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RESEARCH ARTICLE

EVALUATION OF *IN VITRO* ANTIOXIDANT POTENTIAL OF THE METHANOLIC EXTRACTS OF THE FERN, *PSILOTUM NUDUM* (L.) P. BEAUV

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ABSTRACT

Present study reports the antioxidant activities of methanolic extracts of *Psilotum nudum.* The analyses carried out were DPPH radical scavenging activity, Reducing power assay, Ferrous ion chelating assay, $ABTS^{\bullet+}$ assay . From the analyses *Psilotum nudum* was found to have potent antioxidant activity against DPPH with IC^{50}value of 118.81± 1.13. The value of reducing powerassay was 0.769± 0.001 and the value of Ferrous ion chelating assay was 196.28 ± 1.16. TheIC^{50}value of $ABTS^{\bullet+}$ assay30.25± 0.51.Thus the result obtained in the present study that the plant have the potential as natural sources of antioxidant, capable of protecting against free radical damage and may have application in preventing and curing various diseases.

Key words: *Psilotum nudum*, Kolli hills, antioxidant activity.

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INTRODUCTION

Antioxidant are the compounds that protect cells against the damaging effects of reactive oxygen species, such as singlet oxygen, super oxide, peraxyl radical, hydroxylradical and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damages. Oxidative stress has been linked to cancer, aging, ischemic injury, inflammation and neurodegenerative diseases (Parkinson›s and Alzheimer›s) (Saraf *et al.,* 2007). Antioxidant can delay, inhibit or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress (Durackovo, 2010; Reuter *et al.,* 2010). Natural antioxidants have been studied extensively for decades in order to find compounds protecting against a number of diseases related tooxidative stress and free radical- induced damages. To date, many plants have been claimed to pose beneficial health effects such as antioxidant properties (Newman and Cragg, 2007; Kaur and Arora, 2009). However, the investigation of the fern for antioxidant activity is meager. *Psilotum nudum* was the fern reported to have importance in folklore medical practice in Kolli hills. As there is no scientific validation of this species for their medicinal uses, the present study was undertaken to bring out its applications in terms of antioxidant activities.

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MATERIALS AND METHODS

Plant material

The clean and healthy study plant *Psilotum nudum*was collected from the Kolli hills. These fresh materials were washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

Preparation of extract

About 50g of coarsely powdered materials of *Psilotum nudum*was extracted with 250 ml methanol through soxhlet apparatus separately for 8 to 10 hours. The extracts obtained were then concentrated and finally dried to a constant weight.

In vitro antioxidant activities

DPPH radical scavenging activity

The 2, 2-diphenyl-picryl-1-picryl-hydrazyl radical (DPPH) scavenging activity was measured according to the method of Blois, (Blois, 1958). Methanol extract of the samples at various concentrations (50, 100, 150,200 and 250µg/mL,) was added separately to each 5mL of 0.1mM methanolic solution of DPPH and allowed to stand for 20min. Absorbance at 517nm using spectrophotometer was measured. BHT was used as standard. The corresponding blank reading was also taken and DPPH radical scavenging activity was calculated by using the following formula:

DPPH radical scavenging activity (%) = Control OD-Sample OD/Control OD×100

Reducing power assay

Reducing power assay was determined according to the method of Siddhuraj *et al.,* 2002. 50-250µg of the extract was taken in 1 ml of phosphate buffer and 5 ml of 0.2M phosphate buffer (pH 6.6) was added. To this, 5 ml of 1% potassium ferriccyanide solution was added and the mixture was incubated at50^0C for 20 min. After the incubation, adding 5mL of 10% trichloro acetic acid was added. The content was thencentrifuged at 1000rpm for 10min. The upper layer of thesupernatant (5ml) was mixed with 5ml of distilled water and 0.5ml of 1% ferric chloride. The absorbance of the reaction mixture was read spectroscopically at 700nm.

Ferrous ion chelating assay

The chelating of ferrous ions by whole plant methanolic extracts of the two study species were estimated by the method of Dinis *et al.,*1994. Briefly the extract samples (250 µL) were added to a solution of 2 mmol/L FeCl2 (0.05 mL). The reaction was initiated by the addition of 5 mmol/L ferrozine (0.2 mL) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g extract.

$ABTS^{\bullet+}$ assay

The total antioxidant activity of the samples was measured by $ABTS^{\bullet+}$ [2,2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] radical cation decolorization assay according to the method of Re *et al.,* 2005. $ABTS^{\bullet+}$ was produced by reacting 7mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 30˚C to give an absorbance of 0.700±0.02 at 734 nm. The stock solution of the sample extracts was diluted such that after introduction of 10 µL aliquots into the assay, which have been produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10 µL of sample or trolox standards (final concentration 0–15 µM) in ethanol, absorbance was measured at 30˚C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition wascalculated from the blank absorbance at 734 nm and then it was plotted as a function of trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of trolox having equivalent antioxidant activity expressed as µmol/g sample extract on dry matter.

RESULT AND DISCUSSION

***In vitro* antioxidant activity**

DPPH radical scavenging activity: The free radical scavenging activity of the extract is related with hydrogen atoms or election abilities and the conformation of the antioxidant compound of the extracts.DPPH, a relatively stable organic radical with a characteristic strong absorption band at 517 nm was used to evaluate the free radical scavenging effects of the some natural products (Yen *et al.,* 2005). The Galic acid was used as positive control for comparison. The free radical scavenging activity of three study species, *Psilotum nudum*was increased with the increase of concentrations (Table 1).

Table 1. Free radical scavenging activity (dpph) of methanolic extracts of the study plant pilotum nudum

Sample	Concentration (µg/ml)	Percentage activity (%)	IC_{50} (µg/ml)
Psilotumnudum	50	9.16 ± 1.39	118.81± 1.13
	100	18.29 ± 0.86	
	150	26.41 ± 0.86	
	200	32.86 ± 0.15	
	250	37.19 ± 1.31	
Galic acid	4	30.10 ± 0.52	4.46 ± 0.22
	8	43.37 ± 0.12	
	12	56.74 ± 0.37	
	16	75.50 ± 0.62	
	20	86.37± 0.66	

Values are means of three independent analysis ± Standard Deviation (n=3)

Reducing Power assay: Table 2 shows the reductive capabilities of different concentrations of methanolic extracts *Psilotum nudum*in comparison to that of the standard, galic acid. It was found that the reducing power increased with the increasing of the concentrations of the extracts. In the present study, *Psilotum nudum*extract showed the highest reducing ability (absorbance0.769± 0.001 at 700nm) than the other ferns studied.

Table 2. Reducing power activity of methanolic extracts of the study plant pilotum nudum

Sample	Concentration (µg/ml)	Absorbance at 700nm (%)
Psilotumnudum	50	0.153 ± 0.003
	100	0.436 ± 0.035
	150	0.597 ± 0.008
	200	0.669 ± 0.007
	250	0.769± 0.001
Galic acid	20	0.153 ± 0.003
	40	0.386 ± 0.035
	60	0.567 ± 0.008
	80	0.649 ± 0.007
	100	0.739± 0.001

Values are means of three independent analysis ± Standard Deviation (n=3)

Ferrous ion chelating assay: The chelating effect on the ferrous ions by methanolic extract of*Psilotum nudum*is presented in Table 3. All the samples exhibited the ability to chelate metal ions. Among the five extracts*Psilotum nudum* showed higher activity (14.75±2.23 at 5000 µg/mL)

Table 3. Chelating activity of methanolic extracts ofthe study plant pilotum nudum

Sample	Metal chelating activity (mg EDTA E/ g extract)
Psilotumnudum	196.28 ± 1.16
Galic acid	186.52 ± 1.65

Values are means of three independent analysis ± Standard Deviation (n=3)

$ABTS^{\bullet+}$ assay

In the present investigation, **(**Table-4) the methanolic extract of *Pilotum nudum*registered the highest total antioxidant activity (75.60 ± 1.75) in the concentration of100µmol/g).

Table 4. abts.+activity of methanolic extracts of study plantpilotum nudum

Sample	Concentration (µg/ml)	Percentage activity (%)	IC_{50} (µg/ml)
	20	15.05 ± 0.80	30.25± 0.51
Psilotumnudum	40	35.83± 1.75	
	60	52.90 ± 0.60	
	80	66.08 ± 0.64	
	100	75.60 ± 1.75	
Galic acid	10	26.99 ± 0.34	11.65 ± 0.23
	20	34.25 ± 0.75	
	30	59.24 ± 0.67	
	40	69.28 ± 0.94	
	50	96.28± 0.16	

Values are means of three independent analysis ± Standard Deviation (n=3)

$ABTS^{\cdot+}$, a protonated radical has characteristic absorbance maxima at 734nm which decreases with the scavenging of the proton radicals. $ABTS^{\cdot+}$ was generated by incubating it with potassium per sulfate. The presence of chemical compounds in the tested extracts that inhibit the potassium persulfate activity may reduce the production of $ABTS^{\cdot+}$.

Conclusion

The result of the present study that the fern *Pilotun nudum* exhibit strong antioxidant activities The scavenging activity noted against DPPH , reductive capabilities, chelating effect and $ABTS^{\cdot+}$.radicals lead us to propose this species as promising natural sources of antioxidant suitable for the application in nutritional/pharmaceutical fields, in the prevention of free radical-mediated diseases. It also confirms the traditional knowledge of this species on medicinal importance.

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