Antibacterial and antioxidant activities of ethanol extracts from trans Himalayan medicinal plants

Phani Kumar G[1*], Raj Kumar[1], Rupesh Badere[2] and Shashi Bala Singh[1]

[1]Defence Institute of High Altitude Research (DIHAR) Defence R & D Organisation Ministry of Defence Govt. of India, C/o 56 APO, Leh-Ladakh, India [2]RTM Nagpur University, Nagpur, India

ABSTRACT

The antibacterial and antioxidant activity of the ethanol extracts from some trans Himalayan medicinal plants being used in '*Amchi system of medicine*'. These plants were assessed towards selected bacteria as well as in different antioxidant models. Extracts, at concentration between 8 and 250 µg/ml, showed a significant antibacterial effect expressed as minimum inhibition concentration (MIC) against both Gram-negative and Gram-positive bacteria. In particular, samples of *Podophyllum hexandrum* leaf, *Verbascum thapsus* stem against *Bacillus subtilis* and *Salvia sclarea* flower against *Pseudomonas aeruginosa* (MIC = 8 µg/ml) were highly effective. The antioxidant activity was determined by the 2,2- diphenyl -1 picryl hydrazyl (DPPH) method, *Ephedra gerardiana* leaf (13.30 ± 0.6 µg/ml) and *Salvia* sclarea flower (14.97 ± 2.9 µg/ml) were observed with maximum activity. Conclusion of the study supports the use of these plants in traditional medicine to treat various ailments like stomach complaints, wound infections and intestinal disorders etc.

Key words: Antibacterial activity; Anti-oxidant activity; Ethnopharmacology; trans-Himalaya; Amchi system of medicine; Cold desert medicinal plants.

INTRODUCTION

Numerous useful drugs from plants have been discovered by their traditional uses.[1] Medicinal plants contain physiologically active principles include antimicrobial and anti-oxidant properties.[2] Nature is a treasure of diversified plant species possessing multiple usages to mankind. Globally, there is an increasing interest of herbal usages in the living hood set-ups.[3] Today, people all over the world are trying to keep away from chronic stress, pollution and synthetic drugs.[4] The most commonly used anti-xodant at the present time are butylated hydroxyamisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG) and tert-butylhydroxytoluene (TBHQ).[5] However there are suspected of being responsible for liver damage and carcinogenesis in laboratory animals.[6-7] Therefore the developement and utilization of more effective anti-oxidants of natural origin are desire.[8]

Address for correspondence:
Ph: 91+9469164786
E-mail: phani_bot@rediffmail.com

DOI: ****

The Himalayas represent the largest mountain chain in the world, covering about one million sq. km. 'Himalaya' the youngest mountain range of world is famous for its rich plant diversity and varied ecosystem, containing large number of plants. The Trans-Himalayas of Indian cold desert covers under alpine and high alpine zones with peculiar climatic condition featuring and snow covered mountains. The use of plants in curing and healing is as old as man himself.[9] Plants containing beneficial and medicinal properties have been known and used in some form or other by primitive people. Biodiversity of Trans-Himalayas is our natural wealth and its conservation is important for economic, ecological, scientific and ethical reasons. The flora of cold desert areas and their ethnobotanical importance were studied earlier by several authors.[10-12] They focused on plant diversity, its documentation and scattered ethnobotanical uses by the tribal communities. Biodiversity provides us with goods and services fundamental to our survival including food, fodder and medicine. The selection of the species used in this study was mainly based on their ethnomedicinal uses include diarrhea, dysentery, cold, cough, skin infections, healing of wounds, dandruff, respiratory infections, kidney complaints, anti-septic, etc. Some plants without ethnomedicinal prudent but not previously studied were included too.

MATERIALS AND METHODS

Study area

The field work was carried out in Nubra valley, cold desert of Himalaya's in Ladakh region. The Nubra comprises the valley of Shyok river from its acute-angled bend down to its confluence with Nubra and further towards Indus. It is northern most valley of Ladakh. Khardung La pass (18,380 ft) is the gate way of the valley and it includes Siachin glacier. The area lies between two great mountain ranges, i.e. Ladakh (on the south) and Karakoram (on the north). Approximately, 34°15' 45 to 35°30' N latitude and 76°55' to 78°05' E longitude. The topography of the valley is entirely different from other valleys of Ladakh. There is a great variation in altitude and ranges approximately between 8000 ft to 24000 ft mean sea level. The climate of the region is extreme cold desert and characterized by high wind velocity continues throughout the year causes great variation in temperatures. Winter temperatures go below zero (minimum -25°C) and summers as high as 38°C. Precipitation is scanty with less than 80 mm per annum. The valley remains cut off from other parts of country during winter months due to extreme weather conditions.

Collection and identification of plants

Information on the plants used for treatment of several complaints / diseases, other uses were obtained through interviews with traditional healers and conformed by group discussion with local medicinal men called 'Amchi'. The system of medicine being used in Ladakh is Tibetian system of medicine and also called Amchi system of medicine. The plants were identified comparing with authentic specimens at Defence Institute of High Altitude Research (DIHAR) herbarium. Various plant parts such as leaf, stem, flower and roots having ethnobotanical value were separated and shade dried for further analysis. The plants selected were washed thoroughly and dried in shade. Aerial parts of the dried plant material was ground to fine powder and about 250 g of the plant material was collected, and extracted repeatedly in ethanol. The extracts were filtered, concentrated, dried at room temperature and used for determination of antibacterial and antioxidant acitivity.

Determination of antibacterial activity

Four bacterial strains, obtained from the Department of Microbiology, RTM Nagpur University, Nagpur, India. They included the Gram positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus* and the Gram negative bacteria: *Escherichia coli* and *Pseudomonas aeruginosa*). The bacterial strains were grown on Mueller Hinton (MH) agar plates and suspended in MH broth. The MIC values against bacterial strains were performed using the Ericcson and Sherris[13] broth dilution method (MH broth). Inoculum suspensions were prepared form 6 h broth cultures. The extract was sterilized by Millipore filtration (0.45 μm) and added to MH broth medium. The bacterial suspensions were aerobically incubated for 24 h at 37°C. The MIC was defined as the lowest concentration able to inhibit any visible bacterial growth. Negative control cultures, containing only sterile physiological Tris-buffer, were also prepared, while the discs soaked in standard broad spectrum antibiotics i.e. Streptomycin and Amoxicillin were used as positive controls.

Determination of anti-oxidant activity

The anti-oxidant activity was assessed using quantitative 2,2- diphenyl -1 picryl hydrazyl (DPPH).[14] The solution of DPPH was prepared with methanol. The absorbances were read at 517 nm after 30 min of incubation and then percentage of decolourisation determined. Vitamin C was used as the positive control. The IC_{50} values (concentration at which 50% of decolourisation was obtained). DPPH radical's concentration was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

Where A_0 was the absorbance of the control and A_1 was the absorbance in the presence of sample (Oktay et al., 2003).

RESULTS AND DISCUSSION

The Amchi's (medicine men) are still looking after more than 60% public health of tribal communities[15] and they are totally depend upon natural resources for collection of plants and their parts. Traditional and indigenous system of medicine persists in all over the world.[16] The present investigation has highlighted the therapeutic value of some plant species of cold desert Nubra valley to cure cold, cough, fever, stomach problems, kidney disorders, urinogenital complaints, skin diseases, diarrhea, dysentery and problems in menstrual cycle etc. Our findings on antibacterial activity of cold desert plants of Nubra valley justify some ethnobotanical uses such as against diarrhea, dysentery, wounds because we demonstrated strong activity of these plants against some pathogens of the digestive tract as well as septic. Due to the continuous emergence of antibiotic-resistant strains there is continual demand for new antibiotics. In many developing countries about 80% of available drugs come from medicinal plants and in industrialized countries plants make up the raw material for processes, which synthesize pure chemical derivatives.[17] The antibacterial activity results are shown in Table-1. Present findings showed that the ethanol extracts of *Salvia sclarea* flower; *Arnebia euchroma* root against *Pseudomonas aeruginosa* and

Table 1: Minimum inhibitory concentrations (MICs) in µg/ml of antibacterial activity

Leaf samples	*B.s*	*S.a*	*E.c*	*P.a*	Aerial part	*B.s*	*S.a*	*E.c*	*P.a*
Achillea millefolium	32	>250	32	n.a.	*Allium przewalskianum*	>250	>250	32	n.a.
Artemisia dracunculus	128	64	>250	n.a.	*Allium ramosum*	n.a	128	64	>250
Artemisia tournefortiana	>250	n.a	32	>250	**Flower**				
Bidens pilosa	n.a	128	64	16	*Dracocephalum heterophyllum*	128	n.a	64	>250
Ephedra gerardiana	n.a	128	32	64	*Salvia sclarea*	>250	n.a	n.a	8
Mentha royleana	32	32	>250	64	**Underground**				
Podophyllum hexandrum	8	64	16	32	*Allium przewalskianum*	32	32	64	>250
Salvia sclarea	64	64	32	n.a	*Allium ramosum*	n.a	64	32	n.a
Verbascum thapsus	32	32	32	>250	*Arnebia euchroma*	32	n.a	64	8
Stem samples					*Arnebia guttata*	32	128	32	16
Bidens pilosa	n.a	n.a	32	n.a	*Artemisia tournefortiana*	64	128	32	n.a
Mentha royleana	16	>250	32	n.a	*Bidens pilosa*	n.a	32	64	64
Salvia sclarea	n.a	64	n.a	>250	*Dactylorhiza hatagirea*	32	32	32	32
Verbascum thapsus	8	16	32	>250	*Mentha royleana*	>250	n.a	n.a	n.a
					Salvia sclarea	64	n.a	32	32
					Verbascum thapsus	32	128	>250	32

Note: *B.s = Bacillus subtilis; S.a = Staphylococcus aureus; E.c= Escherichia coli; P.a =Pseudomonas aeruginosa*; n.a. = absence of inhibition at 1000 µg/ml.

Table 2: IC 50 values in µg/ml (mean + S.D.; n=3) of anti-oxidant activities

Plant material	Anti-oxidant activity (n=3) µg/ml	Plant material	Anti-oxidant activity (n=3) µg/ml
Leaf		**Aerial parts**	
Achillea millefolium	54.50 + 3.1	*Allium przewalskianum*	18.24 + 0.21
Artemisia dracunculus	98.20 + 7.9	*Allium ramosum*	>100
Artemisia tournefortiana	>100	**Flower**	
Bidens pilosa	>100	*Dracocephalum heterophyllum*	89.76 + 5.1
Ephedra gerardiana	13.30 + 0.6	*Salvia sclarea*	14.97 + 2.9
Mentha royleana	>100	**Underground parts**	
Podophyllum hexandrum	15.94 + 0.2	*Allium przewalskianum*	>100
Salvia sclarea	96.70 + 12.1	*Allium ramosum*	>100
Verbascum thapsus	>100	*Arnebia euchroma*	33.27 + 1.1
Stem		*Arnebia guttata*	15.15 + 0.22
Bidens pilosa	>100	*Artemisia tournefortiana*	>100
Mentha royleana	>100	*Bidens pilosa*	72.33 + 4.61
Salvia sclarea	>100	*Dactylorhiza hatagirea*	97.40 + 7.3
Verbascum thapsus	33.16 + 6.9	*Mentha royleana*	>100
		Salvia sclarea	>100
		Verbascum thapsus	35.21 + 7.5

Podophyllum hexandrum leaf; *Verbascum thapsus* stem against *Bacillus subtilis* had interesting Minimum inhibitory concentrations (MICs) with 8 µg/ml. Inhibiting activity of *Pseudomonas aeruginosa* is particularly interesting from a medical point of view because these microbial agent is responsible for sever opportunistic infections. These findings were also shown in other plants extracts.[18-21]

The anti-oxidant activity of the methanol extracts were analysed for IC_{50} values are displayed in Table-2. (15.15 ± 0.22) *Ephedra gerardiana* leaf, *Salvia sclarea* flower and *Arnebia guttata rhizome* showed highest free radical scavenging activity with 13.30 ± 0.6 µg/ml, 14.97 ± 2.9 µg/ml and 15.15 ± 0.22 µg/ml respectively. Most of samples showed 50% DPPH inhibition in less than 75 µg/ml material. The growing interest in the substitution of synthetic food antioxidants with natural ones has fostered research on plant sources and screening of raw materials to identify new antioxidants. Interest in oxidation reactions is not confined to the food industry, as antioxidants are widely needed to prevent deterioration of other perishable goods, such as cosmetics, pharmaceuticals and plastics. In addition, other biological properties such as anticarcinogenicity have been reported for natural and synthetic antioxidants.[22-23] From these results it can concluded that the crude extracts of cold desert medicinal plants of Nubra valley are promising medicinal value like antibacterial and anti-oxidant activities. Further phytochemical work need to be done on these extracts

including fractionation to isolate active constituent and subsequent pharmacological evaluation.

CONCLUSION

Present results showed interesting antibacterial and anti-oxidant activity of the ethanol extracts from cold desert medicinal plants. The contemporary presence of these medicinal activities suggests that these plants may be source of bioactive substances with multifaceted activity. Further phytochemical work need to be done on these extracts including fractionation to isolate active constituent and subsequent pharmacological evaluation. Farmers should be involved in the cultivation of medicinal plants at least in their barren and fallow land; this would augment their income and in turn help in the conservation of the species. Appropriate research should be carried out in institutions in the hills to develop agro-techniques for the cultivation of medicinal plants on priority basis

ACKNOWLEDGEMENTS

The authors are thankful to the inhabitants of the surveyed areas for their cooperation and help during field study. Thanks to local medicine men (Amchis) for identifying plants and clarifying medicinal uses.

REFERENCES

1. Farnsworth NR, Soejarto DD. Potential consequences of plant extinction in the United States on the current and future availability of prescription drugs. Econ. Bot. 1985;39(3):231-40.
2. Srinivasan D, Nathan S, Suresh T, Peumalsamy O. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. Journal of Ethnopharmacology. 2001;74:217-20.
3. Holmstedt B. Historical perspective and future of thenopharmacology. Journal of Ethnopharmacology, 1991;32:7-24.
4. Benli M, Bingol U, Geven F, Guney K, Yigit N. An Investigation on the antimicrobial activity of some endemic plant species from Turkey. African Journal of Biotechnology, 2008; Vol. 7 (1):1-5.
5. Sherwin FR. 1990. Antioxidant. In: Food Additive (ed. Branen R), 139-193. Marcel Dekker, New York.
6. Grice HC. Safety evaluation of butylated hydroxytoluence (BHT) in the liver, lung and gastrointerstinal tract. Food Chemistry and Toxicology. 1986;24:1127-30.
7. Wichi HP. Enhanced tumor development by butylated hydroxyanisole (BHA) from the prospective of effect on forestomach and oesophageal squamous epithelium. Food Chemistry and Toxicology. 1998;26:717-23.
8. Oktay M, Gulcin I, Kufrevioglu OI, Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extract. Lebensmittel-Wissenchaft und Technologie. 2003;36:263-71.
9. Hedberg I. (1987): Research on Medicinal and Poisonous plnts of tropics: *Past, present and future in medicinal and poisonous plants of the tropics* (Eds. Leewenerg, A.J.M.) International Book distribution, Dehra Dun, India, 9-15.
10. Aswal BS, Mehrotra BN, (1987): Ethnobotanical studies on the flora of Lahaul Valley (North-West-Himalayas). In: Sharma, M. R. and Gupta, B. K. (Eds.) Recent Advances in Plants Sciences. 116-130.
11. Jain, S.K., (1991): *Dictionary of Indian folk-medicine and ethnobotany.* Depp Publications. New Delhi.
12. Kaul, M.K., (1997): *Medicinal plants of Kashmir and Ladakh* (Temperate and cold Himalaya). Indus Publishing Company, New Delhi.
13. Ericcson, H.M., Sherris, J.C., (1971): Antibiotic sensitivity testing: report of an international collaborative study. Acta Pathologica Microbiologica Scandinavica 217,1.
14. Kumar Raj, Kumar GP, Chaurasia OP. *In vitro* antioxidant activity of methanolic extract of *Rhodiola Imbricata* Edgew. Phcog. J.,2010; 7 (2):157-161.
15. Chaurasia OP, Singh B. (1996-2001): *Cold desert flora* (I-V) Field Research Laboratory. Leh- Ladakh.
16. Kalita D, Bikash D, Traditional medicines used by the Sonowal Kacharis of Brahmaputra valley, *Assam,* Plant Architecture, 2004;4:77.
17. Penso G.The role of WHO in the selection and characteristion of medicinal plants. Journal of Ethnopharmacology, 1980;2:183-8.
18. Phani Kumar G, Chaturvedi A, Is *Cleistanthus collinus* Benth. a poisonous plant?. Journal of Phytological Research, 2005a;Vol 18 (1):27-33.
19. Phani Kumar G, Chaturvedi A, Ethnopharmacological and Phytochemical screening of *Putranjiva roxburghii* Wall. Bioinfolet, 2006a;3(1):11-14.
20. Phani Kumar G, Chaturvedi A, Antimicrobial activity of Euphorbiaceae. Indian Drugs, 2006b;43(2):156-9.
21. Phani Kumar G, Chaturvedi A, A Potent medicinal plant: *Securinega virosa* (Willd.) Mull. Arg., Journal of Phytological Research. 2007;Vol 20: (2) 35-41.
22. Moure A, Cruz JM, Franco D, Dominguez JM, Sineiro J, domfinguez H, Nunez MJ, Parajo JC. Natural antioxidants from residual sources. Food Chemistry, 2001;2:145-71.
23. Phani Kumar G, Chaturvedi A, (2005b): Anticancer activities of some members of Euphorbians. *Advances in Ethnobotany,* (Ed. Das, A. P.,) Bisensingh Mahendrapal Singh Publisher, Dehradun, 297-302.