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Evaluation of free radical scavenging activity of methanolic extract of *Pholidota articulata*

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Abstract

Free radical scavenging activity is shown by the methanolic extract of *Pholidota articulata* (Orchidaceae). The inhibitory concentration (Ic50 values) of methanolic extract of *Pholidota articulata* is 196.03. At a concentration of 400mg/ml, the scavenging activity of methanolic extract *Pholidota articulata* reached 82.094%. The Free Radical scavenging activity of methanolic extract is done by diphenyl picryl hydrazine (DPPH) method. Though the DPPH radical scavenging abilities of the plant extracts were observed 400 mg/ml, the study showed that the extracts have the proton donating ability and could serve as free radical inhibitors or scavenging, acting possibly as primary antioxidants. The plant is found to be extensively used in the indigenous system of medicine for the treatment of pain and rheumatic fever, hyperactive states of the gastrointestinal tract, liver diseases, syphilis, infections, fever, pain, inflammations, rheumatism, as a laxative, blood purifier and for the relief of muscular pain.

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)^[4]. 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)^[3, 2]. 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)^[5].

Collection and Identification of Plant Materials

Pholidota articulata whole plants was collected from the Guptakashi (Uttarakhand) India during September-October 2013, and was identified by Taxonomists, Department of Botany, HNB Garhwal University, Srinagar Garhwal Uttarakhand.

Preparation of Crude Extract

The shade dried whole plant was crushed into small pieces and made a coarse powder. This coarse powder was boiled in ethanol at 50-60 °C temp. for 18-20 hours and then ethanol soluble fraction was filtered off. The filtrate was concentrated under vacuum at low temperature (40 °C) with the help of a rotary evaporator to obtain crude extract (400 gm.).

Fractionation

The crude extract was fractionated with petroleum ether and ethyl acetate repeatedly by soxhlet apparatus to yield petroleum ether and ethyl acetate soluble and insoluble masses. Ethyl acetate insoluble crude extract was preserved for the pharmacological/biological activities.

Determination of Antioxidant Activity

Antioxidant assay The antioxidant activity was determined by using the following equation: % Inhibition = $[1-(A_1-A_2)/A_0]x100$

Where A is the absorbance of negative control (original 0 DPPH sample without sample), A is the absorbance of 1 test sample (DPPH sample in presence of sample) and A 2 is the absorbance of sample without DPPH (B. Halliwell, *et al* 1985) ^[1]. The IC 50 value is the concentration (mg/ml) of extract/standard necessary to reduce the absorbance of DPPH by 50% compared to

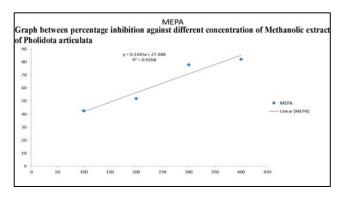
the negative control. The IC was 50 determined by interpolation from linear regression analysis of the antioxidant activity (% Inhibition) against sample concentration (mg/ml) and the IC value decreases as a function of increasing antioxidant activity of samples.

Results and Discussion

The plants have the ability to scavenge free radicals, superoxide and hydroxyl radicals by single-electron transfer. An antioxidant can exert its antioxidant activity through various mechanisms, including chelating ferrous iron, degrading peroxide, and scavenging free radicals. These results showed that the methanolic extract of Pholidota articulata is effective in reducing the stable radical DPPH to the yellow colour diphenyl picryl hydrazine indicating that the extract is active in DPPH radical scavenging. The methanolic fraction of Pholidota articulata had significant scavenging effects with increasing concentrations in the range of 100-400 mg/ml. The antioxidant activity of the methanolic extract as measured by the ability to scavenge DPPH free radicals method. At a concentration of 400 mg/ml, the scavenging activity of methanolic extracts reached 82.094% at the concentration, the study showed that the extract have the proton donating ability and could serve as free radical inhibitors or scavenging, acting possibly as primary antioxidants. The minimum inhibitory concentration (IC₅₀ values) of methanolic extract of Pholidota articulata is 196.03.

Table 1: Percentage inhibition of Methanolic extract of diffirent concentration of *Pholidota articulate* with respect to time.

Concentration	Percentage inhibition			
(µg/mL)	T ₀	T15	T30	T60
100	37.30231	40.88513	42.64888	32.96453
200	39.44029	42.70745	44.0807	51.77026
300	55.67849	79.37195	80.22779	77.94012
400	60.56297	80.32216	82.77904	82.09242



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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