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Potential Antibacterial Activity of *Bergenia purpurascens*

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Abstract

Bergenia purpurascens, an important traditional medicinal plant used as Ayurveda, was collected at 3800 m from the Manaslu Conservation Area. *In vitro* evaluation of the crude extracts of the plant rhizomes using agar well diffusion assay against eight bacteria displayed potential antibacterial activity. The minimum inhibitory concentration values for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium*, *Shiegella flexneri* and *Staphylococcus aureus* were determined. This study supports the traditional use of the plant material for the healing of wounds and antibiotic effect.

***Key words*:** Antibacterial, *Bergenia purpurascens*, Manaslu Conservation Area

Introduction

The Manaslu Conservation Area (MCA), with topographic extremes starts at altitude of 600 meter to top of Mount Manaslu (the 8th highest peak in the world) at 8,163 meter, is a remote Himalayan region in Western Nepal. MCA covers 1663 km^2 land varying physiographic zones from sub-tropical to alpine and lies between latitudes 28˚20′ and 28˚45′ north and longitudes 84˚28′ and 85˚11′ east. MCA is enriched with diversified vegetation of different flora and fauna. Because of remotely set villages, the villagers of this region basically depend on traditional herbal medicinal practices to get remedy from several health diseases. With the help of the experiences gained from the people of the past generations, the traditional medicinal practitioners (*Amchis*), faith healers and ethnic groups have made use of herbal products for various purposes. The high altitude medicinal plants are being consumed by the people for the treatment of various infectious diseases like flue, measles, typhoid, diarrhea, wound, cold, cough and other microbial diseases despite the active principles in the plant materials are not fully established. Recently, the Nepal Academy of Science and Technology (NAST) has envisioned a thematic program for exploration and utilization of biological resources of MCA. We are interested in evaluation of the biological activity of the plant extracts as well as isolation of natural products leading to identification of new medicinal agents.

After the discovery of penicillin, antibiotic chemotherapy has appeared as one of the most important scientific achievements in the twentieth century. According to the chemical structures, the most widely used antimicrobial agents fall under following groups: penicillin, cephalosporin, carbapenem, â-lactamase inhibitor, aminoglycoside, quinolone, tetracycline, lincosamide, sulfonamide, trimethoprim and polypeptide (Yao & Moellering 1991). The misuse of these antibiotics could lead to the emergence of drug-resistant bacteria making threats to the global public health. Thus, finding of new microbial agents is of great concern. Natural products are important sources for finding of new drugs and protected MCA

might be a unique landside to provide us genuine and hidden herbs. Herein the antibacterial property of *Bergenia purpurascens* (local name Pakhanbed), collected from its natural habitat at 3800 m in MCA has been described.

Genus *Bergenia*, flowering plant in the family Saxifragaceae, is the source of drug bergenin. They are distributed in central Asia and the Himalayan region. The genus comprises ten species namely; *Bergenia ciliate*, *Bergenia cordifolia*, *Bergenia crassifolia*, *Bergenia emeiensis*, *Bergenia ligulata*, *Bergenia pacumbis*, *Bergenia purpurascens*, *Bergenia scopulosa*, *Bergenia stracheyi* and *Bergenia tianquanensis*. *Bergenia purpurascens* (Hook. F. & Thomas) Engl. is a perennial herb, 1 to 2 feet tall with thick, stout, creeping rootstock and bears spirally arranged rosette of leaves 6–35 cm long and 4–15 cm broad (Ghimire *et al.* 2008, CSIR 2000). The leaves are glossy green above and purple-red underneath for most of the year but in the winter they turn red or bronze. The leaves are leathery, elliptic to ovate, hairless and often have wavy or saw-toothed edges. The cone-shaped, pink to purplish-red flowers bloom in mid to late spring in terminal cymes. The rhizomes of *Bergenia purpurascens* is considered as astringent, styptic and tonic. The local people in the Himalayan region use rhizome paste in wounds, body ache and bone fracture (Shrestha & Ghimire 1996). Rhizome decoction is said to be safe and effective for treatment of chronic bronchitis, diarrhoea, body ache, and loss of eye sight. It is also used in the treatment of giddiness and general physical feebleness.

Methodology

Plant material

In this study, *Bergenia purpurascens* was selected to investigate which is scientifically much less explored species among genus *Bergenia*. Rhizomes of *Bergenia purpurascens* were collected at 3700-3800 m above sea level from the premises of Kalchuman Lake in MCA in June 2010. The plant material was shade dried and taken to the laboratory. The plant was authenticated at the National Herbarium Laboratory, Lalitpur, Nepal.

Extraction of plant material

The plant material (rhizome of *Bergenia purpurascens*) was chopped, air-dried at room temperature for 2 weeks and coarsely ground. The ground plant material (150 g) was successively extracted with hexane (700 mL, 6 hours), $CHCl_3$ (700 mL, 7 hours) and MeOH (800 mL, 19 hours) in a Soxhlet extractor. The organic extracts were concentrated under reduced pressure using a rotary evaporator and finally dried in a water bath (50 ^{O}C) to obtain hexane, chloroform and methanolic extracts. Upon vacuum distillation of a portion of the methanolic extract afforded pale yellow oil, the constituents of which was analyzed by a gas chromatography-mass spectrometer (GCMS-QP2010, Shimadzu). All the extracts and oil were stored at 4^{O}C until further use.

Phytochemical screening

The phytochemical screening of the hexane, chloroform and methanolic extracts as well as oil was carried out to identify the presence of different groups of natural pigments by utilizing the standard procedures (Ciulei 1982, Harborne 1984).

Bacterial strain

Eight bacterial strains namely *Escherichia coli* (ATCC 25922), *Klebsiella pneumonia* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium* (ATCC 14038), *Shiegella flexneri* and *Staphylococcus aureus* (ATCC 25923) were used for the antimicrobial assay. The bacterial cultures were maintained in nutrient agar slants at 37 ^{O}C. In prior of using, each pathogenic bacterium was reactivated by transferring two-loopful inoculation into a separate nutrient broth and incubated for 24 hours at 37^{O}C.

Antibacterial susceptibility assay

Agar well diffusion assay was used to evaluate the antibacterial potential of crude extract of *Bergenia purpurascens* (Dingle *et al.* 1953, Perez *et al.* 1990). A solution of Mueller Hinton broth (about 25 mL) was distributed in eight Petri dishes (90 mm). The standard culture inoculums were swabbed on the solidified surface of the media separately. Using a sterilized cup-

borer, five wells of 6 mm diameter were bored. The solutions of crude extracts (hexane, chloroform and methanolic extracts) and oil were prepared in dimethyl sulphoxide (DMSO) to obtain concentration of 50 mg/mL. 50 ìL of prepared solutions was separately charged into the well and DMSO was also used as negative control. The plates were incubated at 37 ^{O}C for 24 hours. After complete incubation, the plates were inspected for the zone of inhibition produced due to the antimicrobial activity of the extracts.

Determination of minimum bactericidal concentration

The methanolic extract displayed potential antibacterial activity and was further evaluated for minimum bactericidal concentration (MBC) by standard two-fold microdilution broth methodology (NCCLS 1997). A stock solution of methanolic extract was prepared in DMSO (50 mg/mL) and was serially diluted with Mueller Hinton broth taken in different sterile tubes to obtain a concentration ranging from 25.0 mg/mL to 0.81 mg/mL. A standard culture inoculum of each bacterial strain (50 µL) was added on every tube in a set and incubated at 37 ^{O}C for 24 hours. A loopful of each incubated bacterial solution was sub-cultured by sticking in Mueller Hinton plate and then incubated at 37 ^{O}C for 24 hours. After incubation, the minimum concentration of the extract in which visible growth of bacteria stopped was determined.

Results and Discussion

Soxhlet extraction of the rhizomes of *Bergenia purpurascens* (150 g) gave hexane extract (0.85 g, 0.56%, pale yellow), chloroform extract (0.50 g, 0.33%, yellowish brown) and methanolic extract (58.25 g, 38.83%, dark reddish brown). Furthermore, a portion of methanolic extract under vacuum distillation afforded significant quantity of pale yellow oil, which was soluble in water and boiled at 94 ^{O}C. The phytochemical screening of these organic extracts using different chemical tests showed the presence of steroids, coumarins, polyphenols, reducing compounds and glycosides. GC-MS analysis of the oil indicated that it consisted of phenol (7.4%), 2-cylohexenone (19.4%), 1,2-benzenediol (39.3%), hydroquinone (26.7%), 4-methyl-1,2-benzenediol (2.4%) and 1,2,3-benzenetriol (4.8%).

The antibacterial activity is expressed in terms of the diameter of zone of inhibition (in mm) and the results are summarized in Table 1. The Gram-negative bacteria are in general more resistant against antimicrobial agents compared to the Gram-positive bacteria. The antibacterial susceptibility assay revealed that the methanolic extract has potential antibacterial activity against both Gram-negative and Gram-positive bacteria tested and zone size was ranged from 13 mm to 15 mm. Only methanolic extract was found effective against Gram-positive *Staphylococcus aureus* with zone size 13 mm while the growth of *Pseudomonas aeruginosa* was inhibited by all the extracts. On the other hand, oil displayed no significant zone of inhibition in antibacterial susceptibility assay hence it has no antibiotic value. However, the bactericidal concentrations of antimicrobial agents may vary and the possible errors could not be ignored.

Table 1. Antibacterial susceptibility assay of *Bergenia purpurascens*

S. N.	Pathogenic bacteria used	Zone of inhibition shown by different extracts[a] (in mm diameter)			
		Hexane extract	Chloroform extract	Methanolic extract	Oil
1	*Escherichia coli*	–	–	14	9
2	*Klebsiella pneumonia*	–	–	15	–
3	*Pseudomonas aeruginosa*	11	15	13	7
4	*Salmonella paratyphi*	–	–	15	–
5	*Salmonella typhi*	–	–	15	–
6	*Salmonella typhimurium*	–	–	14	–
7	*Shiegella flexneri*	–	–	14	–
8	*Staphylococcus aureus*	–	–	13	–

[a]Values of the zone of inhibition (in mm) include the diameter of well (6 mm) after 24 hours incubation against different bacterial species in agar well diffusion assay for antibacterial activity of *Bergenia purpurascens*. (–) sign indicates no significant zone of inhibition was observed.

Minimum bactericidal concentration of the methanolic extract of *Bergenia purpurascens* rhizomes, as evaluated against eight bacteria tested by microbroth dilution assay, are presented in Table 2. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were found most susceptible bacteria as they were significantly inhibited at 12.5 mg/mL concentration of methanolic extract, where as MBC of the same extract was found to be 25 mg/mL against *Salmonella paratyphi*, *Salmonella typhi*, *Salmonella typhimurium* and *Shiegella flexneri*.

Table 2. Minimum bactericidal concentration of active methanolic extract of *Bergenia purpurascens* rhizome

S. N.	Test organisms	Concentration of methanolic extract[a] (mg/mL)						MBC (mg/mL)
		25.00	12.50	6.25	3.12	1.56	0.78	
1	*Escherichia coli*	–	–	+	+	+	+	12.5
2	*Klebsiella pneumonia*	–	–	+	+	+	+	12.5
3	*Pseudomonas aeruginosa*	–	–	+	+	+	+	12.5
4	*Salmonella paratyphi*	–	+	+	+	+	+	25.0
5	*Salmonella typhi*	–	+	+	+	+	+	25.0
6	*Salmonella typhimurium*	–	+	+	+	+	+	25.0
7	*Shiegella flexneri*	–	+	+	+	+	+	25.0
8	*Staphylococcus aureus*	–	–	+	+	+	+	12.5

[a]Different concentrations of the methanolic extract evaluated for MBC using microbroth dilution assay as recommended by NCCLS. (–) sign indicates "no bacterial growth observed in the specified concentration" and (+) sign represents "growth observed".

Beside bergenin, rhizomes of *Bergenia purpurascens* of Chinese origin also constitutes 4-*O*-galloylbergenin, 11-*O*-galloylbergenin, 4,6-di-*O*-galloylarbutin, 6-*O*-galloylarbutin, 2,4,6-tri-*O*-galloyl-D-glucose, 1,2,4,6-tetra-*O*-galloyl-â-D-glcose, (+)-catechin, 7-*O*-galloyl-(+)-catechin, procyanidine B-3 and 3-*O*-galloylprocyanidine B-1 (Xin-Min *et al.* 1987). A strong antiviral activity of methanolic extract from *Bergenia ciliata* rhizomes against herpes simplex virus-1 and human influenza virus has been reported (Rajbhandari *et al.* 2001). Antibacterial activity of *Bergenia ciliata* rhizomes also has been recently documented (Kumar *et al.* 2002). However to the best of our knowledge, *Bergenia purpurascens* of Nepalese origin is still an unexplored plant material for its biological activities and chemical composition.

The phytochemical screening of the rhizomes of *Bergenia purpurascens* collected from the Manaslu Conservation Area, Nepal at 3700-3800 m showed the presence of steroids, coumarins, polyphenols, reducing compounds and glycosides. The oil isolated contained phenol, 2-cylohexenone, 1,2-benzenediol, hydroquinone, 4-methyl-1,2-benzenediol and 1,2,3-benzenetriol. The antibacterial susceptibility assay showed that the methanolic extract has potential antimicrobial activity against the test organisms with 13-15 mm size of inhibition zones. This result confirms the traditional use of the plant material for the healing of wounds and antibiotic effect. The isolation and identification of active antimicrobial constitute(s) from the most active methanolic extract is under progress in our laboratories.

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References

Ciulei, I. 1982. *Methods for studying vegetable drugs*. Chemical Industries Branch, Division of Industrial Operations, UNIDO, Bucharest, Romania.

CSIR. 2000. *The wealth of India: A dictionary of Indian raw materials and industrial products. First Supplement Series, Vol I.* Council of Scientific and Industrial Research (CSIR), New Delhi, India.

Dingle, J., W. W. Red and G. L. Solomons. 1953. The enzymatic degradation of pectin and other polysaccharides II: Application of cup assay method to the estimation of enzymes. *Journal of Science, Food and Agriculture* ***4***:149-153.

Ghimire, S. K., I. B. Sapkota, B. R. Oli and R. R. Parajuli. 2008. *Non-timber forest products of Nepal Himalaya: Database of some important species found in the mountain protected areas and surrounding regions*, WWF Nepal, Kathmandu, Nepal.

Harborne, J. B. 1984. *Phytochemical methods*. Chapmann and Hall, London.

Kumar, V., T. Shah, G. B. Shah and N. S. Parrnar. 2002. Antibacterial activity of *Bergenia ciliata* rhizomes. *Indian Journal of Natural Products*, ***18***:22-25.

NCCLS. 1997. *Method for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. Approved Standards M7-A4, National Committee for Clinical Laboratory Standards, Wayne, Pa.

Perez, C., M. Pauli and P. Bazerque. 1990. An antibiotic assay by the agar-well diffusion method. *Acta Biologiae et Medecine Experimentalis* ***15***:113-115.

Rajbhandari, M., U. Wegner, M. Jülich, T. Schöpke and R. Mentel. 2001. Screening of Nepalese medicinal plants for antiviral activity. *Journal of Ethnopharmacology*, ***74***:251-255.

Shrestha, K. K. and S. K. Ghimire. 1996. *Plant diversity inventory of the proposed Kanchanjunga Conservation Area (Ghunsa and Simbua valley), Report Series No. 22.* WWF Nepal Programme, Kathmandu, Nepal.

Xin-Min, C., T. Yoshida, T. Hatano, M. Fukushima and T. Okuda. 1987. Galloylarbutin and other polyphenols from *Bergenia purpurascens*. *Phytochemistry*, ***26***:515-517.

Yao, J. D. C., R. C. Moellering Jr. 1991. In *Manual of clinical microbiology* (Eds. A. Belows, W. J. Hauster Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy). American Society for Microbiology, Washington D. C., 5th edition, chapter 108, pp. 1065-1098.