International Journal of CURRENT PHARMACEUTICAL RESEARCH
ISSN- 0975-1491 Vol 2, Issue 4, 2010

Research Article

PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT ACTIVITY STUDY OF *DRYNARIA QUERCIFOLIA* LINN RHIZOME

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Received: 19 Jun 2010, Revised and Accepted: 17 July 2010

ABSTRACT

In the present research investigation we extracted the powdered rhizome of Drynaria quercifolia linn by soxhlation method using different solvents .Then extracts were subjected to preliminary phyto-chemical investigation followed by evaluation of anti-oxidant activity by DPPH assay method. It was observed that, the methanolic extract was found to be effective anti-oxidant on comparision to the other extracts and has significant activity compared to the standard drug.

Keywords: Drynaria quercifolia, Anti-oxidant, Methanol,

INTRODUCTION

Traditional medicines played an important role in the health care needs of India and other countries for thousands of years. Recently traditional Indian and herbal drugs have gained popularity in international medical, biomedical and pharmaceutical institutions as a potential source of valuable medicinal agents. The effectiveness of Indian tradition and herbal drug products in the treatment of a variety of ailments and diseases has been established empirically over thousands of years of historic use. However scientific data on their efficacy, pharmacological properties and action mechanism as well as on their chemical constituents have so far been lacking. Here in this work we recognize the importance of using established modern scientific methods and criteria to characterize these medicines, so that their full medicinal potential can be harnessed and new insights into diseases process can be uncovered.

Antioxidants are substances, which act as body's first line of defense against unwanted damage by reactive oxygen species. It has been suggested that the ingestion of dietary antioxidants suppress the free radical production or scavenge free radicals and may prevent harmful effect of these radicals. Some phytochemicals i.e., flavanoids, pigments, and antioxidative vitamins are known to be potent antioxidants. Even though there are, so many uses of the rhizome of *Drynaria quercifolia* Linn[1] (Polypodiaceae) has mentioned in the authorative books, but no experimental evidence are reported. Hence in this study an attempt was made to prove the Anti-oxidant activity of rhizome of *Drynaria quercifolia* Linn.

Fig. 1: Plant, Rhizomes and leaves of *Drynaria quercifolia*

Drynaria quercifolia Fam: Polypodiaceae[2] (Asvakatri) is found throughout India, especially in the plains or very low down in the mountains, On trees or rocks. South China, Malaysia and Tropical Australia. An epiphytic fern with short thick fleshy creeping rhizome. 2cm or more thick, the young parts densely scaly, scales very dark brown, to about 2cm long narrowed gradually from the peltate base to the very narrow apes not stiff edges pale and closely and finely toothed. Nest leaves 40 cms long and 30 cm wide, lobed at depth of 2-5cm, lobes are broad and rounded. Stripes of foliage leaf about 30cm long; lamina to about 100cm long and 40cm wide ,lobed to less than 1cm from the mid rib; oblique 25cm long and 4.5cms wide, rather shortly acuminate, separated by rather narrow sinuses, thin but stiffly leathery in texture.

Rhizome is used in diseases like janu roga, sopha, dusta vranam swasa, kasa, sandhi sopha, jwara, siratida, suryavarta.[3]Fronds are used for poultice swellings and the water extract possessed antibacterial properties.[4]The plant is known to have therapeutic uses in tuberculosis, fever, dyspepsia and cough. The fronds have astringent properties. The fronds are pounded and used as a poultice for swelling because of its antibacterial and astringent properties.[5]Rhizome and roots is used as tonic in typhoid fever and dyspepsia[6]Traditional use of this drug in diarrhea, typhoid, cholera, jaundice, fever, headache, skin diseases and syphilis.[7] It single is found to strengthen and promote the repair of sinews, muscles and bones. They are effective for lower back and ligament injuries.[8]In another combination of drug, *Drynaria* is used for expelling rheumatism.[10]*Drynaria* rhizome is used topically in traditional Chinese medicine to stimulate hair growth and to treat baldness. In the treatment of hyperthyroidism. Drynaria along with other drugs are used. In these conditions drynaria is used externally as well as internally.[11]D. quercifolia along with other combination of herbs is used in pain from traumatic injury, such as sprains and contusions with bruising and swelling.[12]

MATERIALS AND METHODS

Collection of plant material

The rhizome of *Drynaria quercifolia* linn (Fam: Polypodiaceae) were collected from Updi District in the month of April 2008 and

authenticated by Dr.Gopalkrishna Bhatt, Professor, Sri poornaprajna college, Udupi, Mangalore, Karnataka, India. The plant material was dried, powdered and stored in air tight containers until further studies.

Proximate analysis[13]

Determination of total ash value

Accurately weighed 5gms of powdered rhizome of *Drynaria quercifolia* Linn (Polypodiaceae) was taken in a dried silica crucible. It was incinerated at temperature 450°C, until free from carbon and then cooled. The weight of total ash was taken and the percentage of it was calculated with reference to the air dried sample.

Determination of acid insoluble ash value

The total ash obtained was boiled for 5 mins with 25 ml of 2N HCI, filtered and the insoluble matter was collected on ash less filter paper. Then it was washed with hot water, ignited in tarred crucible cooled and the residue obtained was weighed. Finally the percentage of acid insoluble ash was calculated with reference to the air dried drug.

Determination of water soluble ash value

The total ash obtained was boiled with 25 ml of water for few mins. The insoluble matter was collected on ash less filter paper, washed with hot water and ignited for 15 mins at temperature not exceeding 450°C. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

Determination of extractive value

Determination of alcohol and water soluble extractive value

20 gms of air dried, coarsely powdered rhizome of *Drynaria quercifolia* linn powdered was macerated with 100 ml of alcohol (90%) in a closed flask for 24 hrs, shaking frequently during the first 6hrs and was allowed to stand for 18 hrs Then it was filtered rapidly and precautions were taken against loss of alcohol. 25m1 of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of alcohol soluble extracts were calculated with reference to the air dried drug. The procedure followed as above using chloroform water instead of alcohol.

Determination of moisture content

Accurately weighed 5gms of powdered rhizome of *Drynaria quercifolia* linn was taken in a china dish. It was kept for 30 mins in a hot air oven at 105 - 110°C. The percentage of moisture content was then calculated with reference to the air dried drug at different times.

Sequential extraction of the drug *Drynaria quercifolia* rhizome

The method is based on the extraction of active constituents present in the drug using various solvents ranging from non-polar to polar. The solvents used are petroleum ether, chloroform, methanol and water.

The successive solvent extraction procedure was adopted for the preparation of various extracts of *Drynaria quercifolia* rhizome. The materials were subjected to successive extraction with solvents in their ascending order of polarity in this process the substance, which is soluble in a solvent with particular range of polarity was extracted in the solvent and remaining marc further extracted with next solvent.

The 2 kg powdered drug was taken and subjected for successive solvent extraction The extraction was carried out for 18 hrs with the following solvents in the increasing order of the polarity i.e. Petroleum ether, chloroform, methanol and distilled water.

Preparations of petroleum ether extract

About 2kg of dried roots powder of *Drynaria quercifolia* rhizome was extracted with 600 ml of petroleum ether using Soxhlet apparatus for 18 hrs at 60-80°C. The extract was concentrated to 1/4 of its original volume by distillation as it was adopted to recover the solvent, which could be used again for extractions.

Preparation of chloroform extract: After pet ether extraction, the remaining dried marc was extracted with chloroform to get chloroform extract.

Preparation of methanol extract: After chloroform extraction, the remaining dried marc was extracted with methanol to get methanol extract.

Preparation of aqueous extract: After methanol extraction, the remaining dried marc was extracted with water to get water extract. For the preparation of aqueous extract, the above dried marc was macerated for 3 days with distilled water and the residue was removed by filtration and filtrate was concentrated to obtain aqueous extractAll the extracts were concentrated by distillation of the solvent and evaporating them to dryness at low temperature. They were then weighed and the percentage of different extractive values was calculated in terms of air dried weight of the plant material.

Preliminary phytochemical screening

The powdered root was subjected to systematic phytochemical screening by successively extracting them in different solvents and testing for the presence of chemical constituents.

Qualitative chemical examination of extracts

Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were tested carefully with alkaloid reagents.

a. **Mayer's Test:** Filtrates were treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow cream precipitate indicated the presence of alkaloids.

b. **Wagner's Test:** Filtrates were treated with Wagner's reagent (iodine in potassium iodide) and observed. Formation of brown or reddish brown precipitate indicated the presence of alkaloids.

Detection of flavonoids

Lead acetate Test: The extracts were treated with few drops of 10% lead acetate solution. The formation of yellow precipitate confirmed the presence of flavonoids

Detection of proteins and amino acids

a. **Millons Test** The e tracts were treated with 2 ml of Millons reagent The formation of white precipitate, which turned to red upon heating, indicated the presence of proteins and amino acids.

b. **Biuret Test**: The extract: were treated with 1ml of 10% sodium hydroxide solution and heated. A drop of 0.7% copper sulphate solution to the above mixtures was added. The formation, of purplish violet color indicated the presence of proteins.

Evaluation of antioxidant activity

D.P.P.H Method [13](Blios MS. 1958, Free radical scavenging potential of extracts was tested against a methanolic solution of DPPH (α, α-diphenyl - β - picryl hydrazyl) antioxidants react with DPPH and convert it to α, α-diphenyl- β - picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity.

α, α- diphenyl - β - picryl hydrazyl` α, α-diphenyl- β - picryl hydrazine

Reagents

1. D.P.P.H Reagent (100μM): 39.4mg of DPPH was dissolved in l000ml of analytical grade methanol.

2. Preparation of extract solution: Stock solution of extracts was prepared in the concentration of 5mg/ml in methanol.

Method Different aliquots of 0.025, 0.05, 0.1, 0.25, 0.5ml of *Drynaria quercifolia* extract solutions were taken in different test tubes. To all these tubes methanol was added and made up to 1ml. To this 4m1 of methanolic DPPH was added and shaken well. The mixture was allowed to stand at room temperature for 20min. The control contains only methanol and DPPH. The readings were noted at 517 nm against methanolic blank. The change in absorbance of the samples was measured.

Free radical scavenging activity was expressed as the inhibition percentage calculated using the formula.(Table 4)

% of anti-radical activity= [A-B/A] x 100

Where, 'A' is absorbance of control & 'B' is absorbance of sample

RESULTS AND DISSCUSION

Proximate Analysis The rhizome of *Drynaria quercifolia* linn was subjected to evaluate its total ash value, acid insoluble ash, water soluble ash, water soluble extractive value, alcohol soluble extractive value and moisture content.

Table 1: Ash value of *Drynaria quercifolia* rhizome linn

Name of plant	Ash value		
	Total	Acid insoluble	Water soluble
Drynaria quercifolia l	9.93%	4.49%	6.96%

Table 2: Extractive value of *Drynaria quercifolia* rhizome

Name of plant	Extractive values (Percentage w/w)	
	Alcohol soluble	Water soluble
Drynaria quercifolia	9.87%	13.94%

Table 3: Moisture content of *Drynaria quercifolia* rhizome

Time (mins)	Moisture content(%)
30	**12.82**
45	**10.94**
60	**7.84**
75	**5.92**
90	**5.53**

Table 4: Results of antioxidant activities by D.P.P.H method

Extracts of *Drynaria quercifolia*	Concentration in PPM	% Scavenging activity
Pet-ether	25	15.98
	50	24.67
	100	38.97
	250	63.99
	500	68.54
Chloroform	25	12.43
	50	22.54
	100	39.55
	250	54.66
	500	66.83
Methanol	25	17.75
	50	37.67
	100	65.99
	250	79.12
	500	94.37
Water	25	13.45
	50	28.56
	100	32.67
	250	44.88
	500	54.76
α-tocopherol	25	14.65
	50	32.87
	100	55.98
	250	70.34
	500	86.27

All the extracts at a concentration of 500ppm have shown very good antioxidant activity. Among the rhizome extracts of *Drynaria quercifolia* only methanolic extract at 500ppm has shown activity above 90%. Higher activity has been shown by the methanolic extract than standard α-tocopherol.

CONCULSION

All the extracts obtained from sequential solvent extraction were subjected to the anti-oxidant activity study. It was observed that, the methanolic extract was found to be effective antioxidant on comparison with the other extracts and has significant activity compared to the standard drug.

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