Analysis on Activity of Antibacterial Eclipta Prostrated L by Gas chromatography and Mass spectroscopy

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Abstract
In the current study attempt to explore phytochemical components from the various solvents such as ethanol, methanol and aqueous extracts of the whole plant of Eclipta prostrata L by Gas chromatography and Mass spectroscopy (GC-MS). 100gms of the powdered sample was exposed to the soxlet extraction monitored by rotary evaporator and investigated using PerkinElmer GC-MS. In which the antibacterial activities were determined by using ethanolic extract of this plant. The GC-MS analysis revealed the existence of various compounds with peak area like 2-cyclopentene-1-tri Dacanoic acid, Octa hydropentalen-1-ol, Tricyclo[6,4,0,027] Dodecane. Dodecanoic acid, tridecanoic acid, Henetricontane, Heptane 2,2,3,3,5,6,6 Hepta methyl, sulfurous acid Hexy Monyl ester. Extracts and metabolites from this plant have been known to possess pharmacological properties and also exhibit antibacterial activity.

Keywords: Ecliptaprostrata L, GC-MS analysis, ethanolic, 2-cyclopentene-1-tri Dacanoic acid, Minimum inhibitory concentration (MIC), Minimum Bactericidal Concentration (MBC), World Health Organization (WHO)


1 INTRODUCTION
Despite the fact that pharmacological industries are provided numerous novel anti-micro bials in most recent thirty years, protection from these medications by microorganisms has extended [1]. In common, bacteria have genetic capability to secure & transmit protection from drugs that are utilized as useful specialists. Such a reality is reason for concern, due to many patients in emergency clinics who have smothered insusceptibility, and due to novel bacterial strains that are multi-safe. Thusly, novel contaminations might occur in emergency clinics resulting in high mortality [2].

The microbial obstruction issue [3] is increasing and standpoint for usage of antimicrobial medications later on is as yet dubious. Along these lines, moves should be made to diminish this problem, for example, to handle the
usage of anti-microbial, create examination to more readily comprehend genetic components of obstruction, and to proceed with surveys to develop novel medications, either manufactured or regular [4]. A definitive objective is to give fitting and “productive antimicrobial medications” to patient [5].

For significant time stretch, plants are main source of natural items for handling human health, principally in latest decade, with more escalated examinations for normal treatments [6]. The plant mixes utilization for drug purposes has continuously extended in Brazil. As designated by WHO (31) restorative plants are better source to develop medications collection. About 80% of people from created nations utilize customary medication that has combinations acquired from therapeutic plants. Subsequently, such plants must to be researched to more readily comprehend their properties, security and productivity [7].

The herb *Eclipta prostrata* L (*Asteraceae*,) commonly known as Bhringraja (Sanskrit), Maka (Marathi) and Bhangra (Hindi) is reported to display protective effect on investigational liver damage in mice & rats. It grows commonly in moist places as a weed in warm temperature to tropical areas whole world [8]. It is widely distributed throughout the India, Thailand, China, and Brazil [1]. The plant is reported for infective hepatitis &liver cirrhosis treatment. The plant is recognized to have few significant pharmacological activities like anti-inflammatory, analgesic, hepatoprotective and also possess antimicrobial activity [1]. *Eclipta prostrata* L is utilized in traditional schemes of medicine & also by traditional healers especially in south section of India for liver treatment since ancient times [9]. The phytochemical screening is more significant in recognizing novel sources of therapeutically & industrially main complexes such as alkaloids, saponins, flavanoids, steroids, phenolic compounds, coumarin, luteolin, wedelolactone, triterpenoids, proteins, amino acids and reducing sugar etc [10]. The current survey aimed to investigate the phytochemical constituent’s existent in methanol, ethanol, and aqueous extracts of entire plant of *Ecliptaprostata*L.

**MATERIALS &METHODS:**
Collection and preparation of plant materials *Eclipta Prostrata* (L.) were collected from Trichy, Tamilnadu, India and confirmed by Dr. S. John Britto, The Rapinat Herbarium, ST. Joseph’s college, Tiruchirappalli, (Ref. No: DND 001/2014) The leaves were thoroughly washed and shade dried and coarsely powdered in a grinder. Then it is sieved and stored in airtight container for further activities.

**Soxhlet Extraction:**
*Eclipta Prostrata* (L.) powder containing phytoconstituents were extracted with 70% Ethanol by soxhlet apparatus [12]. The powdered plant test was pressed in thimble, fixed appropriately with cotton. Ethanol in round lined cup is bubbled up to its breaking point, its fumes goes through the stuffed powder and gathered as consolidated concentrate in the RB jar. After rehashed extraction of about15-20 cycles the concentrate was dissipated to remove solvents, which was then utilized for additional cycle. At long last, unrefined concentrate was gotten. The rough concentrate was put away at 4°C until additional utilization. GC-MS examination 20g of powdered example is splashed with 60ml ethanol short-term and separated through debris less channel paper with sodium sulfate. The concentrate was concentrated to 1ml by percolating nitrogen into the arrangement [13]. The concentrate contains both polar and non-polar phyto-parts. The GC-MS was performed by using column
Elite-5MS (5% diphenyl/95% dimethyl polysiloxane), 30× 0.25 mm × 0.25 µm df, equipment: GC- clarus 500 Perkin Elmer carrier gas: 1ml per minute, split:10.1 detector: Mass detector Turbo mass gold perkin software: turbomass 5.2. 2µl of ethanolic extract of the whole plant of *Eclipta prostrata* L was employed for GC-MS examination. The GC-MS extraction process was maintained at temperature of 110 0C with 30minutes.the injector temperature was set at 250 0C [mass analyzer]. The dissimilar factors were involved in clarus 500MS operation, were standardized. The helium gas was used as the carrier gas at a constant flow rate of 1.0mL/min.MS program [14]: Library used NIST version year 2005 (Inlet line temperature: 200 0C; source temperature: 200 0C). The mass spectra is taken at 70 eV; a scan interval of 0.5s and fragments from 45 to 450 Da. The MS detection was finished in 36 minutes [2]. Phytochemical screening phytochemical analysis of ethanol extract of *Eclipta Prostrata* (L.) L was carried out qualitatively to test for existence of phenols, alkaloids, proteins, amino acids, tannins, carbohydrates, flavonoids, Phytosterols, saponins, etc [15].

All the chemicals reagents were procured from Loba Chemie, India Pvt. Ltd. and microbiological media were obtained from Hi-media Laboratories.

**Collection and authentication of the plant material**

*Eclipta prostrata* were collected from Kaylan (Maharashtra). All these materials were authenticated from Agharkar Research Institute, Pune [16].

**Extraction of plant material**

Plant materials were shade dried, kept in an oven at 37 °C and finally ground to fine powder. Fine powder of *Eclipta prostrata* (sieved through sieve of mesh no.44) and coarse powder of *Eclipta prostrata* was used for extraction. All the 3 materials were extracted by Kinetic Maceration method on a rotary shaker for 24 h using solvents of diverse polarities i.e. Methanol, Ethanol, Ethyl Acetate, Chloroform, pet ether and Hexane17.

On the basis of results of antimicrobial activity (Zone of Inhibition) shown by macerated extracts of above solvents, 2 solvents were chosen for soxhlet extraction [11, 12] for each material.

**Culture and maintenance of microorganisms**

*Staphylococcus aureus* (*S. aureus*) ATCC 25922 and *Bacillus cereus* (*B. cereus*) ATCC 11778 were 2-gram positive bacteria and *Escherichia coli* (*E. coli*) ATCC8739, *Salmonella abony* (*S. abony*) ATCC 6017 and *Shigellaboydii* (*S. boydii*) ATCC 8700 were 3-gram negative bacteria used for this assay. All these bacterial cultures were obtained from Guru Nanak Institute of Research and Development (GNIRD) and Microbiology Department of G. N. Khalsa College, Matunga. All the bacterial cultures were handled on nutrient agar medium at 4 °C till further use [18].

**Preparation of inoculums**

A single colony was transferred in sterile 50 ml of nutrient broth and incubated at 37 °C for 24 h. The concentration of bacterial cells was optimized to 0.5 McFarland standards (that corresponds to 1.5X 10⁸ CFU/ml) at 620 nm for agar well diffusion assay.

**Antimicrobial bioassay**

Antimicrobial activity of all the materials was primarily evaluated by agar well diffusion technique and further quantification was done by MIC and MBC against the selected 5 food pathogens [19].
Agar well diffusion method

Agar well diffusion technique was carried out as per guidelines provided by CLSI. One ml of inoculum prepared was mixed thoroughly into 20 ml of sterile nutrient agar and poured into Petri plate of 9 cm diameter. After solidification of agar, wells were made by using 8 mm of cork borer. Fifty μl of respective plant extracts were included in respective wells. Plates were incubated at 37 °C for 24 h and zone of inhibition was measured in mm. Dimethyl Sulphoxide (DMSO) was used as negative control. Ciprofloxacin (5 ppm) was utilized as positive control.

All experiments were repeated 3 times independently, and the mean and standard deviation of diameter of inhibition zones were estimated. Values attained were specified as mean±SD

ANTIMICROBIAL ASSAY:

The pathogenic bacteria species were collected, and it was determined. Bacterial strains consisted of *Shigella boydii*, *E.coli*, *Klebsilla pneumonia*, *Pseudomonas* Sp. and *Salmonella paratyphi A*. The antifungal effect of *Eclipta prostrate L*. Was determined against fungal strains such as *Aspergiller niger* and *Candida albicans*. The strains were Sub cultured bimonthly and the cultured strains were allowed to grow for one week and stored at 5 0C for further analysis. Muller Hinton agar (MHA) was utilized as media for culturing of bacterial strains [20]. The stock cultures were handled in Sabouraud dextrose broth and two diverse strains of fungal pathogens were maintained in Sabouraud dextrose broth for 24 hours until utilized for antifungal activity. The discs were immersed in different concentrations like 50µg to 250µg/ml allowed evaporating. After that the plates were incubated at room temperature (27 °C ± 2) for 24hours. After incubation, plates were observed for zones of inhibition and recorded in millimeters [3]. Evaluation of Anti- microbial activity. The antimicrobial activity of methanol extracts of various parts and in vitro grown plant of *Eclipta prostrate L* was evaluated through disc-diffusion method [21]. The ethanolic extract of *Eclipta prostrate L*. anti-microbial activity were further tested against all the organisms for the evaluation of its antibacterial and antifungal efficiency at different concentrations (50µg to 250µg/ml) by utilizing filter paper disc diffusion method [22]. The zone of inhibition was estimated in millimeters. Activity index was calculated by comparing the zone of inhibition by plant extract with that of standard drug [4].

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\text{Activity index} = \frac{\text{Test sample inhibition zone (extract)}}{\text{Standard drug inhibition zone}}
\]
Table-1: Represents antimicrobial activity of Eclipta prostrata L.

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>NUMBER OF THE ORGANISMS</th>
<th>ZONE OF INHIBITION(mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Pseudomonas Sp.</td>
<td>1.8</td>
</tr>
<tr>
<td>2.</td>
<td>Shigellaboydii</td>
<td>10.6</td>
</tr>
<tr>
<td>3.</td>
<td>Klebsilla pneumonia</td>
<td>7.1</td>
</tr>
<tr>
<td>4.</td>
<td>Salmonella paratyphi A</td>
<td>5.1</td>
</tr>
<tr>
<td>5.</td>
<td>E. coli</td>
<td>6.5</td>
</tr>
<tr>
<td>6.</td>
<td>Aspergiller niger</td>
<td>5.7</td>
</tr>
<tr>
<td>7.</td>
<td>Candida albicans</td>
<td>4.6</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION:
The individual fragmentations of the components with molecular structure were illustrated in antimicrobial activity was described by estimating the Zone of inhibition diameter [23]. The whole plant extracts (Leaf stem, flower& root) of Eclipta prostrata L. with ethanol as well as aqueous From these results of the various extracts against some pathogenic organisms like Shigella boydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp. and Salmonella paratyphi A, Aspergillerniger and Candida albicans species etc. From the qualitative of Eclipta prostrata L analysis having various chemical constituents by using aqueous and ethanol extracts showed some medicinal properties [6]. In this analysis was carried out to understand the existence of various chemical constituents such as alkaloids, Tannins, glycosides, flavonoids, Terpenoids and steroids [7]. These compounds are shows the plants having antimicrobial activity and also exhibit the pharmacological activity [8]. The different parts of the plants exhibited activity against the pathogenic organisms such as, Shigella boydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp. and Salmonella paratyphi A, Aspergiller niger and Candida albicans species etc [24]. From the Disc diffusion method ethanolic extract of Eclipta prostrata L. shows zone of inhibition in mm represents in Table –I.

CONCLUSION:
The various bioactive constituents revealed from the different parts of an Eclipta prostrata L. Plant by using ethanol extract suggest that the components having pharmacological activity, antimicrobial activity and also possesses antioxidant properties. This medicinal herb indicates that one of the disease curable medicine like liver cancer, Jaundice, hair stimulator, hepatoprotectant and also skin diseases.

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