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In Vitro Antioxidant Activity and Phytochemical Screening of *Pholidota articulata*

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ABSTRACT

The aim of this research was to evaluate antioxidant activity and phytochemical screening of *P. articulata.* The extraction process involved fractionation with various solvents and concentrated using rotary evaporator. The results of antioxidant activity study of *P. articulata* showed maximum activity in the methanolic extracts at different concentrations of 100, 200, 300 and 400 µg/ml. The percent inhibition of writhing response by the extract was 36.18%, 44.72%, 59.21%. 67.08% and 83.39% respectively.

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INTRODUCTION

The genus *Pholidota* (Orchidaceae) belongs to the tribe coelogyneae and comprises 55 species with a distribution from tropical Asia to tropical Australia and China. Among them, 9 species are in India. Commonly distributed from submontane to montane Himalaya. The genus *pholidota* are epiphytic herbs generally grown on rocks and trees [1]. Most plants of the genus *Pholidota* found in India grow as epiphytes. Some are also found growing on moist, moss covered rock structures on large, hilly slopes. On the earth, out of 4,22,127 plant species, about 35,000 to 70,000 species are used as medicinal plants [2]. In the third world countries, 20,000 plant species are believed to be used medicinally [3]. At present, the pharmaceutical sector in India is making use of 280 medicinal plant species, of which 175 are found in the IHR [4]. The plants of the genus *pholidota* are used traditionally for medicinal purposes. The whole plant has long been used as a remedy for acute or chronic bronchitis, toothache, treatment of dysentery, infections, asthma, bronchitis, eczema and duodenal ulcer [5].

MATERIALS AND METHODS

Plant material

The materials included fresh and dry whole plants of *P. articulata* were collected from Guptakashi (Rudraprayag), Uttarakhand district, during September-October 2015. These plants were authenticated by the Taxonomy Laboratory, Department of Botany, HNB Garhwal University, Srinagar.

Preparation of plant extract

The whole plants were first shade dried for a week. Then the crushed plant material was ground into coarse powder with the help of a mechanical grinder and soxhlet extracted with petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water using the soxhlet apparatus [6]. Each extract was evaporated to dryness under reduced pressure using a rotary evaporator. The extracts thus obtained were stored in airtight container at 4^{o}C until further analysis.

Chemicals

All the chemicals and reagents used were of analytical grades such as DPPH (2, 2-Diphenyl-1-picrylhydrazyl), sodium hydroxide, methanol, ethyl alcohol, hydrochloric acid and sulphuric acid (Merk India Ltd).

Phytochemical analysis

The qualitative phytochemical analysis of all samples was carried out using standard methods. The extracts obtained as above are then subjected to qualitative tests for the identification of various plant chemical constituents. In addition, 50 gm of air dried or fresh plant material is also subjected to hydrodistillation to detect the presence of volatile oil. The plant material may be subjected to preliminary phytochemical screening for the detection of various plant constituents on the following lines [7].

DPPH radical scavenging assay

The ability of the plant extract to scavenge DPPH free radicals was assessed by the standard method and adopted with suitable modifications. The stock solutions of extracts were prepared in methanol to achieve the concentration of 1 mg/ml. The dilutions were made to obtain concentrations of 100, 200, 300 and 400 µg/ml. The diluted solutions (1 ml each) were mixed with 2 ml of methanolic solution of DPPH in concentration of 1 mg/ml. After 30 min incubation in darkness at room temperature (23^{o}C), the absorbance was recorded at 517 nm. The control sample contained all the reagents except the extract and the percentage inhibition was calculated using equation 1, whilst IC50 values were estimated from the % inhibition versus concentration plot, using a non-linear regression algorithm [8].

$$\text{Inhibition (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Statistical analysis

The data are expressed as the mean ± SEM analyzed by one-way analysis of variance (ANOVA) and Tukey's t-test was used as the test of significance. P value<0.05 was considered as the

minimum level of significance. All statistical tests were carried out using SPSS statistical software [9].

RESULTS

Table 1: Absorbance and inhibition percentage at various concentrations of *in–vitro* antioxidant activity of standard BHT.

Sr. No	Concentration	BHT (µg/ml)	Pet. ether Extract	Ethyl Acetate Extract	Methanolic Extract
		Inhibition %	Inhibition %	Inhibition %	Inhibition %
1	100	42.94	07.07	39.93	42.18
2	200	44.52	11.21	63.14	51.72
3	300	63.62	19.68	67.78	79.21
4	400	81.97	21.50	81.03	82.08

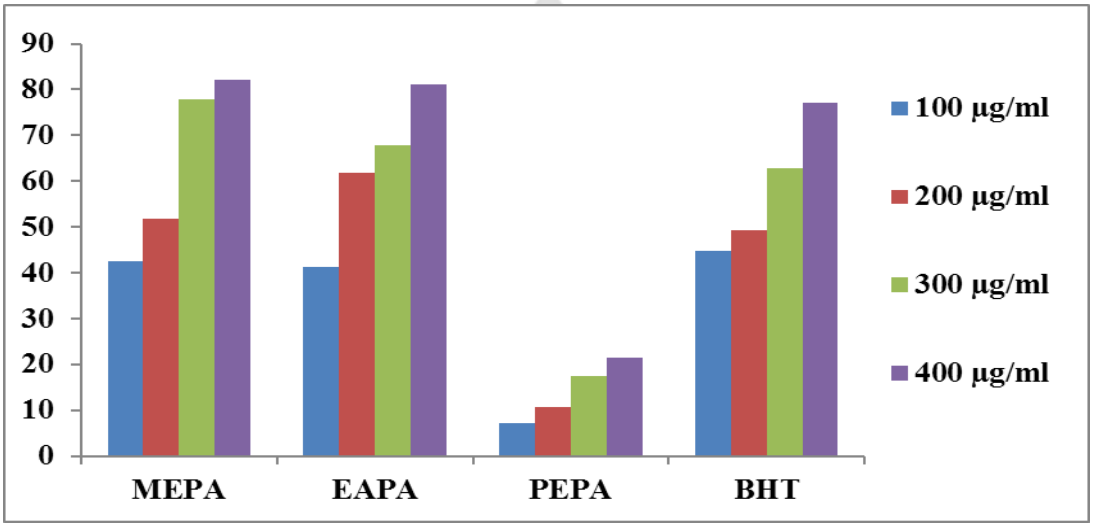

Figure 1. DPPH scavenging activities of the different solvent extracts of *P. articulata.*

Table 2: Inhibitory Concentration (IC_{50}) Of *P. articulata* extracts fractions

Concentration (400µg/ml)	BHT Standard	Pet. Ether *P. articulata*	Ethyl Acetate *P. articulata*	Methanolic *P. articulata*
IC_{50}	172.90	188.78	146.88	196.03

DISCUSSION

Antioxidant activity (free radical scavenging activity)

The free radical scavenging activity of the methanolic extract of different fraction has been tested by DPPH radical method using BHT as a reference standard. The concentration ranged from 100–400 µg/ml. DPPH is very stable free radical. The antioxidant activity of Standard and different plant species in terms of inhibition (%). The highest inhibition percentage of BHT standard is 81.97% in 400 µg/ml concentration. The methanolic and ethyl acetate fractions were found strong DPPH scavenging activity. At a concentration of 400 µg/ml, the scavenging activity of methanolic extract of *P. articulata* and ethyl acetate reached 82.08% and 81.03% respectively.

CONCLUSION

It can be concluded that the different extracts of the whole plant of *P. articulata* possess potent antioxidant activity and rich sources of different traditional uses. The present study was attempted for the first time to investigate the antioxidant and phytochemical activity of *P. articulata* to search for newer, safer and more potent antioxidant agent and we herein delineate the results of our study.

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