Research Paper

A STUDY OF ANTIBACTERIAL ACTIVITY OF ESSENTIAL SEED OIL OF BRYONIA LACINOSA PLANTS

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ABSTRACT
An essential oil was extracted from the ripe seeds of highly medicinal indigenous plant Bryonia lacinosa. GLC and GLC-MS of the oil revealed altogether 10 components, the main constituents being palmitic acid (30.6%), stearic acid (22.9%) oleic acid (17.8%) linoleic acid (13.7%) and butyric acid (11.5%). The oil has been evaluated for the antimicrobial activity against pathogenic strains of Gram positive (Staphylococcus aureus) and Gram negative (Escherichia Coli, Pseudomonas aeruginosa) bacteria in vitro. It showed strong activity against Pseudomonas aeruginosa and Staphylococcus aureus. Seed oil with MBC concentration of 5 mg/ml inhibited the growth of Staphylococcus aureus, while for inhibiting the growth of Pseudomonas aeruginosa and Escherichia coli, the required MBC concentrations for the oil were 1.25 mg/ml and > 5 mg/ml respectively. These results show that Bryonia lacinosa seed oil could be used as a natural alternative to traditional food preservatives.

Keywords:- Bryonia lacinosa, essential oil, chemical composition, anti-bacterial activity
INTRODUCTION -

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents. The gram positive bacterium such as *Staphylococcus aureus* is mainly responsible for post operative wound infections, toxic shock syndrome, endocarditis, osteomyelitis and food poisoning (Mylotte, 1987). The gram negative bacterium such as *Escherichia coli* is present in human intestine and causes lower urinary tract infection, coleocystis or septicemia (Levine, 1987; Singh et al., 2000). *Pseudomonas* is an aerobic, nonfermentative, oxidase positive bacillus which mainly causes urinary tract infection, wound or burn infection, chronic otitis media, septicemia in human (Bodey, 1983) and several diseases in fishes (Bullock et al., 1965). The effects of herbal compounds and their phytochemical effects on pathogenic and economically important bacteria have been well studied (Sato et al., 1996; Khalid et al., 1997; Binutu, 1997; Sampietro et al., 1997).

*Bryonia lacinosa* (N.O. Cucurbitaceae) syn *Bryonopsis lacinosa* plant locally known as ‘Shivlingi’ and ‘Gargumaru’ is distributed throughout India, an annual climber with bright red fruits and is reported to be highly medicinal (Kirtikar & Basu, 1987). Plant as a whole is bitter, tonic and mild laxative. Its leaves are used on inflammations. Roots with roots of *Michelia champaca* is given against asthma and promotes conception. Plant as a whole is also used against snake-bite though the active part not specified. A bitter principle *Bryonin* from the leaves, fatty acid and sugars from the fruit are reported (Paul & Hemraj, 1960). The arabinoglucomannan present in the polysaccharide of its fruit berries is reported to be active against *E. coli* bacteria (Singh et al, 2009). Locally in India its seeds are being used for promoting conception in women (Chopra et al, 1956) and are highly medicinal and its aqueous extract has been subjected to phytochemical investigations where a nonionic glucomannan was reported (Singh & Malviya, 2006).

In view of high medicinal importance of the seeds, composition and antimicrobial activity of the essential oil isolated from its petroleum ether extract was undertaken for
the first time in the present study. Antibacterial activity of the essential oil from *Bryonia lacinosa* was studied by Broth dilution method; the strains tested were *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*.

**MATERIALS AND METHODS**

1. **Plant material**

   Seeds of *Bryonia lacinosa* (N.O. Cucurbitaceae) were locally collected in Allahabad, India, and were identified at Botanical survey of India, Allahabad. All the solutions were concentrated under reduced pressure and GCMS was carried out on ULBON HR-1 column equivalent to OC-1, on Shimadzu QP-2000 instrument using helium as carrier gas (helium) at flow rate of 2 ml per minute.

2. **Isolation of essential oil**

   The essential oil from 1 kg dried and ground seeds of *B. lacinosa* was collected by hydrodistillation for 4 h using a Clevenger-type apparatus. The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate. The oil thus obtained was dissolved in acetone and decolorized using small amount of charcoal and filtering through fuller’s earth. The purified oil did not give test for N & S. The purified oil was stored in tightly closed dark vials and covered with aluminum foil at 4°C until further analysis. The essential oil was obtained as a light yellow transparent liquid and had unpleasant aroma with a 10% (w/w) yield.

3. **Preparation of methyl ester of essential oil**

   A mixture of the oil and 7.8% ethanolic KOH was refluxed for 1 hour and the resulting solution was poured in cold water and the unsaponifiable matter was removed.
by extracting with ether. Aqueous layer containing the potassium salt of the fatty acids was acidified with sulfuric acid. The mixed acids were extracted with diethyl ether. The fatty acid mixture thus obtained was esterified by refluxing it for 6 h with anhydrous methanol in presence of sulfuric acid. The reaction mixture was poured in water and the methyl esters were extracted with diethyl ether. Preliminary examination of the mixture revealed the presence of five different methyl esters and finally the mixture was subjected to GC-MS.

4 GC-MS analysis

The analysis of the essential oil was performed using a Hewlette Packard 5890 II GC, equipped with a DB-5 MS capillary column (30 m x 0.25 mm; film thickness, 0.25 mm) and a HP5972 mass selective detector for the separation. The mass selective detector was operated in electron impact ionization (EI) mode with a mass scan range from m/z 50 to 350 at 70 eV. GC conditions were the same as described above. The retention indices were calculated, for all volatile constituents using a homologous series of n-alkanes C8-C22. The essential oil constituents were identified by comparing their GC retention indices, mass spectra with publish data (Adams, 2001, pp. 1-40). Essential oil components are reported as a relative percent of the total oil by peak area.

5. Antimicrobial activity assay

All pure cultures of the microorganisms were taken from the microbiology laboratory of Motilal Nehru Medical College, Allahabad. The seed oil was tested to anti-bacterial activity using the macrobroth dilution method in solid media (Koneman et al, 1997). In these experiments, the seed oil (5 mg/ml) was
dissolved in the dimethyl sulfoxide and the media peptone water (containing peptone-sodium chloride-water) was prepared. All the samples, test tubes and glass wares were autoclaved at 120° C before use. Antibacterial activity was tested for three bacteria’s- *Staphylococcus aureus*, *pseudomonas aeruginosa* and *Escherichia coli*. 1 ml of susceptibility test broth containing serial one fold dilution of the seed oil was added in the glass test tube (13 × 100 mm) fitted with loose plastic non screw caps. All tubes were incubated in air at 37° C for 24 h before being read. The MIC was considered as the lowest concentration of the sample that prevented visible growth. Minimum bactericidal concentrations (MBCs) were determined by sub-culturing from each negative tube and from the positive growth control. MBCs were defined as the lowest concentration yielding negative sub-culture or only one colony. All samples were examined in duplicate in three separate experiments.

**OBSERVATIONS**

![Fig. 1 Growth of the Bacteria relative to different concentrations of oil.](image)

No growth of *P. aeruginosa* in regions 1 (5.0 mg/ml), 2 (2.5 mg/ml), 3 (1.25 mg/ml), growth of *P. aeruginosa* in regions 4 (0.62 mg/ml), 5 (0.31 mg/ml), 6 (0.15 mg/ml).
<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Retention time</th>
<th>% total</th>
<th>Retention area</th>
<th>Bacteria Serial dilution in mg/ml</th>
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<tbody>
<tr>
<td>1.</td>
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<td>11.5</td>
<td>37.5</td>
<td>Control (00 5 25 6 (5)</td>
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<td>2.</td>
<td>21.13</td>
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<td>3.5</td>
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<td>3.</td>
<td>21.56</td>
<td>0.5</td>
<td>1.6</td>
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<tr>
<td>4.</td>
<td>28.86</td>
<td>0.7</td>
<td>2.4</td>
<td>4</td>
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<tr>
<td>5.</td>
<td>46.10</td>
<td>30.6</td>
<td>100.0</td>
<td>)</td>
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<tr>
<td>6.</td>
<td>55.06</td>
<td>13.7</td>
<td>44.8</td>
<td>Staphylococcus aureus + _ + + + +</td>
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<tr>
<td>7.</td>
<td>55.46</td>
<td>17.8</td>
<td>58.1</td>
<td>Escherichia coli + _ _ _ _ _ +</td>
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<td>8.</td>
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<tr>
<td>9.</td>
<td>60.46</td>
<td>0.6</td>
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<td>Pseudomonas aeruginosa + _ _ _ _ _</td>
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<td>10.</td>
<td>62.16</td>
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Table 1- Relative amount of identified and unidentified fatty acids

Table 2- Anti microbial activity of seed oil of *Bryonia lacinosa*

(+)- Growth

(-)- No growth
RESULTS

The Gas chromatographic analysis of the essential oil of B. lacinosa showed a very diverse composition with 10 constituents reported in Table 1. The oil was dominated by palmitic acid (30.6%), stearic acid (22.9%) oleic acid (17.8%) linoleic acid (13.7%) and butyric acid (11.5%). The present study indicated the presence of a high percentage of saturated fatty acid in comparison to unsaturated fatty acids. The identification of each compound was carried out by comparison of RRT (Relative Retention Time) and mass spectral data obtained with standard methyl esters preparation of palmitic, stearic, oleic, linoleic, butyric acids. Six major fatty acid esters have been detected on the basis of m/z peaks and retention time. The assessment of fatty acid profile (percent composition) is given in Table-1.

Scan no. 2, 3, 4, 9, 10 were found in very small amount and were neglected, while the identified fractions are as under- Scan no. 1) Butyric acid methyl ester (C₃H₇COOCH₃)
Scan no. 5) Palmitic acid methyl ester (C₁₅H₃₁COOCH₃)
Scan no. 6) Linoleic acid methyl ester (C₁₇H₃₃COOCH₃)
Scan no. 7) Oleic acid methyl ester (C₁₇H₃₅COOCH₃)
Scan no. 8) Stearic acid methyl ester (C₁₇H₃₇COOCH₃)

The result of antimicrobial activity of the essential oil of B. lacinosa seeds has been presented in Table 2, Fig. 1. Different concentrations of oil showed antibacterial activity against S. aureus and strong antibacterial activity against P. aeruginosa. The seed oil inhibited S. aureus at a MBC concentration of 5 mg/ml. Pseudomonas was
inhibited by seed oil with MBCs at 1.25 mg/ml. Gram negative bacteria, *Escherichia coli* was inhibited by the seed oil at concentrations greater than 5 mg/ml. The minimum concentration of antimicrobial necessary to kill an organism, MBC, should be equal to or greater than the MIC for that microbe. MBCs for all bacterial tests are reported in Table-2.

**DISCUSSION**

Volatile oils of many plants are known to have antimicrobial activity. This activity could act as chemical defense against plant pathogenic diseases. Pathogens can readily penetrate at wound sites caused, for example, by herbivores. Wounding of leaves which are covered with volatile oil glands results in the rupture of glands causing the oil to flow over the wound. The existence, therefore, of antimicrobial activity in the oil, would be of considerable benefit to the plant. Indeed, a good majority of aromatic and medicinal plants do not succumb too many of the commonest diseases.

The results support the notion that the essential oils and extracts may have a role as pharmaceuticals and preservatives. Because of the appearance of bacterial resistance to antimicrobial agents, more effort is being made to find alternative antimicrobial components. It had been suggested that natural products are preferable to synthetic ones.

**CONCLUSIONS**

Present study revealed the role of essential oil of seed of *B. lacinosa* as a strong antibacterial agent against *P. aeruginosa* and *S. aureus* in laboratory condition. These results showed that Bryonia lacinosa seed oil could be considered as a natural alternative to traditional food preservatives and pharmaceuticals.
REFERENCES


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