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Evaluation of *in vitro* antioxidant activity of rhizome extract of *Drynaria quercifolia* L.

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Abstract: The aim of this study was to screen the *in vitro* antioxidant activity of methanol extract of *Drynaria quercifolia* rhizome. Three different concentrations of plant extract were evaluated for their antioxidant activity by *in vitro* models such as DPPH radical scavenging assay and ferric reducing antioxidant power (FRAP) assay. The results showed the dose dependent response in both assay that is when the concentration of plant extract increases, antioxidant activity was also increased. From the results, it was concluded that the studied plant *Drynaria quercifolia* has potent source of radical scavenging and antioxidant activity and this is strongly support the traditional use of this plant for the treatment of diseases associated with oxidative damage.
Key words: Antioxidant, DPPH, *Drynaria quercifolia,* FRAP, Methanol extract.

Introduction

Reactive oxygen species (ROS) are produced as a natural byproduct/intermediates in biological processes in body by the normal oxygen metabolism. The role of free radical reactions in disease pathology is well established and is known to be involved in many acute and chronic disorders in human beings, such as diabetes, atherosclerosis, aging, immune suppression and neurodegeneration[1]. An imbalance between ROS and the inherent antioxidant capacity of the body, directed the use of dietary and /or medicinal supplements particularly during the disease attack. Human body has an inherent antioxidative mechanism and many of the biological functions such as the anti mutagenic, anti-carcinogenic, and anti-aging responses originate from this property[2, 3]. Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells[4].

Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity[5]. Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities[6]. Therefore, it is important to assess antioxidant activity of the plants used in the herbal medicine either to elucidate the mechanism of their pharmacological action or to provide information on antioxidant activity of these herbal plants. Hence, the present study has been designed to evaluate the *in vitro* antioxidant activity of the medicinal fern *Drynaria quercifolia* L. rhizome.

Drynaria quercifolia L. belonging to the family of Polypodiaceae, is used in typhoid fever, phthiriasis, dyspepsia, cholera, cough, arthralgia, cephalalgia, diarrhea, chronic jaundice, foul ulcers, and inflammation and also used to treat skin diseases[7,8,9]. Different extracts of *D. quercifolia* were screened for their antibacterial activity against several bacterial pathogens[10]. 30 compounds have been identified in methanol extract of

Drynaria rhizome by GC-MS analysis[11]. The compounds friedelin, epifriedelinol, amyrin, β-sitosterol, β-sitosterol-3-β-D-glucopyranoside, naringin have been isolated from the rhizome of *D. quercifolia* [9]. The rhizome is reported to have anti fertility[12], anti inflammatory[13] and antipyretic[14], antimicrobial[15] and antiulcer[16] properties.

Materials and Methods

Collection of plant material

The rhizome of *Drynaria quercifolia* L. was 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 (Voucher No:001) was deposited in the department of Biochemistry, S.T.E.T Women's College, Mannargudi.

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 water. 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 *in vitro* antioxidant activity.

In vitro antioxidant study

Methanolic extract of *Drynaria quercifolia* was tested for its antioxidant property using *in vitro* models such as DPPH and FRAP assay. All the experiments were performed thrice and the results averaged.

DPPH free radical scavenging activity

The ability of the extracts to annihilate the DPPH radical (1,1-diphenil-2-picrylhydrazyl) was investigated by the method described by Blois, 1958[17]. Stock solution of extracts was prepared to the concentration of 1mg/ml. 100µg of each extracts were added, at an equal volume, to methanolic solution of DPPH (0.1mM). The reaction mixture is incubated for 30min at room temperature; the absorbance was recorded at 517 nm. The experiment was repeated for three times. BHT was used as standard controls. The annihilation activity of free radicals was calculated in % inhibition according to the following formula

% of Inhibition = (A of control – A of Test)/A of control * 100

FRAP Assay

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay[18]. FRAP assay uses antioxidants as reductant in a redox-linked colorimetric method, employing an easily reduced oxidant system present in stoichiometric excess. At low P^H, reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has intense blue color) can be monitored by measuring the change in absorption at 593nm. The change in absorbance is therefore, directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. 100µl, 200µl and 300µl of sample is mixed with 1.5ml of working FRAP reagent and incubated at 37°C for 4 minutes. After incubation the absorbance at 593nm was measured. Ferrous sulphate standard was processed in the same way and the FRAP value was calculated from the standard graph.

Calculation

FRAP value of sample in µM.

(Change in Absorbance of sample from 0 to 4 mins)/ (Change in Absorbance of standard from 0 to 4 mins) * FRAP value of standard (1000µM).

Results

In the present study, methanol extract of *Drynaria quercifolia* rhizome has been evaluated for their antioxidant activity by *in vitro* models such as DPPH radical scavenging activity and FRAP method. For both the experiments, three samples were analyzed and the assays were carried out in triplicate. The results were expressed as mean ± standard deviation.

DPPH radical scavenging activity

Table 1 shows the result of DPPH radical scavenging activity different concentration of (50, 100 and 150 µl**)** of *Drynaria quercifolia* rhizome. Among the three concentrations, highest % of inhibition (49.58 0.03) was found in 150 µl followed by 100 µl **(**22.71±1.00**)** and 50 µl **(**17.13±0.16**)**. From the results, dose dependent response was observed, that is the concentration of extract increased, the scavenging activity was also increased.

Table 1: DPPH free radical scavenging activity of *Drynaria quercifolia* rhizome

S.No	Concentration of plant extract (µl)	% Inhibition
1	50	17.13±0.16
2	100	22.71±1.00
3	150	49.58±0.03

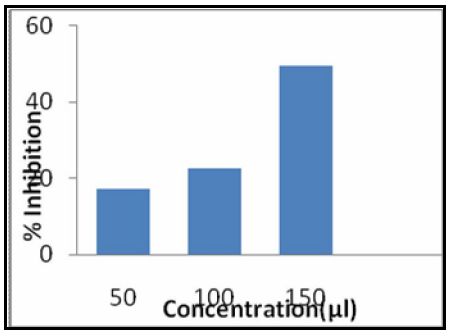

Figure 1: DPPH free radical scavenging activity of *Drynaria quercifolia* rhizome

FRAP assay

Table 2 shows the result of ferric reducing antioxidant power (FRAP) assay of *Drynaria quercifolia* rhizome. Different concentration of (100, 200 and 300 µg) plant extract were investigated and the results showed the dose dependent responses at concentration of 100 to 300 µg. Highest FRAP value was observed in 300µg (2.78±0.01 µM/mg) followed by 200 µg (0.44±0.005 µM/mg) and 100 µg (0.27±0.01 µM/mg).

Table 2: FRAP assay of *Drynaria quercifolia* rhizome

S.No	Concentration of plant extract (µg)	FRAP value (µM/mg)
1	100	0.27±0.01
2	200	0.44±0.005
3	300	2.78±0.01

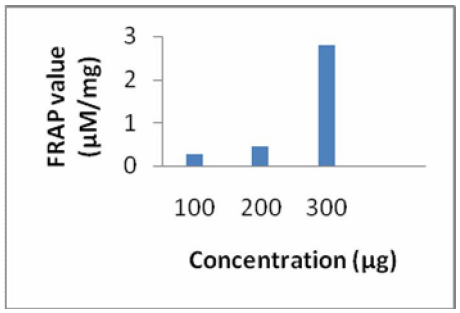

Figure 2: FRAP assay of *Drynaria quercifolia* rhizome

Discussion

The search for novel natural antioxidants of plant origin has ever since increased. It is not known which constituents of plant are associated in reducing the risk of chronic diseases, but antioxidants appear to play a major role in the protective effect of plant medicine. The present study was designed to investigate the antioxidant activity of *Drynaria quercifolia* rhizome by DPPH and FRAP method. Good stability, credible sensitivity, simplicity and feasibility are the advantages of DPPH assay[19, 20, 21]. This assay is often used to evaluate the ability of antioxidants to scavenge the free radicals from the supplied samples, whereby the free radicals cause biological damage through oxidative stress and such processes leads to many disorders like neurodegenerative disorders, cancer and AIDS[22]. Therefore, DPPH assay is an effective method to measure their scavenging power. The principle of the DPPH is based on the color changes from purple (DPPH solution) to yellow[23]. The color changes can be measured quantitatively at the absorbance 517nm. In the present study, dose dependent response was observed in DPPH assay. 150 µl of plant extract was found to be more significant than other two concentrations. As concentration of extract goes on increasing, antioxidative potential also shows positive response which revealed the potent antioxidant activity of *Drynaria quercifolia* rhizome.

The ferric reducing antioxidant potential (FRAP) assay is another simple and inexpensive procedure which deal the total antioxidant levels in plants. FRAP assay also be used to analyze antioxidant status in humans after hyperbaric oxygen therapy[24]. It has also been used to compare antioxidant activity in plants and mammals[25, 26]. In the present study, the higher the FRAP value was observed at high concentration of plant extract which indicates the greater antioxidant activity of *Drynaria quercifolia* rhizome. The radical scavenging and inhibition of lipid peroxidation by the extracts was due to the quenching free radicals or reduction of Fe3+ to Fe2+, which can be attributed to the presence of number of polyphenolics such as flavonoids, anthocyanins etc., [27]. Previous study has also demonstrated that the concentration dependent antioxidant effects of methanoilc extract of *Drynaria quercifolia* rhizome in the models such as total antioxidant capacity, reducing power ability, nitric oxide radical scavenging activity, Hydrogen peroxide radical scavenging activity[28].

In conclusion, the results of our study have shown that the studied plant *Drynaria quercifolia* rhizome is the good source of free radical scavenging compounds and therefore it has good antioxidant property. Hence, the studied plant *Drynaria quercifolia* rhizome will be use as potential source for pharmaceutical application in term of antioxidant drug to cure various ailments.

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