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EVALUTION OF ANTI-OXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *PHOLIDOTA ARTICULATA*(ORCHIDACEAE)

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ABSTRACT
Free radical scavenging activity is shown by the methanolic extract of *Pholidota articulata* (Orchidaceae). That the various plant extract of *P. articulata* is effective at reducing the stable radical DPPH to the yellow coloured diphenyl picryl hydrazyl indicating that the extract is active in DPPH radical scavenging. The *P. articulata* methanolic fraction show significant scavenging effect with increasing concentration in the range of 100-400 µg/ml. At a concentration of 400µg/ml. the scavenging activity of *P. articulata* methanolic extract reached 82.09%. The scavenging ability of *Pholidota articulata* methanolic fraction was found to be better than BHT(Ahmad, W et al., 2003; Rong, S,et al.,2005; Singh, Gurjaran et al.,2004; Harrington,et al.,2005).

KEYWORDS: *Pholidota articulata*, Orchidaceae, Antioxidant activity.

INTRODUCTION
The genus *Pholidota* (Orchidaceae) belongs to the tribe coelogyneae, and comprises 55 species with a distrubution from tropical asia to tropical australia and china. Among them 9 species in India. Commonly distributed from submontane to montane Himalaya. The plant *P.articulata* are epiphytic herbs generally grown on rocks and trees and at altitude of 1,500 to 2,800 meter. (Gaur. R.D et al., 1999). The whole plant has long been used mostly in folk medicine for the treatment of various ailments (X.S.Lin et al., 1985; Jiangsu et al., 1986). 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 (Zhong Hua et al., 1999).

MATERIALS AND METHODS
Collection and Identification of Plant Materials
Plant material (whole plant) was collected from Guptakashi and Chopta district Rudraprayag Uttarakhand, India during August 2013. The plant species was identified by Taxonomists, Department of Botany, H.N.B. Garhwal University Srinagar (Garhwal), Uttarakhand.

Preparation of Crude Extract
The shade dried whole plant was crushed and boiled in ethanol at 40–50ºC for 16–18 hrs and then EtOH soluble fraction was filtered off. The filtrate was concentrated under reduced pressure with the help of evaporator (Perfit India). A crude extract (400gm) was obtained from the filtrate.

Determination of Antioxidant Activity
Antioxidant Assay
The radical-scavenging capacity of Crude extract and its various fractions of *P. articulata* was determined using the DPPH radical method. A 2.0ml liquid of test solution was added to 2ml of 2 $\times 10^{-4}$ mol/l ethanolic DPPH solution. The mixture was shaken vigorously and the absorbance was measured at 517 nm immediately. All the tests were performed in triplicate and mean values calculated. The antioxidant activity was expressed according to the ability of an extract to scavenge DPPH free radical and determined using the following equation:

$$\% \text{ Inhibition} = [1-(A_1-A_2)/A_0] \times 100$$

Where A_0 is the absorbance of negative control (Original DPPH sample without sample), A_1 is the absorbance of test sample (DPPH sample in presence of sample) and A_2 is the absorbance of sample without DPPH. The IC_{50} is the concentration (µg/ml.) of extract/standard necessary to reduce the absorbance of DPPH by 50% compared to the negative control. The IC_{50} was determined by interpolation from linear regression analysis of the antioxidant activity (% Inhibition) against sample concentration (µg/ml.) and the IC_{50} values decreases as a function of increasing antioxidant of sample.

Calculation of Inhibitory Concentration (IC_{50}) value of different extract of *P. articulata* by linear regression analysis

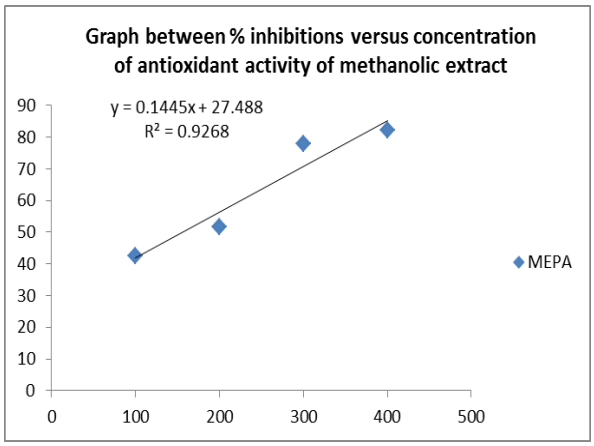

Figure 1: Graph between % inhibitions versus concentration of antioxidant activity of methanolic extract.

RESULTS AND DISCUSSION

From the above data it was observed that the various plant extract of *P. articulata* is effective at reducing the stable radical DPPH to the yellow coloured diphenyl picryl hydrazyl indicating that the extract is active in DPPH radical scavenging. The *P. articulata* methanolic fraction show significant scavenging effect with increasing concentration in the range of 100-400 µg/ml. At a concentration of 400µg/ml. the scavenging activity of *P. articulata* methanolic extract reached 82.09%. The scavenging ability of *Pholidota articulata* methanolic fraction was found to be better than BHT. The IC_{50} values (µg/ml.) of different extract of are given in (Table 1).

Table 1: Inhibitory concentration (IC_{50}) of *P. articulata* methanolic extract fractions.

Concentration (400µg/ml.)	MEPA	BHT
IC_{50}	196.03	172.90

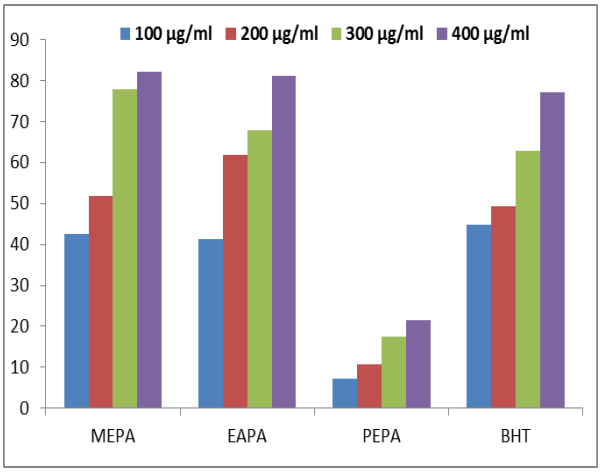

Figure 2: Comparative antioxidant study of *P. articulata* methanolic extract fractions with BHT.

CONCLUSION

Strong antioxidant properties was observed in methanolic extract of *Pholidota articulata*. Thus from above study it was analysed that it is an important plant from the medicinal point of view and can be a potentially used for bio-assays purposes, which would lead the preparation and also synthesis of safe eco-friendly herbal drugs of global interests.

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