	St	Pa	Reference drug(Ciprofloxacin25 µg).
Acetone	480	500	125	500	500	250	250	250	18.5
Ethanol	490	180	125	500	250	250	125	500	18.6
Methanol	485	500	800	500	155	500	500	125	18.5
AqueouS	>700	500	250	160	>1000	>1000	>1000	130	18.9
Hexane	90	240	300	90	125	130	125	150	19.0

Sa **-** *S.aureus*, ***Sp***-*S.pyogenes*, ***Bc***- *B. cereus*, ***Ec***- *E.coli*, ***Kp***- *K.pneumoniae*, ***Pm***- *P.mirabilis*, ***St***-*S.typhi*, ***Pa***- *P.aeruginosa*, **Reference drug** - Ciprofloxacin (25 µg).

DISCUSSION

The present study was conducted to investigate the *in vitro* antibacterial activity of some folklore medicinal plant used by people of India, to evaluate the scientific basis of their applications. All the extracts evaluated in the study showed antibacterial activity against the gram positive strains *S.aureus*, *S.pyogenes*, *B.cereus* and also the gram negative strains *K.pneumoniae*, *S.typhi E.coli*, *P.aeruginosa*, and *P.mirabilis* causing serious infections in human beings and animals. *S.aureus* causes localized abscesses, superficial skin lesions and food poisoning, while the gram negative strains *E.coli*, *K.pneumoniae*, *S.typhi.*, *P.aeruginosa*, and *P.mirabilis* cause Pimples, typhoid, food borne infections, UTI, Sore throat and nosocomial infections (Topley, 1998, Khan and Khan, 2008). *Eclipta alba* has significant antimicrobial activity against common pathogens due to the wedelolactone components (Dalal, 2009). Similar studies (Uddin et al, 2010, Chitravadivu et al, 2009) elsewhere also recorded that the ethanol aerial parts extract of *Eclipta alba* revealed high antibacterial activity for *S.aureus*, *E.coli*, and *S.typhi*.

From the present investigation it was clear from this study that the solvent of extraction and method of extraction affected the degree of antimicrobial activity. Others factors such as the environmental and climatic conditions of the plants also affected the degree of antimicrobial activity. Successful predication of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use primarily water as solvent but in our studies we found

that plant extract in organic solvent provided more consistent antimicrobial activity compared to those extracted in water.

CONCLUSIONS

The results of the present study showed that the selected plant *Eclipta alba* extracts was effective against the bacterial spp. tested. This can be used to treat various diseases like pimples, typhoid, food borne infections, UTI, sore throat 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 studied extracts can be used as antimicrobial agents in new drugs for therapy infectious diseases caused by pathogens. The most active extract 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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