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Free radical scavenging activity of *Aspidium cicutarium* rhizome

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Abstract

Objective: To evaluate the free radical scavenging activity of methanolic extract of *Aspidium cicutarium* rhizome. Materials and methods: Preliminary phytochemical analysis, estimation of total phenolic content and total tannin content was carried out as a part of phytochemical evaluation of *Aspidium cicutarium* rhizome. Free radical scavenging activity was studied using two *in vitro* models *viz.,* DPPH assay and superoxide scavenging assay. Results: Preliminary phytochemical screening showed the presence of phenols, tannins, flavonoids and sterols. Total phenolics and total tannin content was found to be 17.35% (*w/w*) and 7.98 % (*w/w*) respectively. Methanolic extract of *A. cicutarium* rhizome showed concentration dependent free radical scavenging activity comparable to that of the positive controls pyrogallol and ascorbic acid in the respective models. EC_{50} was found to be 30.63 µg/ml and 85.19 µg/ml in DPPH assay and superoxide scavenging assay respectively. Conclusion: The results indicate that methanolic extract of *Aspidium cicutarium* rhizome exhibited good free radical scavenging activity.

Key words: *Aspidium cicutarium*, free radical scavenging activity, DPPH assay, superoxide scavenging assay.

1. Introduction

Rhizome of *Aspidium cicutarium* Sw. (Family-Polypodiaceae) is a traditionally reputed herbal drug belonging to *Pteridophyte*, found to be popular amongst the Vaidyas of Maharashtra. This drug is sold in the market under the common name of *Kukkutnakhi* or *Kombadnakhi* (*Kukkut* or *Kombad* = cock and nakhi = nail) as rhizomes are covered with curved frond bases which resemble the curved foot paw attached with nails of the cock. The fern is found throughout India from the plains up to 5000 feet height and in the tropics throughout the world [1, 2]. In folklore medicine, it is used in clinical conditions like tonsillitis, arthritis, mental disorders, rheumatism and obesity by Vaidyas of Maharashtra.

Free radicals of different forms are constantly generated for specific metabolic requirement and quenched by an efficient antioxidant network in

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the body. When the generation of these species exceeds the levels of antioxidant mechanism, it leads to oxidative damage of tissues and biomolecules, eventually leading to disease conditions, especially degenerative diseases [3].

Free radicals are believed to be involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorders, atherosclerosis, ageing and neoplastic diseases [4]. Antioxidant agents of natural origin have attracted special interest because they can protect human body from damage due to free radicals [5]. Literature survey showed that no work has been reported on this plant.

The present study was undertaken to conduct preliminary phytochemical evaluation and free radical scavenging activity of *A. cicutarium* rhizome, to check if it is one of the underlying mechanisms for its activity as claimed traditionally, especially in arthritis and rheumatism.

2. Materials and Methods

2.1. Plant material

Rhizome of *A. cicutarium* was collected from Pune, Maharashtra. The sample was authenticated at the Botanical Survey of India (B.S.I.), Pune and a voucher specimen has been preserved in our department. Rhizome was dried initially under shade and subsequently in hot air oven at 50°C. It was preserved in air-tight container and powdered to 40# as per requirement.

2.2 Chemicals

1, 1-Diphenyl-2-picryl hydrazyl (DPPH) was purchased from Sigma Ltd. Ethylene diamine tetra acetate (EDTA) and Folin ciocalteu reagent were purchased from SD Fine Chemicals, India. Riboflavin, nitroblue tetrazolium (NBT) and pyrogallol were purchased from HiMedia Ltd. All the other chemicals used for the experiment were of analytical grade.

2.3 Equipment

UV/VIS Spectrophotometer (Elico-India; SL – 164).

2.4 Preparation of extract

5 g of *A. cicutarium* rhizome powder was extracted with methanol (4 x 50 ml) under reflux. The extract was filtered, pooled and evaporated to dryness under reduced pressure.

The methanolic extract was subjected to preliminary phytochemical testing for the presence of different classes of compounds [6, 7]. The total phenolic content was estimated according to the method described by Singleton & Rossi [8] and total tannin content of the drug was estimated as per AOAC method [9].

2.5 Free radical scavenging activity

2.5.1 Diphenyl-picryl-hydrazyl (DPPH) assay

Free radical scavenging activity of methanolic extract of *A. cicutarium* rhizome was tested by its ability to bleach the stable 1, 1-diphenyl 2-picryl-hydrazyl (DPPH) radical [7-10]. A stock solution of DPPH (1.5 mg/ml of methanol) was prepared such that 75 µl of it in 3 ml methanol gave initial absorbance of 0.9. This stock solution

Table 1
Preliminary phytochemical screening methanolic extract of *Aspidium cicutarium* rhizome.

Tested for	Observation
Phenols	+++
Tannins	+++
Steroids/terpenoids	++
Alkaloids	-
Anthraquinones	-
Flavonoids	++

(-) = Absent, (+) = Traces, (+++) = Abundant

was used to measure the antiradical activity. Decrease in the absorbance in presence of methanolic extract of *A. cicutarium* rhizome at different concentrations was noted after 15 min. EC_{50} was calculated from percentage inhibition. Pyrogallol was used as a positive control.

2.5.2 Superoxide radical scavenging assay

The assay was based on the capacity of the methanolic extract of *A. cicutarium* rhizome to inhibit formazon formation by scavenging the superoxide radicals generated in riboflavin-light system [11]. The reaction mixture contains 50 mM psosphate buffer pH 7.6, 20 µg riboflavin, 12 mM EDTA, NBT 0.1 mg/3ml, added in that sequence. Reaction was started by illuminating the reaction mixture with different concentrations of sample extract for 90 sec. Immediately after illumination, the absorbance was measured at 590 nm. Ascorbic acid was used as a positive control.

3. Results and Discussion

Free radicals such as superoxide radical, hydroxyl radical, peroxyl radical and singlet oxygen play an important role in the genesis of various diseases such as inflammation, atherosclerosis, arthritis, rheumatism, cirrhosis of liver, cataract, and ischemia [12]. Herbal drugs containing antiradical constituents are gaining importance in prevention and treatment of such diseases and free radical scavengers like phenolics are well known for their therapeutic activity [13].

In the present study, preliminary phytochemical screening of methanolic extract of *A. cicutarium* rhizome showed the presence of a large amount of tannins and phenolics (Table 1). Subsequent analysis showed 7.98 % (*w/w*) tannins and 17.35% (*w/w*) phenolics in the rhizome. Presence of large amount of phenolic compounds prompted us to test the free radical scavenging activity of *A. cicutarium* rhizome.

Antiradical activity of methanolic extract of *A. cicutarium* rhizome was tested by its ability to bleach the stable DPPH radical. This method is based on the reduction of alcoholic DPPH solution at 517 nm in the presence of hydrogen donating antioxidant (AH) due to the formation of non-radical form DPPH-H by the reaction:

$$DPPH + AH \longrightarrow DPPH - H + A$$

The remaining DPPH measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant [14]. The sensitivity of the method is determined by the strong absorption of DPPH. This method is rapid, sample analysis takes only 15 min and little manpower, no expensive reagents or sophisticated instruments are required [14].

This assay is being used widely as a preliminary test which provides information on the reactivity of test compound with a stable free radical since odd electron of DPPH gives strong absorption band at 517 nm (violet colour) and when it is quenched by the extract, there is a decrease in absorbance. Methanolic extract of *A. cicutarium*

Table 2
Antiradical activity of methanolic extract of *Aspidium cicutarium* rhizome.

Sample	Concentration (µg/ml)	% inhibition*	EC_{50} (µg)
Methanolic extract	10	17.41± 2.16	
	20	33.74 ± 0.34	
	40	58.68 ± 1.55	30.63
	80	83.25 ± 1.21	
	100	85.63 ± 0.43	
Pyrogallol			4.85

* Mean ± % CV (n=3)

Table 3
Superoxide anion scavenging activity methanolic extract of *Aspidium cicutarium* rhizome.

Sample	Concentration (µg/ml)	% inhibition*	EC_{50} (µg)
Methanolic extract	20	13.41 ± 1.21	
	40	30.80 ± 0.00	
	60	46.42 ± 0.155	85.19
	100	53.52 ± 1.89	
	200	74.34 ± 1.68	
Ascorbic acid			45.39

* Mean ± % CV (n=3)

rhizome showed excellent antiradical activity by quenching DPPH radical, with an EC_{50} value of 30.63 µg/ml (Table 2). Superoxide radical is known to be very harmful to cellular components as a precursor of more reactive oxygen species [15]. The extract was found to be an efficient scavenger of superoxide radical generated in riboflavin-NBT-light system *in vitro*, with an EC_{50} of 85.19 µg/ml (Table 3), which is compared with the activity of ascorbic acid (EC_{50}: 45.39 µg/ml).

In conclusion, from the above investigation, methanolic extract of *A. cicutarium* rhizome was found to possess free radical scavenging activity. The activity was due to several components present in the drug including tannins and other phenolic compounds.

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