ANTIBACTERIAL ACTIVITY OF SOME PLANT EXTRACTS ALONG WITH ANTIOXIDANT ACTIVITY OF POTENT ONES
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Abstract: The in vitro antibacterial activity of 14 methanolic extracts was investigated by disc diffusion method against a gram negative bacteria Staphylococcus aureus. Amongst the extracts tested, the extracts of Datura stramonium, Ocimum basilicum, Cymbopogon citratus and Eucalyptus sideroxylon showed significant antibacterial activity against the bacterial pathogen. D. stramonium showed highest antibacterial activity followed by O. basilicum extract. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was used to determine the antioxidant activity of potent extract. D. stramonium showed the appreciable antioxidant activity. The highest antioxidant activity was observed at 60 mg/ml concentration with a percent inhibition of 71.66 and IC_{50} value 9.71 mg/ml. Moreover, the radical scavenging activity of extract was lower than that observed for the synthetic antioxidant BHA and BHT. The results provide evidence that the extract of D. stramonium and O. basilicum can be further recommended in the treatment of the infections caused by the bacterial pathogen and D. stramonium is a potential source of natural antioxidants.

I. INTRODUCTION
From antiquity, nature has been a rich store of remedies for relief from various ailments affecting mankind. Plants have been used for thousands of years in traditional medicine. The use of plants for treating diseases is as old as the human species. Plants produce a wide variety of secondary metabolites such as vitamins, terpenoids, tannins, flavonoids, alkaloids and other metabolites, which are rich in antimicrobial and antioxidant activities [1] [2]. Popular observations on the use and efficacy of medicinal plants significantly contribute to the disclosure of their therapeutic properties, so that they are frequently prescribed, even if their chemical constituents are not always completely known.

A number of plants have been documented for their biological [3] [4] and antimicrobial properties [5] [6]. It can be assumed, that although the bulk of traditional antibiotics can still manage drug-resistant bacteria, many commonly used antibiotics are no longer effective [7] [8]. Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Drug resistance can be described as a state of decreased sensitivity to drugs that ordinarily cause growth inhibition or cell death. More strains of pathogens have become antibiotic resistant, and some have become resistant to several antibiotics and chemotherapeutic agents, the phenomenon of multidrug resistance. Limited treatment options for infections caused by such multiresistant microorganisms prompted the search for novel compounds with a broad spectrum of activity and new therapeutic strategies. In an effort to expand the spectrum of antimicrobial agents from natural resources, ten medicinal plants belonging to seven families, have been selected to assess their antibacterial potential.

II. MATERIALS AND METHODS

Plant materials: The leaves 15 plants were collected from different regions of Gorakhpur district. The leaves were plucked and packed in polythene bags. Plants were initially identified by morphological features and then confirmed from the herbarium database present in the herbarium of DDU Gorakhpur University Gorakhpur. The scientific names and family of the 16 plant materials are detailed in Table 1.

Preparation of Plant material: The fresh leaves were washed with tap water and then with 90 per cent alcohol, chopped into smaller pieces with a knife and then kept in the shade for 14 days to dry and then crushed using pestle and mortar and further reduced to powder using electric blender and then stored in airtight closed bottles until tested and analyzed.

Extraction procedure: 10 g of the powdered sample of the plant was soaked in 100 ml of methanol in a 250 ml conical flask at room temperature with shaking after every 4 for 24 h. The extract was filtered using muslin cloth and then Whatman no.1 filter paper. The filtrates were then evaporated to dryness in a rotary evaporator maintained to remove residual solvents and then stored in screw capped bottles for further use. The extracted powder was resuspended in the methanol at desired concentrations before it was tested for the antibacterial activity.
**Microbial strains and Preparation of inoculums:** Gram positive bacteria *Staphylococcus aureus* (MTCC No. 9542) was used for evaluation of antibacterial assay. The stock culture was maintained in nutrient agar (NA) slant at 4°C and sub-cultured monthly. Working cultures were prepared by inoculating a loopful of each test microorganism in 10 ml of nutrient broth (NB) from NA slants. Broths were incubated at 37°C for 18-20 hours. The suspension was diluted with sterile distilled water to obtain approximately 10^6 CFU/ml.

**Determination of antibacterial activity:** The antibacterial activity of plant extracts was evaluated using disc diffusion method [9]. 10ml of sterilized nutrient agar medium was poured in 80mm Petridishes and was allowed to solidify. The plates were seeded by spreading 0.1 ml of overnight inoculum and allowed to set for 20-25mins. For, screening, sterile, 6mm diameter filter paper discs were soaked in plant extracts at 500 µg/ml concentration and placed on the surface of inoculated media agar plates using sterile forceps and then gently pressed down onto the agar surface. Disk soaked with the solvent was used as control. The positive control plates were inoculated with test organism. All the plates were incubated at 35-37°C for 24h. Clear inhibition zones around the discs indicated the presence of antibacterial activity. Diameter of inhibition zones were measured in millimeters. An inhibition zone of 10mm or more was considered as high antibacterial activity.

**Determination of MIC (Minimum inhibitory concentration) values:** The minimum inhibitory concentration value for bacterial pathogen was determined by agar dilution technique of CLSI with slight modifications [10]. A series of twofolds dilution of extract concentrations (25 µg/ml- 3200 µg /ml) was prepared in Petridishes. 10ml of sterilized and molten nutrient agar medium was poured in each dish already containing 100µl amount of extracts. Plates were dried at 35°C for 30minutes prior to spot inoculation with 5µl of overnight bacterial culture (adjusted to 0.5 MacFarland standard) containing approximately 10^6 CFU/spot using an sterilized inoculating loop. Nutrient agar with solvent was used as positive control. The inoculum spots were allowed to dry at room temperature and plates were incubated at 35-37°C for 24h. MICs were determined as the lowest concentration of oil inhibiting the visible growth of microorganisms on agar plate disregarding the presence of 1 or 2 colonies.

**Determination of MBC (Minimum bactericidal concentration) values:** The MBC of the extracts was determined as described by Mishra *et al.*, [11]. Fresh nutrient agar medium was poured into Petriplates and allowed to solidify. Inoculum from various poisoned plates of MIC experiment showing no growth was submitted to subculture on freshly prepared plates. The lowest concentration of antimicrobial agent from which bacteria do not recover on fresh medium was treated as MBC.

**DPPH free radical scavenging activity:** Effect of extracts on DPPH radical was estimated using method of Güllüce *et al.* [12] with slight modifications. 0.004% of DPPH (Hi Media) was prepared in methanol and 2ml of this solution was mixed with different concentrations of extracts (10, 20, 30, 40, 50 and 60 mg/ml) dissolved in methanol. Reaction mixture was vortexed thoroughly and left for 30mins. After 30mins absorbance of the mixture was measured at 517nm in an UV spectrophotometer (Hitachi) against a blank (pure methanol). Control sample was also prepared as above without any oil. Ascorbic acid, BHT (Butylated hydroxytoluene) and BHA (Butylated hydroxyanisole) was taken as reference standards. Experiments were performed in triplicate and averaged. IC_{50} value was determined from percent inhibition versus concentration graph. Percent inhibition was calculated from control using following equation:

\[
\text{Radical scavenging activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100
\]

Where,

- \(\text{Abs}_{\text{control}}\) = Absorbance of DPPH radical + methanol
- \(\text{Abs}_{\text{sample}}\) = Absorbance of DPPH radical + sample oil/standard

**III. RESULTS**

Antibacterial activity:

Results from antibacterial disc diffusion assay are summarized in Table 1. Some of the extracts showed moderate to high inhibiting activity while most of the extracts did not found effective against the tested bacterial pathogen. The zones of inhibition ranged from 10-30mm. Results showed that *Datura stramonium* and *Ocimum basilicum* extracts showed significant antibacterial activity against the bacteria tested. Additionally, the extracts of *Citrus aurantifolia*, *Cymbopogon citratus* and *Eucalyptus sideroxylon* also showed moderated inhibitory activity. *D. stramonium* showed highest activity forming 30mm zone of inhibition against *Staphylococcus aureus* followed by *O. basilicum* which formed 16.67mm inhibition zone. The zone of inhibition formed by other extracts was negligible. Furthermore, the antibacterial activity of most effective extract against *Staph. aureus* quantitatively was assayed by determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).
Table 1: Antibacterial activity of different plant extracts against *Staph. aureus* based on Disc Diffusion Method

<table>
<thead>
<tr>
<th>Plant extracts</th>
<th>Family</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cymbopogon citratus (DC.) Stapf</td>
<td>Poaceae</td>
<td>11.33±0.46</td>
</tr>
<tr>
<td>Datura stramonium (Linn.)</td>
<td>Solanaceae</td>
<td>30.66±0.45</td>
</tr>
<tr>
<td>Eucalyptus sideroxylon (Cunn.)</td>
<td>Myrtaceae</td>
<td>14±0.81</td>
</tr>
<tr>
<td>Euphorbia hirta Linn.</td>
<td>Euphorbiaceae</td>
<td>-</td>
</tr>
<tr>
<td>Haemelia patens (Jacq.)</td>
<td>Rubiaceae</td>
<td>-</td>
</tr>
<tr>
<td>Hyiptis suaveolens (Linn.) Poit.</td>
<td>Lamiaceae</td>
<td>-</td>
</tr>
<tr>
<td>Ocimum basilicum Linn.</td>
<td>Lamiaceae</td>
<td>16.67±0.94</td>
</tr>
<tr>
<td>Ocimum canum Linn.</td>
<td>Lamiaceae</td>
<td>-</td>
</tr>
<tr>
<td>Ocimum gratissimum Linn.</td>
<td>Lamiaceae</td>
<td>-</td>
</tr>
<tr>
<td>Ocimum sanctum Linn.</td>
<td>Lamiaceae</td>
<td>-</td>
</tr>
<tr>
<td>Piper longum Linn.</td>
<td>Piperaceae</td>
<td>-</td>
</tr>
<tr>
<td>Piper methysticum G.Forst.</td>
<td>Piperaceae</td>
<td>-</td>
</tr>
<tr>
<td>- no visible zone of inhibition</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC):

*D. stramonium* exhibited strong action against *Staph. aureus* with MIC value of 800 µg/ml followed by the extract *O. basilicum* with 1600 µg/ml MIC value (Table 2). MBC values were found to be 1600 µg/ml and 3200 µg/ml for *D. stramonium* and *Ocimum basilicum* respectively (Table 2).

Table 2. MIC and MBC data of *D. stramonium* and *O. basilicum* extracts against *Staph. aureus* µg/ml.

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th><em>D. stramonium</em></th>
<th><em>O. basilicum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>MBC</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>800</td>
<td>≤1600</td>
</tr>
</tbody>
</table>

DPPH radical scavenging assay: The DPPH radical scavenging activity of most potent extract, *D. stramonium* and references are shown in Figure 1. *D. stramonium* methanolic extract notably reduced the concentration of DPPH free radical, with an efficacy lower than that of reference BHA (Butylated hydroxy anisole) and BHT (Butylated hydroxytoluene). The results showed significant decrease in the concentration of DPPH free radical due to the scavenging ability of extract and reference. Decrease in concentration of DPPH was observed with the increase in concentration of extract. The highest antioxidant activity was observed at 60µg/ml concentration (75.66%). IC₅₀ value of extract was found to be 9.17 mg/ml.

IV. DISCUSSION

The present study was designed to obtain preliminary information on the antibacterial activity of some methanolic plant extracts. Disc diffusion method was used in this study. Out of 12 extracts tested, only methanolic extracts of *D. stramonium* and *O. basilicum* exhibited good antibacterial activity and gave zone of inhibition followed by the methanolic extracts of *C. aurantifolia*, *C. citratus* and *E. sideroxylon* against *Staph. aureus* Reference 13 reported that methanol was the most effective solvent for plant extraction than any other solvents. Reference 14 also found methanol as the most effective solvent. *D. stramonium* showed highest inhibitory activity against bacterial pathogen. The present study is comparable with the reports of Sharma and Sharma [15] and Johnson et al., [16] and Sreenivasa et al., [17]. *O. basilicum* extract was proved good in inhibiting *E. coli* after *D. stramonium* as reported by Hossain et al., [18]. It has been hypothesized that the inhibition involves phenolic compounds, because these compounds sensitize the phospholipid bilayer of the microbial cytoplasmic membrane causing increased permeability, unavailability of vital
intracellular constituents and impairment of bacterial enzymes. Though the minimum inhibitory concentration is high, nevertheless it showed that plant extract under invito study has broad antibacterial activity. Generally, it is well known that Gram negative bacteria are more resistant than Gram positive bacteria.

Many studies demonstrated correlation between phenolis content and antioxidant activity [19]. On the other hand Bajpai et al., [20] reported no correlation between total phenolic content and antioxidant capacities of a number of medicinal plant extracts. The phenolic compounds may contribute directly to the antioxidative action. DPPH is a stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule, which is widely used to investigate radical-scavenging activity. In DPPH radicalscavenging assay, antioxidants react with DPPH, and convert it to yellow coloured a,a-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the radical-scavenging potential of the antioxidant activities [21]. Figure1. Shows DPPH scavenging activity of D. stramonium extracts at different concentrations in comparison with well known synthetic antioxidants. The antioxidant activity reflected by the DPPH radical scavenging assay was clearly observed in the methanolic leaf extract of D. stramonium.

D. stramonium extract was found to have good antioxidant activity as well promising antibacterial activity. The DPPH assay proved that the antioxidant activity of the extract was appreciable. It can be used as potential source of natural antioxidants.

References

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