

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 extracts 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 by villagers. 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, Kotdwara,

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 in refrigerator for further processes. Five Gram - negative (*Escherichia coli*, *Enterobacter aerogenes*, *Protues vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and two Gram - positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria strains were commercially purchased from Bangalore Genei and maintained at laboratory conditions under 37±2°C (Table 1). 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

Table 3. Showing antimicrobial screening of leaves of the plants in terms of average zone of inhibition (1 g/ml) [+++ very high; ++ moderate; + low].

S. No.	Name of the plants	Average zone of inhibitions (mm) from best of three						Standard 30 µg/ml	
		<i>B. s.</i>	<i>S. a.</i>	<i>K. p.</i>	<i>P. v.</i>	<i>E. c.</i>	<i>E. a.</i>		<i>P. a.</i>
1.	<i>C. coggygria</i>	++	++	++	++	++	++	++	26
2.	<i>A. vasica</i>	+	+	+	+	+	+	+	24
3.	<i>A. mexicana</i>	++	++	++	++	++	++	++	24
4.	<i>Z. armatum</i>	+	+	+	+	+	+	+	24
5.	<i>C. opaca</i>	+++	+++	+++	+++	+++	++	++	24
6.	<i>B. asiatica</i>	+++	+++	+++	+++	+++	++	++	26
7.	<i>E. hirta</i>								24
8.	<i>R. communis</i>	+++	+++	+++	+++	+++	++	++	24
9.	<i>C. fistula</i>	++	++	++	++	++	++	++	24

Abbreviations: *B. s.* (*Bacillus subtilis*), *S. a.* (*Staphylococcus aureus*), *K. p.* (*Klebsiella pneumoniae*), *P. v.* (*Proteus vulgaris*), *E. c.* (*Escherichia coli*), *E. a.* (*Enterobacter aerogenes*), *P. a.* (*Pseudomonas aeruginosa*)

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 leaves extracts of different medicinal plant species occurring in Uttarakhand. Out of nine plant species, only three (*C. opaca*, *B. asiatica* and *R. communis*) 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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