Journal of Applied and Natural Science 4 (1): 47-50 (2012)

JANS

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

Suneel Kumar Singh*, Ritu Vishnoi, Gulshan Kumar Dhingra[1] and Kunal Kishor[2]

Department of Biotechnology, Modern Institute of Technology, Dhalwala, Rishikesh – 249 201 (Uttarakhand), INDIA
[1]Department of Botany, Govt. P.G. College, Uttarkashi (Uttarakhand), INDIA
[2]Department of Microbiology, S.G.R.R. (ITS), Dehradun (Uttarakhand), INDIA
*Corresponding author. E-mail: drsuneelkumarsingh@gmail.com

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, Enterobactor 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, Enterobactor 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±2ºC (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

ISSN : 0974-9411 (Print), 2231-5209 (Online)

maintained on slants of medium and transferred to a fresh slant once in a week. The slant was incubated at 37ºC 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^{0}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

Table 1. Showing antimicrobial screening of leaves of the plants interms of average one of inhibition (10 µg/ml) [+++ very high; ++ moderate; + low].

S. No.	Name of the plants	Average zone of inhibitions (mm) from best of three							Standard 30 µg/ml
		B. s.	***S. a.***	***K. p.***	***P. v.***	***E. c.***	***E. a.***	***P. a.***	
1.	*C. coggygria*	++	++	++	++	++	++	++	22
2.	*A. vasica*	+	+	+	+	+	+	+	20
3.	*A. mexicana*	++	++	++	++	++	++	++	20
4.	*Z. armatum*	+	+	+	+	+	+	+	20
5.	*Carissa opaca*	+++	+++	+++	+++	+++	++	++	20
6.	*B. asiatica*	+++	+++	+++	+++	+++	++	++	22
7.	*E. hirta*								20
8.	*R. communis*	+++	+++	+++	+++	+++	++	++	20
9.	*C. fistula*	++	++	++	++	++	++	++	20

Table 2. Showing antimicrobial screening of leaves of the plants interms of average one of inhibition (20 µg/ml) [+++ very high; ++ moderate; + low].

S. No.	Name of the plants	Average zone of inhibitions (mm) from best of three							Standard 30 µg/ml
		B. s.	***S. a.***	***K. p.***	***P. v.***	***E. c.***	***E. a.***	***P. a.***	
1.	*C. coggygria*	++	++	++	++	++	++	++	24
2.	*A. vasica*	+	+	+	+	+	+	+	22
3.	*A. mexicana*	++	++	++	++	++	++	++	22
4.	*Z. armatum*	+	+	+	+	+	+	+	22
5.	*C. opaca*	+++	+++	+++	+++	+++	++	++	22
6.	*B. asiatica*	+++	+++	+++	+++	+++	++	++	24
7.	*E. hirta*								22
8.	*R. communis*	+++	+++	+++	+++	+++	++	++	22
9.	*C. fistula*	++	++	++	++	++	++	++	22

Table 3. Showing antimicrobial screening of leaves ofthe plants interms of average one 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
		B. s.	***S. a.***	***K. p.***	***P. v.***	***E. c.***	***E. a.***	***P. a.***	
1.	*C. coggygria*	++	++	++	++	++	++	++	26
2.	*A. vasica*	+	+	+	+	+	+	+	24
3.	*A. mexicana*	++	++	++	++	++	++	++	24
4.	*Z. armatum*	+	+	+	+	+	+	+	24
5.	*C. opaca*	+++	+++	+++	+++	+++	++	++	24
6.	*B. asiatica*	+++	+++	+++	+++	+++	++	++	26
7.	*E. hirta*								24
8.	*R. communis*	+++	+++	+++	+++	+++	++	++	24
9.	*C. fistula*	++	++	++	++	++	++	++	24

Abbreviations: B. s. (Bacillus subtilis), S. a. (Staphylococcus aureus), K. b. (Klebsiella pneumonia), P. v. (Proteus vulgaris), E. s. (Escherichia coli), E. a. (Enterobactor aerogenes), P. s. (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. cummunis*) 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 bas 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.

ACKNOWLEDGEMENTS

The authors are thankful to the Director (Modern Institute of Technology, Rishikesh) for providing the laboratory facilities; to Dr. G. S. Rajwar (Deputy Director, Higher Education, Uttarakhand) and to Director, Forest Research Institute (F.R.I.) and Botanical Survey of India (B.S.I.), Dehradun (Uttarakhand), for identification of plants.

REFERENCES

Ahsan, M.R., Islam, K.M., Haque, M.E. and Mossaddik, M.A. (2009). *In vitro* antibacterial screening and toxicity study of some different medicinal plants. *World J. Agric. Sci*., 5(5): 617-621.

Bauer, R.W., Kirby, M.D.K., Sherris, J.C. and Turck, M. (1996). Antibiotic susceptibility testing by standard single disc diffusion method. *Am. J. Clin. Pathol.*, 45 : 493 – 496.

Bhattacharjee, I., Chatterjee, S.K., Chatterjee, S. and Chandra, G. (2006). Antibacterial potentiality of argemone Mexicana solvent extracts against some pathogenic bacteria. *Memorias do Instituto Oswaldo Cruz*, 101(6): 645-648.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microb. Rev.*, 12: 564-582.

Hoffman, B.R., Delas, A.H., Blanco, K., Wiederhold, N., Lewis, R.E. and Williams, L. (2004). Screening of antibacterial and antifungal activities of ten medicinal plants from Ghana. *Pharmaceutical Biol.*, 42(1): 13-17.

Jigma, P., Rathish, N. and Sumitra, C. (2005). Preliminary screening of some folklore medicinal plants from western India for potential antimicrobial activity. *Indian J. Pharmacol*., 37(6): 408-409.

Kumar, N. (2006). Medicinal plants: future source of pesticides. *Asian J Microb, Biotech & Env. Sciences*, 8(1) : 57 – 59.

Rahman, M.M., Jahangir, Alam M., Sharmin, S.A., Rahman, M.M., Rahman, A. and Alam, M.F. (2009). *In vitro* antibacterial activity of *Argemone Mexicana* L. (Papaveraceae). *CMU J. Nat. Sci*., 8(1): 77-84.

Nair, R. and Chanda, S. (2006). Activity of some medicinal plants against certain pathogenic bacteria strains. *Indian J. Pharmacol*., 38(2): 142-144.

Osho, A. and Adetunji, T. (2010). Antimicrobial activity of the essential oil of *Argemone mexicana* Linn. *J. Med. Plants. Res*., 4(1): 19-22.

Panthi, M.P. and Chaudhary, R.P. (2006). Antimicrobial activity of some selected folklore medicinal plants from west Nepal. *Scientific World*, 4(4): 16-21.

Rahman, M.M., Alam, M.J., Sharmin, S.A., Rahman, M.M., Rahman, A. and Alam, M.F. (2009). *In vitro* antibacterial activity of *Argemone mexicana* L. (Papaveraceae). *CMU. J. Nat. Sci*., 8(1): 77-84.

Rios, J.L., Recio, M.C. and Villar, A. (1988). Screening methods for natural products with antibacterial activity: a review of the literature. *J. Ethanopharmacol*., 23: 127-149.

Singh, M., Srivastava, S. and Rawat, A.K.S. (2007a). Antimicrobial activities of Indian Berberis species. *Fitoterapia*, 78: 574-576.

Singh, S.K., Kumar, M., Kohli, S., and Rajwar, G.S. (2007b). Screening of methanolic leaf extracts of *Eupatorium adenophorum* against different bacteria under *in vitro* condition. *Annals of Med. Entomol*., 16(1 & 2): 1-6.

Srinivasan, K., Dheen, M.A.N., Perumal, G., Mohanasundari, C. and Natarajan, D. (2005). Screening of methanolic leaf extracts of some medicinal plants against pathogenic bacteria. *Ad. Plant Sci*., 18(II): 605-607.

Uniyal, S.K., Singh, K.N., Jamwal, P. and Lal, B. (2006). Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. *J. Ethnobiol. Ethnomed.*, 2: 1-14.