

Free radical scavenging activity of an aerial part of *Aconitum heterophyllum* (wall) from Kashmir Himalayas, India

Ayaz Ahmed^{*1}, Ranjana Singh² and Ravi Upadhyay³

*Corresponding author:

Ayaz Ahmed

¹Govt. Narmada Mahavidhyalya
Hoshangabad-461001, M.P, India

²Govt. Motilal Vigyan Mahavidhyalya
Bhopal-462008, M.P, India

³Govt. Narmada Mahavidhyalya
Hoshangabad-461001, M.P. India

Abstract

The plant *Aconitum heterophyllum* native to Kashmir has been widely used as a traditional medicine to cure diseases associated inflammations, cardiovascular, neurodegenerative and other ailments, microbial, bronchitis and joint pains since times. Keeping this in view the present study was carried out to investigate the antioxidant potential and the phytoconstituents composition of the plant extract. Various compounds were detected in the preliminary screening of the leaf extract of the plant, among them were the dominance of alkaloids and flavonoids. Wide range of antioxidant activity was detected in the plant compared to the standard antioxidants.

Keywords: *Aconitum heterophyllum*, Phytoconstituents, Antioxidant activity

Introduction

Since times, herbal medications have been used for relief from various diseases [1]. Despite the great advancement observed in modern medicine, plants still make an important contribution to health care. Much interest, in medicinal plants however, emanates from their long use in folk medicines especially in developing countries. Large number of medicinal plants has been investigated for their potential antioxidant properties. Natural antioxidants are very effective to prevent the destructive processes caused by oxidative stress [2]. Although the toxicity profile of most medicinal plants have not been thoroughly evaluated, it is generally accepted that medicines derived from plant products are safer than their synthetic counterparts [3,4]. Substantial evidence has indicated key roles for reactive oxygen species (ROS) and other oxidants in causing numerous disorders and diseases. The evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases, and maintenance of human health [5]. Human body has an inherent antioxidative mechanism and many of the biological functions. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells [6]. Recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance [7].

Aconitum heterophyllum Wall ex Royle (Ranunculaceae) is a plant of temperate region mainly distributed over temperate parts of Western Himalaya extending from Kashmir to Kumaon. The plant is considered as a necessary component in the traditional medicines. It has many pharmaceutical characteristics like, cardio

tonic effect, analgesic, anesthetic, anti-inflammatory effect and blood pressure elevation [8]. The plant has been evaluated for various pharmacological activities since times it the phytoconstituents varies with the geographical locations and the habitat composition. Since the plant has potential to cure various diseases, it's illegal extraction from natural habitat has resulted over exploitation resulting the plants to be at the verge of extinction. The present study was aimed at looking for the antioxidant potential of crude extract from the aerial part (Leaves) of *Aconitum heterophyllum* and explore the importance to the audience for sustainable utilization and conservation in wild.

Materials and Methods

The leaves of the plant (Plate. 1) were collected from higher reaches of Baderwah, Jammu and Kashmir (Figure. 1), and were dried in shade. The dried plant material was subjected to extraction in 50% methanolic solvent for five days in separating funnels. The extract was oven dried at 50 °C and the crude drug was subjected for further preliminary screening for phytoconstituents [9] and antioxidant potential [10,11]. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was purchased from Sigma-Aldrich (USA). Ascorbic acid and butylated hydroxytoluene (BHT) were purchased from E. Merck (India). Stock solutions of DPPH were prepared in methanol. The solvents and other chemicals were of analytical grade. The reaction tubes, in triplicates, were wrapped in aluminum foil and kept at 30 °C for 30 min in dark. All measurements were done under dim light. Spectrophotometric measurements were done at 517 nm using spectrophotometer. Ascorbic acid in different concentrations was used as a standard. The OD was calculated and the % age inhibition was also calculated with the standard formula [10].

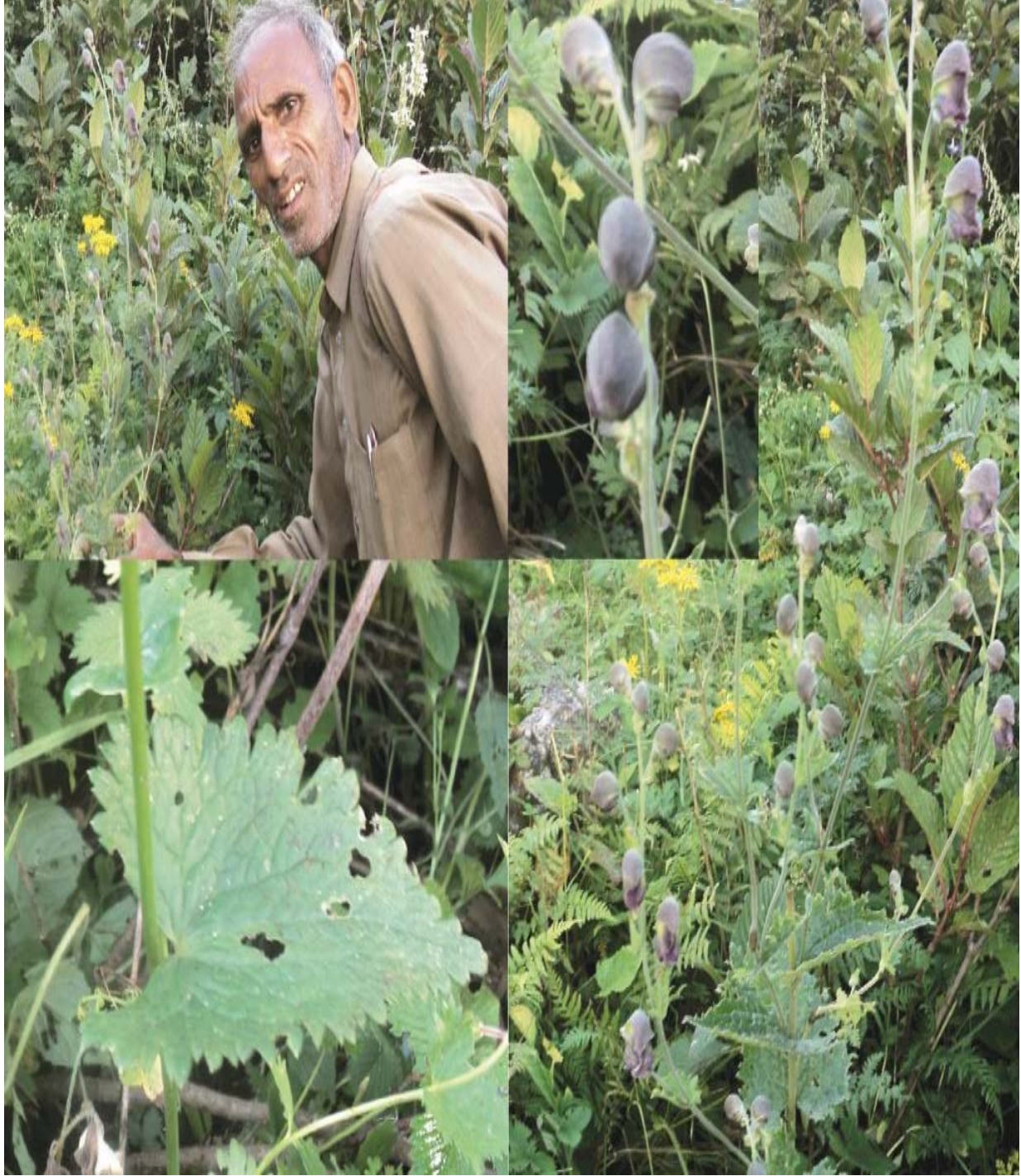


Plate. 1 *Aconitum heterophyllum* Wall. In natural habitat





Figure. 1 Map of Kashmir Himalayas showing site of plant collection

Results and discussion

The present study is focused on the detection of bioactive compounds from leaves of an endangered medicinal herb *Aconitum heterophyllum*. Preliminary screening of the crude drug is presented in Table. 1. Methanolic extract showed presence of

maximum concentration of phytoconstituents as compared to the water and methanolic extract. Further based on the presence of maximum constituents, the methanolic extract was studied for antioxidant potential along with the standard antioxidants, ascorbic acid and butylated hydroxyl toluene.



Table. 1 Phytochemical screening of an aerial part of *Aconitum heterophyllum*

Phytoconstituents	Extract		
	WE	ME	EE
Alkaloides	–	**	*
Steroids	–	**	*
Glycosides	–	**	*
Flavonoides	*	**	*
Saponin	*	**	*
Phenolic compound	*	**	*
Proteins	*	**	*

–: indicates absence, *: indicates low concentration, **indicates high concentration

The antioxidant potential in terms of inhibitory concentrations IC_{50} of the plant and the standard antioxidants and the calibration curve of DPPH are presented in Figure. 2. The IC_{50} concentration ascorbic acid and BHT varied between 10-15 μ M and 50-70 μ M. A wide range of concentrations of plant extract (10-800 μ M) recorded

the inhibition between 34-80.5% with IC_{50} concentration between 20-50 μ M. IC_{50} indicates that all the concentrations tested during the present work, antioxidant activity of *Aconitum heterophyllum* is lesser that ascorbic acid and comparable to BHT. Earlier study also reported antioxidant activity of *Aconitum heterophyllum* to be lesser that ascorbic acid [12].

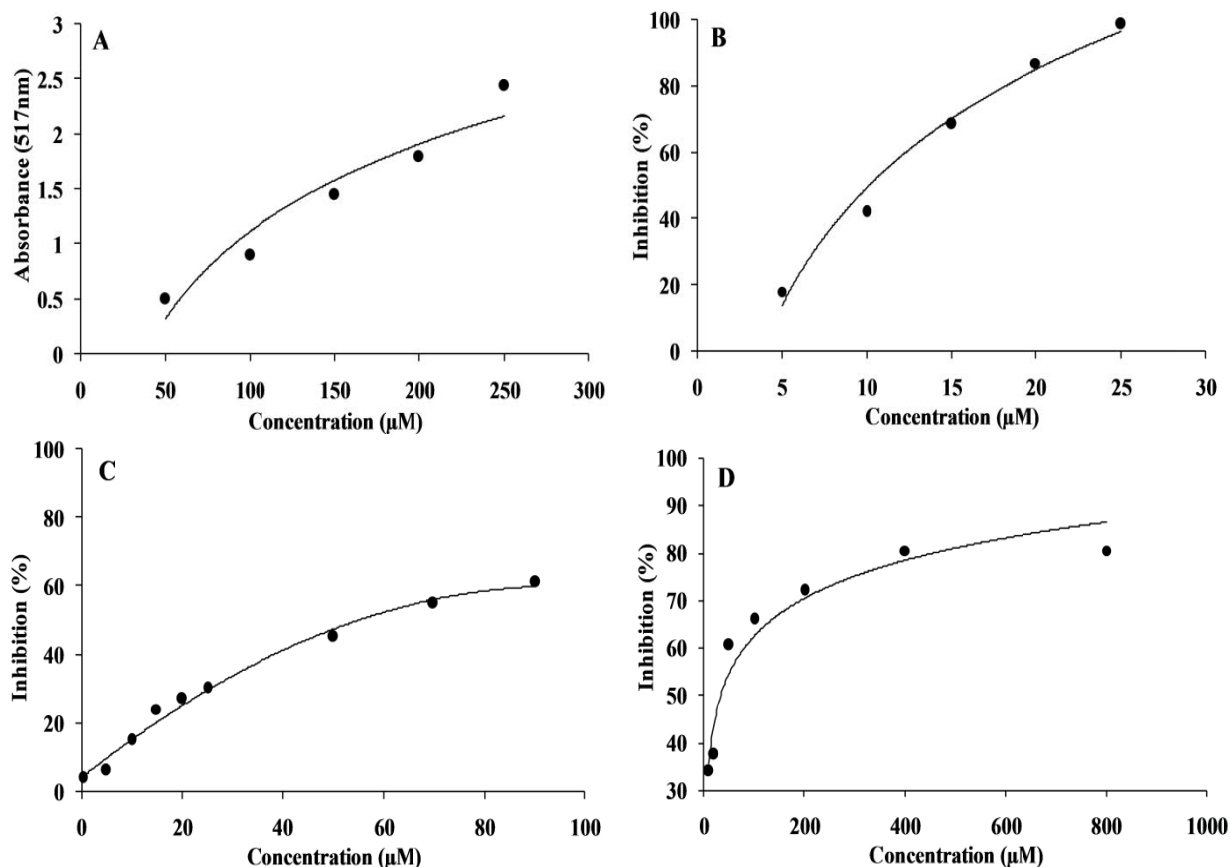


Figure. 2, A: Absorbance of methanolic DPPH solution; Scavenging of DPPH radical by B: Ascorbic acid, C: by BHT and D: by crude methanolic extract from leaves of *Aconitum heterophyllum*



Preliminary phytochemical analysis performed in the study gives an idea about the chemical nature of the active constituents present in that extract. The phytoconstituents quantified in the present study have a great value in human health care system. Phenols have been reported as an active, quenching of oxygen-derived free radicals by donating hydrogen atom or an electron to the free radicals [13]. The property of flavonoids in increasing capillary permeability has been widely used for the treatment of various cardiovascular diseases and is regarded as a agents having potential antioxidant and anti-inflammatory activity [14]. Alkaloids have a wide range of medicinal importance such as in treatment of cancer, malaria, pain, inflammation, Parkinsonism, hypertension and number of central nervous system disorders [15]. In human body free radicals such as nitric oxide, hydroxyl radical and hydrogen peroxide get bind to DNA nucleotides thus, causing damage to various biological systems which may result in carcinogenesis, mutagenesis, and cytotoxicity. Therefore, plants rich in phenolic, tannins and flavonoids are considered as a potential antioxidant agent as they neutralizes the free radicals via

donation of hydrogen atom, quenching of oxygen and by chelation of metals thus minimizing oxidative stress [16-18].

The present study reveals that the consumption of these medicinal plants could be beneficial to mankind by virtue of their effective antioxidant potential. Plant extracts are almost safe at certain level; hence, they could be exploited sustainably as additional source of antioxidant and nutritional supplements. Further the findings also confirm the use of these herbs as traditional medicine and may be used as effective and potential source of novel antioxidant and other drugs.

Acknowledgment

Authors are thankful to the principal, Govt. NMV Hoshangabad and HOD department for providing necessary laboratory facilities. We are also thankful to Kh. Gulsher Sheikh for providing assistance during collection of plant material in the Himalayan range.

References

- [1]. Maqsood S, Singh P, Samoon MH, Balange AK. Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio* challenged with *Aeromonas hydrophila*. *Inter Aqua Res.* 2010; 2:77-85.
- [2]. Zengin G, Cakmak YS, Guler GO, Aktumsek A. Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei*. *Rec Nat Prod.* 2011; 5:123-132.
- [3]. Vongtau HO, Abbah J, Chindo BA, Mosugu O, Salawu AO, Kwanashie HO, Gamaniel KS: Central inhibitory effects of the methanol extract of *Neorautanenia mitis* root in rats and mice. *J Pharm Biol.* 2005; 43:113-120.
- [4]. Oluyemi KA, Okwuonu UC, Baxter DG, Oyesola TO. Toxic effects of methanolic extract of *Aspilia africana* leaf on the estrous cycle and uterine tissues of Wistar rats. *Int J Morphol.* 2007; 25:609-614.
- [5]. Halliwell B, Gutteridge JMC. Formation of thiobarbituric acid reactive substances from deoxyribose in the presence of iron salts: the role of superoxide and hydroxyl radicals. *FEBS Lett.* 1981; 128:347-352.
- [6]. Nunes XP, Silva FS, Almeida JR GDS, Barbosa Filho JM, de Lima JT, de Araujo Ribeiro LA, and Junior LJQ. Biological oxidations and antioxidant activity of natural products. INTECH Open Access Publisher. 2002.
- [7]. Wannas WA, Mhamdi B, Sriti J, Jemia MB, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B: Antioxidant activities of the essential oil and methanol extracts from myrtle (*Myrtus communis* var. *italica* L.) leaf, stem and flower. *Food Chem Toxicol.* 2010; 48:1362-1370.
- [8]. Zhao K, Penttinen P, Guan T, Xiao J, Chen Q, Xu J and Strobel GA. The diversity and anti-microbial activity of endophytic actinomycetes isolated from medicinal plants in *Panxi plateau*, China. *Current microbiology.* 62(1):182-190.
- [9]. Khandelwal KR. Pune: Nirali Prakashan; Practical Pharmacognosy. 2007; 149-53.
- [10]. Sharma Om P and Tej K Bhat. "DPPH antioxidant assay revisited." *Food chemistry.* 113.4 (2009): 1202-1205.
- [11]. Braca A, Fico G, Morelli I, De Simone F, Tome F and De Tommasi N. (2003). Antioxidant and free radical scavenging activity of flavonol glycosides from different *Aconitum* species. *Journal of Ethnopharmacology.* 2003; 86 (1): 63-67.
- [12]. Prasad SK, Kumar R, Patel DK, Sahu AN, and Hemalatha S. Physicochemical standardization and evaluation of in-vitro antioxidant activity of *Aconitum heterophyllum* Wall. *Asian Pacific Journal of Tropical Biomedicine.* 2012. 2 (2):526-531.
- [13]. Wanasundara UN, Shahidi F. Antioxidant and pro-oxidant activity of green tea extracts in marine oils. *Food Chem.* 1996; 63:335-342.
- [14]. Prasad SK, Singh PN, Wahi AK, Hemalatha S. Pharmacognostical standardization of *Withania coagulans* Dunal. *Pharmacog J.* 2010; 2: 386-394.



- [15]. Rathbone DA, Bruce NC. Microbial transformation of alkaloids. *Curr Opin Microbiol.* 2002; 5: 274-281.
- [16]. Patel DK, Kumar R, Prasad SK, Sairam K, Hemalatha S. Antidiabetic and in vitro antioxidant potential of *Hybanthus enneaspermus* linn f. muell in streptozotocin-induced-diabetic rats. *Asian Pac J Trop Biomed.* 2011; 1:316-322
- [17]. Patel DK, Kumar R, Laloo D, Sairam K, Hemalatha S. Evaluation of phytochemical and antioxidant activities of the different fractions of *Hybanthus enneaspermus* (Linn.) F. Muell. (Violaceae). *Asian Pac J Trop Med.* 2011; 4: 391-396.
- [18]. Govindarajan M, Jebanesan A, Reetha D, Amsath R, Pushpanathan T, Samidurai K. Antibacterial activity of *Acalypha indica* L. *Eur. Rev. Med. Pharmacol. Sci.* 2008; 12: 299-302.

