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IN VITRO ANTIOXIDANT ACTIVITY OF *DRYNARIA QUERCIFOLIA* L. RHIZOME

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ABSTRACT: Antioxidant activity of methanolic extract of *Drynaria quercifolia* rhizome at different concentrations (100, 200 and 300µg/ml) were studied in various *in vitro* models. Results were compared with standard ascorbic acid. Antioxidant activity of extract was increased with the increasing concentration. The order of antioxidant potential according to models were found to be highest in nitric oxide scavenging activity followed by total antioxidant activity, reducing power assay and hydrogen peroxide scavenging activity. IC50 values were found to be 180, 230, 230 and 240µg/ml respectively in nitric oxide scavenging activity, total antioxidant assay, reducing power assay, and hydrogen peroxide scavenging activity. In conclusion, the results of present analysis demonstrated that *Drynaria quercifolia* rhizome possess potential antioxidant activity, and could be used as a viable source of natural antioxidants.

INTRODUCTION: Free radicals play a major role in the development of chronic and degenerative diseases such as cancer, arthritis, autoimmune disorders, aging, cardiovascular and neurodegenerative diseases [1-3]. Imbalance in the formation and neutralization of free radicals generates a condition called oxidative stress, which affect the cell membranes and major biomolecules [4-6]. Oxygen free radical can initiate peroxidation of lipids, which in turn stimulates glycation of protein, inactivation of enzymes and alteration in the structure and function of collagen basement and other membranes, and play a role in the long-term complication of diabetes [7, 8].

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Antioxidants are radical scavengers which are an important in the prevention of human diseases. Naturally occuring antioxidants possess the ability to reduce the oxidative damage associated with many diseases [9-11]. These natural antioxidants may have free radical scavengers, reducing agents, potential complexes of pro-oxidant metals, quenchers of singlet oxygen etc.[12]. Antioxidant compounds like phenolic acids, polyphenols and flavanoids scavenge free radicals such as peroxide, hydroperoxide or lipidperoxyl and thus inhibit the oxidative mechanism that lead to degerative diseases [13].

The plant based antioxidants can be derived from any part of plant's like bark, leaves, flowers, roots, fruits etc that is any part of the plant may contain active compounds [14]. Therfore, the importance of search for natural antioxidants has greatly increased in recent years. The researchers have focused on natural antioxidants and numerous crude extracts and pure natural compounds have been reported to possess antioxidant properties [15]. In the current

study, *in – vitro* experiments were conducted to determine antioxidant effect of methanolic extract of *Drynaria quercofolia* rhizome.

Drynaria quercifolia (Asvakatri) belongs to the Family of Polypodiaceae 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. It is an epiphytic fern with short thick fleshy creeping rhizome. Traditionally, the fronds of plant are reported to be used by tribal communities of Tamil Nadu and Kerala in treatment of diverse ailments including typhiod fever, chronic jaundice. It is used as an antiinflammatory and antifertility agent [16-19]. Rhizome decoction or drink of *Drynaria quercifolia* used as an antipyretic preparations[20]. Various phytochemicals like friedelin, epifriedeinol, β amyrin, β-sitosterol, 3-β-glucobyranoside and naringin has been isolated from the plant [21].

MATERIALS AND METHODS:

Collection of plant material:

The rhizome of *Drynaria quercifolia* linn were collected from Kollimalai, Namakkal district, Tamil Nadu, India. The collected samples were carefully kept in polythene bags. These plant samples were authenticated by Dr. S. Johnbritto, The Director, The Rabinet Herbarium Centre for Molecular Systematic, St. Joseph's College, Tiruchirappalli and a voucher specimen was deposited in the department of Biochemistry, S.T.E.T Women's College, Mannargudi (Voucher No:001).

Processing and preparation of plant extract:

The rhizome is covered with small brown coloured hair like strutures. They were removed using sterile scalpel and washed with sterile distilled waer. They were cut into small pieces and dried in shade and made into coarse powder, using blender, and stored in air tight containers. 50g rhizome powder of *Drynaria quercifolia* was weighed and macerated in methanol in the ratio of 1:6. They were kept at room temperature for 72 h. The mixture was stirred every 24h using a sterile glass rod. Then it was filtered through the Whatmann No: 1 filter paper. Extraction procedure was done further twice for complete extraction of bioactive compounds. The obtained filtrate was combined together and concentrated in vacuum using rotary evaporated. The dried residue was used for evaluating invitro antioxidant activity.

In vitro antioxidant study:

Methanolic extract of *Drynaria quercifolia* was tested for its free radical scavenging property using different invitro models. Standard ascorbic acid was also analysed to compare the efficacy on antioxidant activity of plant extract. All the experiments were performed thrice and the results averaged.

Total antioxidant capacity:

For total antioxidant capacity assay, 1ml of the plant extract at different concentration (100, 200, 300 µg/ml) were dissolved in water and mixed with 3ml of reagent solution (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in Eppendorf tube. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After 90 min, the mixture was cooled to room temperature, the absorbance was measured at 695nm against reagent blank. Ascorbic acid was used as the standard and the total antioxidant capacity is expressed as equivalents of ascorbic acid [22].

Reducing power ability:

Reducing ability of plant extract was measured by mixing 1.0ml extract at different concentration (100, 200, 300 µg/ml) to 2.5ml of phosphate buffer (0.2m, pH6.6) and 2.5ml of 1% potassium ferricyanide and incubated at 50ºC for 30 minutes. After that 2.5ml of trichloroacetic acid (10%) were added to the mixture and centrifuged for 10min at 3000g. 2.5ml from the upper part were diluted with 2.5ml water and shaken with 0.5ml fresh 0.1% ferric chloride. The absorbance was measured at 700nm using UV-spectrophotometer [23].

Nitric oxide radical scavenging activity:

Nitric oxide radicals were generated from sodium nitro prusside solution at physiological p^H. Sodium nitro prusside (1 ml of 10Mm) was mixed with 1ml of plant extract of different concentrations (100, 200 and 300µg/ml) in phosphate buffer (pH 7.4). The mixture was incubated at 25ºC for 150min. To 1ml of the incubated solution, 1ml of Griess' reagent (1% sulphnilamide, 2% o-phosphoric acid

and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was read at 546nm and percentage inhibition was calculated[24].

Hydrogen peroxide radical scavenging activity:
A solution of H_2O_2 was prepared in phosphate buffer (p^H7.4). H_2O_2 concentration was determined spectrophotometrically by measuring absorption with extinct coefficient for H_2O_2. Methanolic extract of plant (100, 200, 300 µg/ml) in distilled water was added in H_2O_2 solution (0.6 ml, 40µM/). After 10 min, absorbance of H_2O_2 was measured at 230 nm against blank solution containing the phosphate buffer without H_2O_2. The % of scavenging effect of extracts and standard ascorbic acid were calculated [25].

RESULTS AND DISCUSSION: Free radicals are chemical entries that can exist separately with one or more unpaired electrons. The propagation of these radicals can bring about thousands of reactions and thus may cause extensive tissue damage. Lipids, proteins and DNA are all susceptible to attack by free radicals [26, 27]. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals, inhibiting lipid peroxidation etc. In the present study, *in vitro* antioxidant activity of methanolic extract of *Drynaria quercifolia* rhizome were analysed by various *in vitro* models. Results were compared with standard ascorbic acid.

Total antioxidant activity:
The total antioxidant activity of the methanolic plant extract were concentration dependent (100, 200, 300µg/ml), with the increasing concentration, the activity is also increased. Highest antioxidant activity of plant extract and standard were found to be 54.88% and 54.53% respectively at 300µg/ml. IC_{50} value for plant extract and standard drug were 230 and 150µg respectively. These findings shows that plant extract exhibited good antioxidant activity which was similar to that of standard (**Table 1**).

Total antioxidant capacity by Phosphomolybdenum method assay has been routinely used and it is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid[14].

TABLE 1: TOTAL ANTIOXIDANT ACTIVITY

Concentration (µg /ml)	Antioxidant Activity in %	
	Plant extract	Standard drug
100	31.65±0.68	45.68±3.18
200	38.48±1.18	53.49±2.14
300	54.88±0.17	54.53±0.57

All values are expressed as mean ± SEM for three determinations

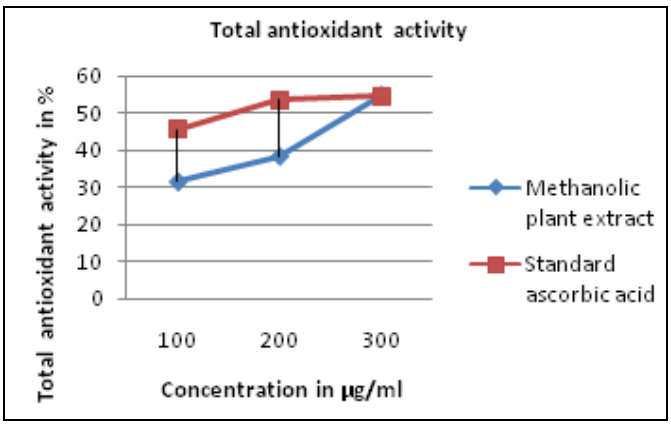

FIG.1: IC 50 VALUES: FOR PLANT EXTRACT =230 µg/ml, FOR STANDARD=150 µg/ml

Reducing power assay:
The reducing ability of the extract served as a significant indicator of its potential antioxidant activity of plant extract and standard ascorbic acid were used at dose range of 100-300µg/ml (**Table 2**). The reducing power of extract increased at the concentration of 300µg/ml (58.07 %) and for standard ascorbic acid, reducing power was 62.80% at 300µg/ml. IC_{50} value of plant extract and standard drug were found to be 230µg and 220µg respectively.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability is generally associated with the presence of reductones, which breaks the free radical chain by donating a hydrogen atom [28].

In the present study, the extract exhibited a moderate reducing power which was comparably less than that of the standard ascorbic acid.

TABLE 2: REDUCING POWER ASSAY

Concentration (µg /ml)	Reducing Power in %	
	Plant extract	Standard drug
100	36.13±0.97	38.68±1.15
200	39.71±0.53	47.22±1.02
300	58.07±1.97	62.80±2.02

All values are expressed as mean ± SEM for three determinations

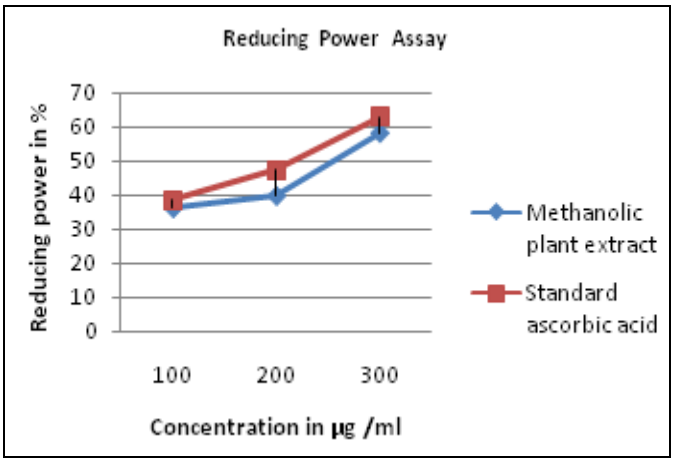

FIG.2: IC 50 VALUES: FOR PLANT EXTRACT =230 µg/ml, FOR STANDARD=220 µg/ml

Nitric oxide scavenging activity:

Incubation of solutions of nitroprusside in phosphate buffer saline at 25^0C for 150min resulted in generation of NO. The plant extracts at various concentrations effectively reduced the NO generation. Scavenging activity was dose dependent and shown in **Table 3**. Extract showed potent nitric oxide scavenging (62.77%) at 300µg/ml where as ascorbic acid showed 54.60% at 300µg/ml. IC50 value for both plant extract and standard were found to be 180 and 240µg/ml respectively.

Nitric Oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons etc and involved in the regulation of various physiological prosesses [29]. Excess concentration of NO is associated with several diseases [30, 31]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrate anions, which act as free radicals [26, 32]. In the present study, the extract competes with oxygen to react with nitric oxide and thus inhibit the generation of the anions.

Hydrogen peroxide radical scavenging activity:

Methanolic extract of *Drynaria quercifolia* was capable of scavenging H_2O_2 in a dose dependent manner. The scavenging ability of extract and standard were shown in **Table 4**. Maximum activity was observed at 300µg/ml of plant extract (44.09%) and ascorbic acid (57.87%). IC 50 values for plant extract and standard drug were found to be 240µg and 200µg respectively. Comparison of hydrogen peroxide radical scavenging activity of plant extract with standard, extract showed mild antioxidant activity.

Hydrogen peroxide itself is not very reactive, but it may be toxic to cell due to increase in hydroxy1 radical concentration in the cells [33]. Thus removal of H_2O_2 as well as O_2 leads to survival of the cell life and its components. In the present study, the scavenging ability of extract on H_2O_2 is comparably less potent than standard ascorbic acid.

TABLE 3: NITRIC OXIDE SCAVENGING ACTIVITY

Concentration (µg /ml)	% of Inhibition	
	Plant extract	Standard drug
100	47.13±2.60	44.25±0.25
200	58.76±0.60	42.11±0.95
300	62.77±0.58	54.60±0.45

All values are expressed as mean ± SEM for three determinations

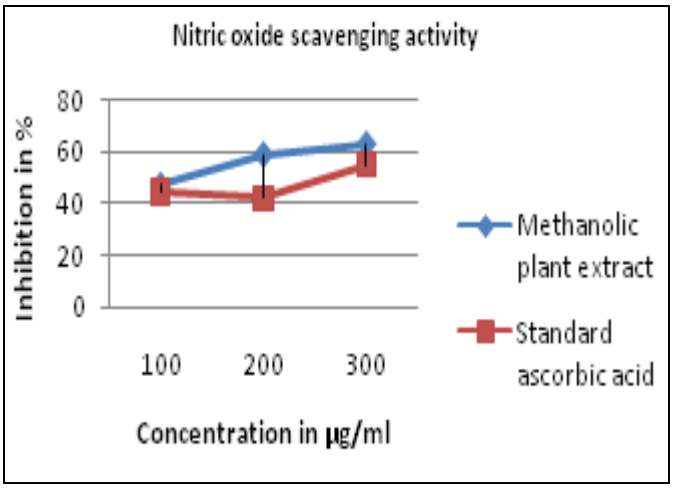

FIG. 3: IC 50 VALUES: FOR PLANT EXTRACT =180 µg/ml, FOR STANDARD=240 µg/ml

TABLE 4: HYDROGEN PEROXIDE SCAVENGING ACTIVITY

Concentration (µg /ml)	% of Inhibition	
	Plant extract	Standard drug
100	31.77±1.5	33.13±0.93
200	32.78±0.75	44.49±2.07
300	44.09±1.03	57.87±0.71

All values are expressed as mean ± SEM for three determinations

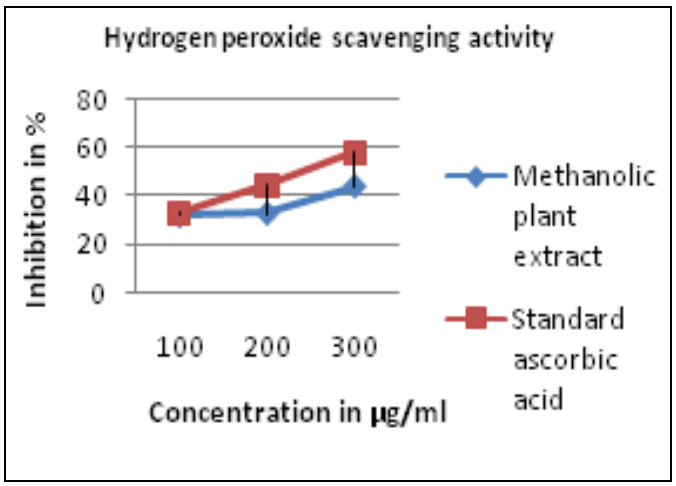

FIG. 4: IC 50 VALUES: FOR PLANT EXTRACT =240 µg/ml, FOR STANDARD=200 µg/ml

CONCLUSION: This study has demonstrated the antioxidant effects of methanoilc extract of *Drynaria quercifolia* rhizome. The extract showed concentration dependent activity in all the models. These findings suggest that this plant is a potential souece of natural antioxidant that could have great impoatance as therapeutic agents for various diseases.

REFERENCES:

1. Gujkl.Valko M, Izakovic M , Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem. 2004; 266:37-56.
2. Valko M, Leibfritz D, Moncola J, Cronin MD. Free radicals and antioxidants in normal physiological functions and human disease. Review. Int J Biochem Cell Biol. 2007; 39: 44-84.
3. Chatterjee M, Saluja R, Kanneganti S. Biochemical and molecular evaluation of neutrophil NOS in spontaneously hypertensive rats. Cell Mol Biol. 2007; 53: 84-93.
4. Droge W. Free radicals in the physiological control of cell function. Review. Physiol Rev. 2002; 82: 47-95.
5. Willcox JK, Ash SL, Catignani GL. Antioxidants and prevention of chronic disease. Review. Crit Rev Food Sci Nutr. 2004; 44: 275-95.
6. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiol Rev. 2007; 87:315-424.
7. Saha MN, Alam MA, Aktar R, Jahangir R. Bangladesh Journal Pharmacology 2008; 3: 90-96.
8. Prior RL. American J Clin Nut 2003; 78: 570S-578S.
9. Pietta P, Simonetti P and Mauri P. Antioxidant activity of selected medicinal plants. J Agric Food Chem. 1998; 46: 4487-4490.
10. Lee KG, Mitchell AE and Shibamoto T. Determination of antioxidant properties of aroma extracts from various beans. J Agric Food Chem. 2000; 48: 4817-4820.
11. Middleton E, Kandaswamy C and Theoharides TC. The effects of plant flavonoids and mammalian cells: implications for inflammation, heart disease and cancer. Pharmacol Rev. 2000; 52: 673-751.
12. Andlauer W ans Furst P, Antioxidative power of phytochemicals with reference to cereals. Cereal Foods World. 1998; 43:356-359.
13. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenols, flavonoid contents of selected Iranian medicinal plants. S.Afr. J. Biotechnol. 2006; 5"1142-1145.
14. Halliwel B. Free radicals, antioxidants and human diseases curiosity, cause or consequence. 1994; 344: 505.
15. Jayaprakasha G K, Selvi T and Sakariah K K. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extract. Food Res Int. 2003; 36: 117-122.
16. Dixit RD, Vohra JN. A Dictionary of pteridophytes of India. In: Botanical survey of India. Howrah: India; 1984.
17. Irudayaraj V, Senthamarai R. Pharmacognostical studies on a medicinal fern, *Drynaria quercifolia* (L) J. Sm. (Polypodiaceae: Pteridophyta). Phyto pharmacology. 2004; 54: 193-200.
18. Shokeen P. Preliminary Studies on activity of O.Sanctum, *D.Quercifolia* and *A.Squamosa* against *Neisseria gonorrhoeae*. Sexually Transmitted Disease. 2005; 32: 106-111.
19. Rajendran A, Rajan S. *Drynaria quercifolia*: an antifertility agent. Ancient science of Life. 1996; 15: 286-287.
20. ASEAN centre for for Biodiversity. The gateway to biodiversity information in South East Asia. 2004; http://www.aseanbiodiversity.org/medicinal-plants/page3.htm.
21. Ramesh N, Viswanathan MB, Saraswathy A Balakrishna K, Brindha P, Lakshmanaperumalasamy P, Pyhtochemical and antimicrobial studies on *Drynaria quercifolia*. Fitoterapia. 2001; 72: 934-936.
22. Kumaran A and Karunakaran JR. *In-vitro* antioxidant activities of methanolic extracts of five Phyllathaus species from India. LWT-Food Science and Teahnology. 2007; 40(2): 344-352.
23. Yildirim A, Oktay M, Bilaloglu V. The antioxidant activity of the leaves of *Cydonia vulgaris*. Turk J Med Sci. 2001; 31:23-27.
24. Streejayan, Rao MNA. Nitric oxide scavenging by curcuminoids. Pharma Pharmacol. 2002; 79: 325 – 329.
25. Ruch R J, Cheng S J and Klaunig J E. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogen 1989; 10: 1003-1008.
26. Cotran R S, Kumar V and Collins T in Robbin's Pathological basis of diseases. 6th edn. (Thomson Press (I) Ltd, Noida, India). 1999; 1

27. Yu B P, Suescun E A and Yang S Y. Effect of age related lipid peroxidation on membarance fluidity and phopholipase A_2: modulation by directory restriction. Mech Ageing Dev. 1992; 65:17.
28. Subhashini N, Thangathirupathi A and Lavanya N. Antioxidant activity of *Trigonella boenum graecum* using various *In vitro* and *Ex vivo* models. I J Pharm and pharm Sciences. 2011; 3(2): 96-102.
29. Lata H, Ahuja G K, Role of free radicals in health and disease. Ind J physio of Allied. 2003; 57:124.
30. alenti A, Moncada S & Di Rosa M. Modulation of adjuvant arthritis by endogenous nitric oxide. Br J Pharmacol. 1993; 110:701.
31. Ross R. The Pathogenesis of artherrosclerosis: a perspective for the 1990's. Nature. 1993; 362: 801.
32. Sainani G S, Manika J S & Sainani R G. Oxidative Stress: a key factor in pathogenesis of chronic diseases. Med update.1997; 1:1.
33. Halliwell B. Reactive Oxygen Species in living Systems: Source, Biochemistry, and role in human disease. Am J Med. 1991; 91: 14-22.

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