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Evaluation of Antioxidant Activity of Some Pteridophytes

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The present study was undertaken to find the antioxidant value of certain Pteridophytes in Garhwalregion. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in cardiovascular and anti-inflammatory diseases to treat of burn and wounds. The methanolic crude extracts of some commonly used Pteridophytes were screened for their free radical scavengingproperties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The overall antioxidant activity of *Diplazium esculentum* was the strongest, followed in descending order by *Adiantum lunulatum*, *Pteris vittata*, *Equisetum ramosissimum* Desf. and *Ampelopteris prolifera* (Retz.). All the *methanolic* extracts exhibited antioxidant activity significantly. The IC50 of the methanolic extracts ranged between 0.32 ± 0.12 and 0.81 ± 0.21 mg/ml. The study reveals that the consumption of these spices would exert several beneficial effects by virtue of their antioxidant activity.

Keyword: *Diplazium esculentum, Adiantum lunulatum, Pteris vittata, Equisetum ramosissimum, Ampelopteris prolifera (Retz.),* Pteridophytes, Antioxidant, DPPH, Free Radical Scavenging Activity.

1. Introduction

The many number of medicinal plants are used in the cellular and metabolic disease treatment such as diabetes, obesity and cancer etc. There are some speculations that the generation of free radicals inside the body in some physiological conditions is resulted in the cellular changes and development of cancer etc. and this could be neutralized by the antioxidants from different medicinal plants. Several studies have shown that plant derived antioxidant neutraceuticals scavenge free radicals and modulate oxidative stress-related degenerative effects[1]. Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging. Previous research reports suggest that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer[2].

The pteridophytes considered to be the primitive vascular plant group which are scattered all over the world. More than 1200 species of fern and fern allies have been reported from India, though new genuine findings are made from time to time[3,4]. The free radical neutralizing property of several plants was reported by previous studies. The extracts from number of medicinal plants which are known to have some biologically active principles are used

in ayurvedic preparations and these extracts are prepared in bulk for commercial purpose. In this present study we have measured antioxidant activity of various extracts like *Diplazium esculentum*, *Adiantum lunulatum*, *Pteris vittata*, *Equisetum ramosissimum* and *Ampelopteris prolifera (Retz.)* employing various *in vitro* assay methods, such as scavenging activity of DPPH, superoxide radical, inhibitionof microsomal lipid peroxidation and reducing power.

Table 1.IC50 Values of the Respective Extracts in Different *in vitro* models. Each Values Represents SD± mean

Extract	IC $_{50}$ in mg/mL	
	DPPH radical scavenging activity	Super oxide radical scavenging assay
Diplazium esculentum	0.32±0.12	0.4±0.14
Adiantum lunulatum	0.4±0.23	0.52±0.18
Pteris vittata	0.65±0.11	0.7±0.22
Equisetum romosissimum.	0.73±0.09	0.76±0.02
Ampelopteris prolifera (Retz.)	0.81±0.21	0.79±0.32

2. Materials And Methods

2.1 Plant Material

The leaves of *Diplazium esculentum*, *Adiantum lunulatum*, *Pteris vittata*, *Ampelopterisproliferaand whole plant of Equisetum romosissimum* were air dried, powdered, pulverized and passed through 16 no. Sieve the powder was extracted sequentially with methanol using soxhlet extractor and concentrated under reduced pressure using flash evaporator and stored in caped vials.

2.2 Antioxidant Assay

The antioxidant activity of plant extracts was determined by different in-vitro methods such as, the DPPH free radical scavenging assay and reducing power methods. The different extracts were dissolved in methanol at the concentration of 2mg/ml. all the assays were carried out in triplicate and average value was considered.

2.3 DPPH Radical Scavenging Activity

DPPH radical scavenging activity was done using the reported method [5]. The reaction mixture containing 1 mL of DPPH solution (0.1 mmol /L, in 95% ethanol v/v) with different concentrations of the extract was shaken and incubated for 30 min at room temperature and the absorbance was read at 517 nm UV-Visible Spectrophotometer against a blank. Blank was prepared with the addition of DPPH and for control 0.2 ml of methanol (without plant extract) was added. The radical scavenging activity was measured as a decrease in the absorbance of DPPH. Percentage of DPPH scavenging activity determined as follows

$$\% \text{ DPPH radical-scavenging} = \frac{(\text{Absorbance of control} - \text{Absorbance of test Sample})}{\text{Absorbance of control}} \times 100$$

Decreased absorbance of the reaction mixture indicates stronger DPPH radical-scavenging activity.

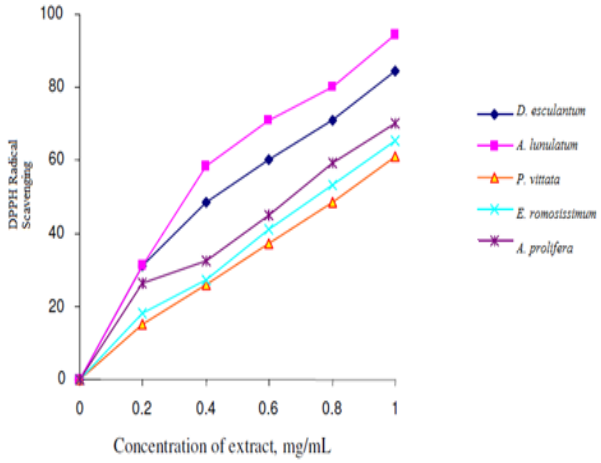

Fig 1: DPPH Radical Scavenging Assay

2.4 Superoxide Radical Scavenging Assay

The superoxide radical scavenging activity of the extracts was measured according to the literature method[6]. The reaction mixture containing Phenazine Methosulphate (PMS) (0.1 mmol/L), Nicotinamide adenine dinucleotide-reduced (NADH) (1 mmol/L), Nitrobluetetrazolium (NBT) (1 mmol/L) in phosphate buffer (0.1 mol/L, pH 7.4) with different concentrations of the extract was incubated at room temperature for 5 min and the color was read at 560 nm against a blank. The scavenging effect was calculated using the following equation:

Effect of scavenging (%) = [1-A sample (560nm) /A control (560nm)] ×100

3. Results and Discussion

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing thedeleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present paper, we have evaluated the free radical scavenger activity of methanolic extract of *Diplazium esculentum, Adiantum lunulatum, Pteris vittata, Ampelopteris prolifera (Retz.) and Equisetum romosissimum.*

Extracts were subjected for the evaluation of antioxidant activity by using various *in vitro* model systems. DPPH radical scavenging activity was observed in all the extracts, the *Diplazium esculentum* extract showed dominant activity followed by *Adiantum lunulatum* extract. The results indicate that the antioxidant activity of the crude extract of *Diplazium esculentum* is higher than that of ascorbic acid. The IC50 values were calculated and are depicted in (Table 1).

Proton radical scavenging action is an important attribute of antioxidants, which is measured by DPPH radical scavenging assay. Hydrogen donating ability of the antioxidants molecules contributes to its free radical scavenging nature. Superoxide radical scavenging activity was shown by all extracts and was concentration dependent with an IC50 value of 0.4±0.14 and 0.79±0.32 mg /mL respectively. *Diplazium esculentum* extract was markedly a more potent scavenger of superoxide anion than followed by the others.

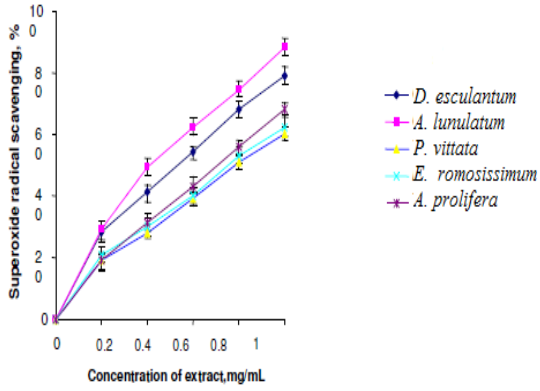

Fig 2: Superoxide Radical Scavenging Activity

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