Antibacterial activity of leaf extracts of some selected traditional medicinal plants of Uttarakhand, North East India

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Abstract: Screenings of methanolic leaf extracts of nine medicinal plants (Cotinus coggygria, Adhatoda vesica, Argemone mexicana, Zanthoxylum armatum, Berberis asiatica, Corissa opaca, Euphorbia hirta, Cassio fistula and Ricinus communis), belonging to selected areas of Uttarakhand, were tested against seven bacterial strains (Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris and Pseudomonas aeruginosa) by disc diffusion method. Leaf extracts of R. communis, B. asiatica and C. opaca showed high (13 – 23) effect on all the bacterial strains while E. hirta, Z. armatum and A. vesica exhibits minimum (6 – 15) effects. Remaining leaf extract of plants were found moderately (10 - 19) effective.

Keywords: Antibacterial activity, Medicinal plants, Methanol extracts

INTRODUCTION

The effects of plant extracts on different bacteria have been studied from time to time (Hoffman et al., 2004; Jigma et al. 2005; Srinivasan et al. 2005; Kumar, 2006; Nair and Chanda, 2006; Panthi and Chaudhary, 2006; Uniyal, et al. 2006; Singh et al. 2007a,b; Ahsan et al., 2009 and Osho and Adetunji, 2010). Now-a-days, herbal drugs are prescribed widely even when their biologically active compounds are unknown because of their effectiveness, minimal side effects in clinical experience and relatively low cost. Many of plants have been used traditionally in India. In case of Uttarakhand (north east part of India), many plants from high altitude are still to be tested. However, these plants are in use of ailments traditionally in India. In case of Uttarakhand (north east part of India), many plants from high altitude are still to be tested. However, these plants are in use of ailments traditionally in India. Keeping this view in mind, leaves of nine easily available medicinal plants (Cotinus coggygria, Adhatoda vesica, Argemone mexicana, Zanthoxylum armatum, Berberis asiatica, Corissa opaca, Euphorbia hirta, Cassio fistula and Ricinus communis) collected from different parts of Uttarakhand, were selected for antimicrobial activity against seven bacteria namely Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris and Bacillus subtilis.

MATERIALS AND METHODS

Fresh leaves from healthy plants (Cotinus coggygria, Adhatoda vesica, Argemone mexicana, Zanthoxylum armatum, Berberis asiatica, Corissa opaca, Euphorbia hirta, Cassio fistula and Ricinus communis) have been collected from different study areas Srinagar, Kotdwar, Pauri, Tehri, Chamoli and Uttarkashi) of Uttarakhand due to availability of these plants in hilly areas. These all listed areas are located above 500 mean sea levels (MSL) and also floral specific. The leaves of all the nine medicinal plants were plucked/detached, washed thrice in running tap water, dried away from direct sunlight in the shade place under room temperature, crushed to coarse powder in electric grinder and used for further studies. Fifteen gram of the dried and powdered plant material (leaves) was extracted with 250 ml methanol by continuous hot extraction using Soxhlet apparatus for 8 hours. The extract was filtered in Whatman filter paper no. 1, concentrated by removing the solvent and then dried on rotovap. The residue was kept in sample tubes under room temperature, crushed to coarse powder in electric grinder and used for further studies.

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Antibacterial Streptomycin disc of the concentration of 30 µg/ml was used as a standard. Nutrient broth were used and prepared in distilled de-ionized water. The prepared media (Singh et al, 2007a, b) were autoclaved at 15 lbs per square inch (PSI) pressure at 121°C for 20 minutes. Plants, species-wise and part-wise extract (10 µg, 20 µg and 1 g/ml) were suspended in 1000 ml of dimethyl sulphoxide (DMSO) in a conical flask. The suspended solution was then heated on a water bath to dissolve the medium completely. The test organisms were
maintained on slants of medium and transferred to a fresh slant once in a week. The slant was incubated at 37°C for 24 hrs. Using 3 ml of saline solution, the organisms were washed from the agar slant on to a large agar surface (medium) and incubated for 24 hrs at 37°C. The growth from the nutrient surface was washed using 50 ml of distilled water. The amount of suspension to be added to each 100 ml nutrient broth was determined by use of test broth. The test organisms were stored under refrigeration.

The antimicrobial activity of soluble and insoluble compounds is normally investigated using the standard methods like disc diffusion technique (Rios et al., 1988; Bauer et al., 1996). Serial dilutions of the agent are prepared in a suitable broth or agar medium and a standard inoculum of the test organism is added. The culture to be examined over the surface of paper discs (4 mm in size) individually impregnated with antibiotic is spaced evenly over the inoculated plate. Antibiotic diffuses outwards from each disc into the surrounding media and produces a diminishing gradient of concentration. On incubation, the bacteria grow on areas of the plate except those around the standard to which they are sensitive. The width of the zone of inhibition is a rough measure of the degree of sensitivity to the test organisms. A highly standardized version of the disc-diffusion method (the Bauer-Kirby test) is very popular in different countries. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no bacterial growth around the disc. An average of three independent determinations was recorded.

RESULTS

The results of antimicrobial activity of different concentrations of selected plants on different bacteria have been shown in Tables 1, 2 and 3. The methanol extracts of leaves of R. communis, B. asiatica and C. opaca exhibited high inhibitory effects on S. aureus, K. pneumoniae, E. coli, B. subtilis and P. vulgaris in all the concentration. C. coggygria, A. mexicana and C. fistula showed moderate impact on all the bacterial strain while E. hirta, Z. armatum and A. vesica were found to have lower affect or least affect on all the strains. Concentration of 10 μg/ml of leaves extract showed various zone of inhibition in different plants and bacterial strains (6 - 9 very low; 10 - 12 moderate and 13 - 16 very high). Similarly, the concentrations of 20 μg/ml showed more or less similar results (9 - 12 very low; 13 - 16 moderate and 17 - 19 very high). Concentration of 1g/ml of same extract affected all the bacterial strain (13 - 15 very low; 16 - 19 moderate and 20-23 very high). Thus, higher concentration checked the growth of all the bacterial strains (Table 3).

DISCUSSION

A number of workers have approved that medicinal plants...
represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. A wide range of medicinal plant parts are being used for extract as raw drug and they possess varied medicinal properties. The different parts are roots, stems, flowers, fruits, leaves, twigs exudates and modified plant organs. The zone of inhibition produced by the test organisms indicated their susceptibility to the plant extracts; it was observed that the zones of inhibition varies from one organisms to another and from one plant part extract to another. The present study is the preliminary reports on testing of another and from one plant part extract to another. The present study is the preliminary reports on testing of different medicinal plant species occurring in Uttarakhand. Out of nine plant species, only leaves extracts of different medicinal plant species were found very effective on two gram positive (B. subtilis and S. aureus) and three gram negative (E. coli, P. vulgaris and K. pneumoniae) bacterial strain, while E. aerogenes and P. aeruginosa exhibited moderate affect. Similarly, leave extract of C. coggygria, A. mexicana and C. fistula moderately inhibited all the seven bacterial strain. E. hirta does not showed any effect in all the concentration for any bacteria. Various workers have already shown that gram positive bacteria are more susceptible towards plants extracts as compared to gram negative bacteria. In the present study, similar trend has been noted as both the gram positive bacteria were affected by leave extracts of all the eight plant species. Bhattacharjee et al. (2006), Ahsan et al. (2009) Rahman et al. (2009); Osho and Adetunji (2010) have reported that A. mexicana is very effective against several bacterial strains, but in our study extract of A. mexicana showed moderate effect. Likewise, Cowan (1999) reported that extract of R. communis checked the growth of bacteria which is further supported by present study. However, before coming to conclusive statement, further studies like gas chromatography - mass spectroscopy (GCMS), infrared (IR) and nuclear magnetic resonance (NMR) essential for identification of present active constituents, responsible for the observed activity are under consideration to perform. Perfect prediction of antibacterial activity of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Methanol extracts was found to much better in present investigation. Crude extracts and their interaction with different active fractions of the plant need to be explored the exact mechanism of the interaction among the active phyto-constituents. Similarly, the efficacy of crude extracts preparation required to be studied in more detail.

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REFERENCES


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<th>S. No.</th>
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<th>Average zone of inhibitions (mm) from best of three</th>
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<td>1.</td>
<td>C. coggygria</td>
<td>++ ++ ++ ++ ++ ++ ++</td>
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<td>A. vasica</td>
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<td>4.</td>
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<td>5.</td>
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<tr>
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<td>7.</td>
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<tr>
<td>8.</td>
<td>R. communis</td>
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<tr>
<td>9.</td>
<td>C. fistula</td>
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Abbreviations: B. s. (Bacillus subtilis), S. a. (Staphylococcus aureus), K. b. (Klebsiella pneumonia), P. v. (Proteus vulgaris), E. c. (Escherichia coli), E. a. (Enterobacter aerogenes), P. s. (Pseudomonas aeruginosa)