Antibacterial activity of Eclipta alba (L.) Hassk

Manoj Kumar Pandey, G.N.Singh, Rajeev Kr Sharma and Sneh Lata

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 antitymotoxicanalgesic, anti inflammatory, antihypertensive, 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. Main active principles consist of coumestans like wedelolactone, desmethywedelolactone (Wanger et al, 1986) furanocoumarins, ecalbatin (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 Mengi, 2005) and popularly used to enhance memory and learning (Jadav, 2009). The plant has a reputation as an anti 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 coumeostans-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 antiaging 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), Escherichia 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 37°C 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 10⁶ 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 inhibitory activity was considered as good. If in moderate concentration range of 100-500µg/ml the antimicrobial activity was considered to be the best, if in moderate (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.pyphi (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.

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Zone of inhibition (in mm)</th>
<th>Reference drug(Ciprofloxacin 25 µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus</td>
<td>AE 6.6 ET 6.2 MT 6.3 AQ 5.5 HE 13.5</td>
<td>23.4</td>
</tr>
<tr>
<td>S.pyogenes</td>
<td>6.0 9.0 6.2 5.1 11.6 19.1</td>
<td></td>
</tr>
<tr>
<td>B. cereus</td>
<td>8.4 8.1 4.3 9.0 12.1 26.1</td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>6.9 5.0 9.7 3.2 11.5 25.0</td>
<td></td>
</tr>
<tr>
<td>K.Pneumonia</td>
<td>7.0 7.2 8.8 3.6 11.4 22.1</td>
<td></td>
</tr>
<tr>
<td>P.mirabilis</td>
<td>8.3 7.9 6.7 4.1 3.5 24.1</td>
<td></td>
</tr>
<tr>
<td>S.typhi</td>
<td>8.4 9.5 4.9 4.2 12.3 19.4</td>
<td></td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>7.8 6.1 9.9 8.8 9.8 22.9</td>
<td></td>
</tr>
</tbody>
</table>


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

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sa</td>
<td>Sp</td>
</tr>
<tr>
<td>Acetone</td>
<td>480 500 125 500 250</td>
</tr>
<tr>
<td>Ethanol</td>
<td>490 180 125 500 250</td>
</tr>
<tr>
<td>Methanol</td>
<td>485 500 800 500 155</td>
</tr>
<tr>
<td>Aqueous</td>
<td>&gt;700 500 250 1500 &gt;1000 &gt;1000 &gt;1000</td>
</tr>
<tr>
<td>Hexane</td>
<td>90 240 300 90 125</td>
</tr>
</tbody>
</table>


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 B. aeruginosa, 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 Pipples, 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 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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