

Antioxidant and Cytotoxic activities of Methanolic extract of *Dryopteris filix-mas* (L.) Schott Leaves

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Abstract

Dryopteris filix-mas (L.) Schott traditionally used for the treatment of various ailments by the indigenous people of the hills areas in Bangladesh. So, our present study was designed to evaluate the antioxidant and cytotoxic activities of methanolic extract of leaves. A dose dependent scavenging activity of DPPH radical was observed with good reducing power of the extract. In DPPH radical scavenging assay, the IC₅₀ value of the extract was 275.50µg/ml while the IC₅₀ value for the reference ascorbic acid was 110.13µg/ml and good reducing power activity showed by the extract with increasing the concentration and a total phenol content in the was 238.10 mg/gm of gallic acid and was found to contain 126.80mg/gm of quercetin in flavonoid assay. The extract also possesses potent cytotoxic activity with LC₅₀ 25.94µg/ml where the LC₅₀ value of standard vincristine sulphate was 10.00µg/ml.

Key words:

Dryopteris filix-mas; Antioxidant; Cytotoxic; Brine Shrimp.

How to Cite this Paper:

M. Sekendar Ali*, Kawsarul Mostafa¹, M. Obayed Raihan², M. Khalilur Rahman¹, Dr. M. Aslam Hossain³, M. Shah Alam⁴ "Antioxidant and Cytotoxic activities of Methanolic extract of *Dryopteris filix-mas* (L.) Schott Leaves", Int. J. Drug Dev. & Res., April-June 2012, 4(2): 223-229

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Article History:-----

Date of Submission: 04-04-2012

Date of Acceptance: 11-04-2012

Conflict of Interest: NIL

Source of Support: NONE

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INTRODUCTION

Oxidation is a chemical reaction that produce free radicals such as the hydroxyl radical ($\cdot\text{OH}$) and the superoxide anion (O_2^-) and reactive oxygen species in the cells include hydrogen peroxide (H_2O_2), hypochlorous acid (HClO) [1]. In turn, these radicals and reactive oxygen species can start chain reactions such as lipid peroxidation, or by oxidizing DNA or proteins [2] and produce oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease [3-4] Parkinson's disease [5] the pathologies caused by diabetes [6-7] rheumatoid arthritis [8] and neurodegeneration in motor neuron diseases [9]. Damage to DNA can cause mutations and possibly cancer, if not reversed by DNA repair mechanisms [10-11] while damage to proteins causes enzyme inhibition, denaturation and protein degradation [12]. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They do this by being oxidized themselves [2]. Antioxidants are widely used as ingredients in dietary supplements or as natural dietary compounds [13] and have been investigated for the prevention of diseases such as cancer, coronary heart disease, stroke neurodegenerative diseases and even altitude sickness. Antioxidants are used as food additives to help guard against food deterioration. It is also frequently added to industrial products. A common use is as stabilizers in fuels and lubricants to prevent oxidation, and in gasolines to prevent the polymerization that leads to the formation of engine-fouling residues [14].

Cytotoxicity assays are widely used to screen for cytotoxicity of plant extract either looks for cytotoxic compounds to develop a therapeutic that targets rapidly dividing cancer cells, for instance; or to screen for unwanted cytotoxic effects before investing in the development as a pharmaceutical.

Dryopteris filix-mas (L.) Schott belongs to the family Dryopteridaceae known as Male Fern. The half-evergreen leaves have an upright habit and reach

a maximum length of 1.5 m, with a single crown on each rootstock. The bipinnate leaves consist of 20-35 pinnae on each side of the rachis. The leaves taper at both ends, with the basal pinnae about half the length of the middle pinnae. The pinules are rather blunt and equally lobed all around. The stalks are covered with orange-brown scales. On the abaxial surface of the mature blade 5 to 6 sori develop in two rows. It is one of the most common ferns of the temperate Northern Hemisphere, occurring throughout much of Europe, Asia, and North America. It favours damp shaded areas and is common in the understory of woodlands, but is also found in shady places on hedge-banks, rocks, and screes. Its rhizome contains several toxic and irritant compounds such as flavaspidic acid, a phloroglucinol derivative which are anthelmintic used to expel tapeworms.

According to our knowledge there are no any scientific detailed reports on leaves as antioxidant or cytotoxic activity. So, our present investigation was to evaluate the antioxidant and cytotoxic activities of methanolic extract of leaves of *D. filix-mas*.

MATERIALS & METHODS

Chemicals: DPPH (1, 1-diphenyl, 2-picryl hydrazyl) trichloroacetic acid, ferric chloride, gallic acid and quercetin were obtained from Sigma chemical Co. USA. Ascorbic acid was obtained from SD Fine chem. Ltd., Biosar, India and potassium ferricyanide from May and Backer, Dagenham, UK. Methanol, Folin-ciocalteu reagent, Sodium carbonate and Potassium ferricyanide were purchased from Merck, Germany. All chemicals used were of analytical reagent grade.

Plant Materials: The fresh leaves of *D. filix-mas* were collected from local area of Chittagong, Bangladesh in September, 2011 and were authenticated by Dr. Sheikh Bokhtear Uddin, Associate Professor, Department of Botany, University of Chittagong, Bangladesh. The leaves were dried at room temperature in shade and away

from direct sunlight for 5 days and in hot air oven for 2 days.

Preparation of crude extract: The dried leaves were coarsely powdered and extracted keeping 7 days with methanol. The sediments were filtered and filtrates were dried at 40°C in a water bath. The solvent was completely removed by filtering with Hartman filter paper and obtained dried crude extract which was used for investigation.

Antioxidant Activity

DPPH Radical Scavenging Activity: The free radical scavenging activity of the extract, based on the scavenging activity of the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was determined by the method of Braca *et al.* 2001, [15]. Plant extract (0.1 ml) was added to 3 ml of a 0.004% methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min. and the percentage inhibition activity was calculated by using the following equation:

$$\% \text{ scavenging activity} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the extract. The inhibition curves were prepared and an IC_{50} value was calculated using linear regression analysis.

Reducing Power: The reducing power of extract was determined according to method of Srinivas *et al.* 2007, [16]. Different concentrations of extract (125, 250, 500 and 1000 µg/ml) in 1 ml of distilled water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide- $K_3Fe(CN)_6$ (2.5 ml, 1% w/v). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as

the reference and phosphate buffer (pH 6.6) was used as blank solution.

Total Phenol Content: The total phenol content of extracts was determined using Folin-Ciocalteu reagent using the method of Singelton *et al.* 1999, [17]. Extract (200 µg/ml) was mixed with 400 µl of the Folin-Ciocalteu reagent and 1.5 ml of 20% sodium carbonate. The mixture was shaken thoroughly and made up to 10 ml using distilled water. The mixture was allowed to stand for 2 hours. Then the absorbance at 765 nm was determined. The concentration of total phenol content in extract was then determined as mg of gallic acid equivalent by using an equation that was obtained from standard gallic acid graph.

Total Flavonoids Content: The flavonoids content was determined using a method of Kumaran *et al.* 2007, [18] using quercetin as a reference compound. 1 ml of plant extract in methanol (200 µg/ml) was mixed with 1 ml aluminium trichloride in methanol (20 mg/ml) and a drop of acetic acid, and then diluted with methanol to 25 ml. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 1 ml of plant extract and a drop of acetic acid, and then diluted to 25 ml with ethanol. The total flavonoid content was determined using a standard curve of quercetin (12.5-100 µg/ml) and expressed as mg of quercetin equivalent (QE/gm of extract).

Cytotoxicity screening: Brine shrimp lethality bioassay is widely used in the bioassay for the bioactive compounds [19-20]. Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. The eggs of the brine shrimp were collected from an aquarium shop (Chittagong, Bangladesh) and hatched in artificial seawater (3.8% NaCl solution) for 48 hours to mature shrimp called nauplii. The cytotoxicity assay was performed on brine shrimp nauplii using method of Meyer *et al.* 1982 [19]. The test sample (extract) were prepared by dissolving them in DMSO (not

more than 50 µl in 5 ml solution) plus sea water (3.8% NaCl in water) to attain concentrations of 6.25, 12.5, 25, 50, 100, 200 and 400 µg/ml. A vial containing 50 µl DMSO diluted to 5 ml was used as a control. Standard vincristine sulphate was used as positive control. Then matured shrimps were applied to each of all experimental vials and control vial. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of mortality of the brine shrimp nauplii was calculated for each concentration using the following formula:

$$\% \text{ Mortality} = \frac{N_t}{N_o} \times 100$$

Where, N_t = Number of killed nauplii after 24 hours of incubation,

N_o = Number of total nauplii transferred i.e 20.

The LC_{50} (Median lethal concentration) was then determined using Probit analysis

RESULTS

Antioxidant Screening

DPPH radical scavenging activity: The DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity of *D. filix-mas* is shown in **Figure 1**. This activity was found to increase with increasing concentration of the extract. DPPH antioxidant assay is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants.

The IC_{50} value of the extract was 275.50µg/ml as compared to that of ascorbic acid (IC_{50} 110.13 µg/ml) which is a well known antioxidant.

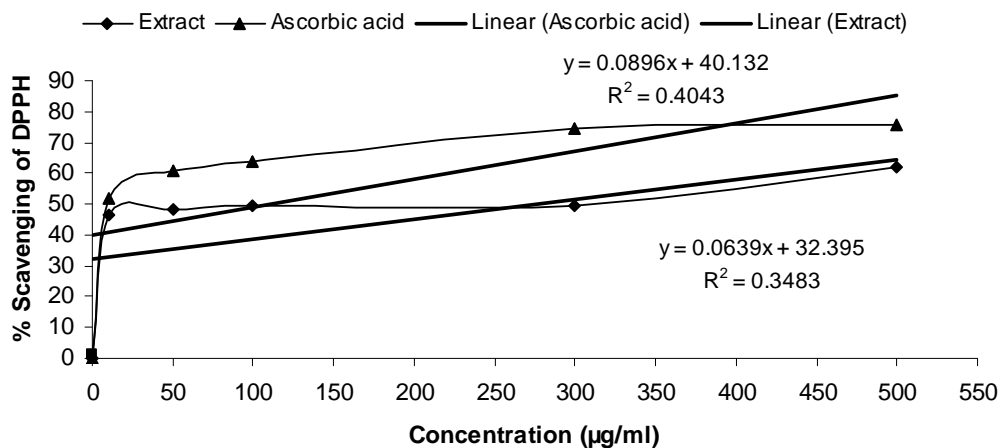


Figure 1: DPPH radical scavenging activity of the methanolic extract of *D. filix-mas* leaves.

Reducing Power: The methanol extract displayed significant reducing power activity which was found to increase with the increasing concentration as shown in **Figure 2**.

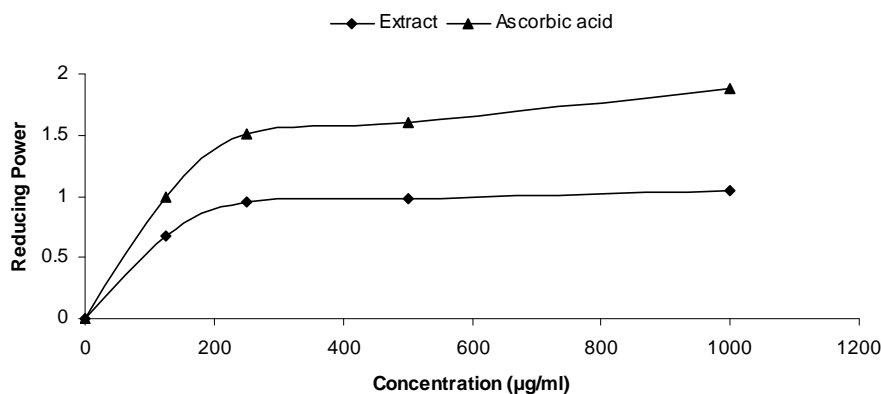


Figure 2: Reducing power of the methanolic extract of *D. filix-mas* leaves.

Total phenol and Flavonoid Content: The total amount phenol and flavonoid which are obtained in separate experiment have been shown in the **Table 1**.

Table 1: Total amount of phenol, flavonoid content of methanolic extract of *D. filix-mas* leaves

Extract	Total phenol(mg/gm, Gallic acid equivalent)	Total flavonoid (mg/gm, quercitin equivalent)
<i>D. filix-mas</i>	238.10±0.42	126.80±0.45

Values are the average of duplicate experiments and represented as mean ± SD.

Brine shrimp lethality bioassay: Following the procedure of Meyer, the lethality of the crude extract of *D. filix-mas* leaves to brine shrimp was determined on *Artemia salina* after 24 hours of exposure the samples, the positive control and vincristine sulphate. This technique was applied for the determination of general toxic property of the plant extract. From the **Figure 3**, we have observed that the LC₅₀ value of the extract was 25.94 µg/ml and that for standard vincristine sulphate was 10.00 µg/ml. No mortality was found in the control group, using DMSO and sea water.

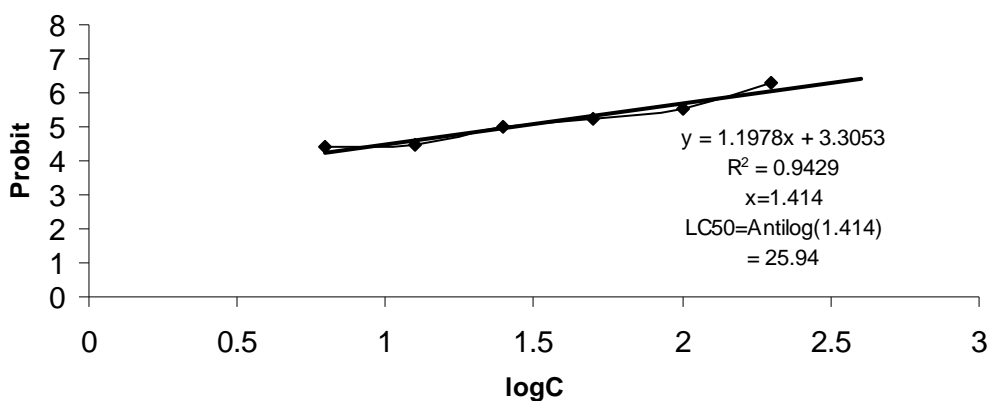


Figure 3: Cytotoxic activity of methanolic extract of *D. filix-mas* leaves.

DISCUSSION

Traditional use of medicines is recognized as a way to learn about potential future medicines. In 2001, researchers identified 122 compounds used in mainstream medicine which were derived from "ethnomedical" plant sources; 80% of these compounds were used in the same or related manner as the traditional ethnomedical use [21]. Plants have evolved the ability to synthesize chemical compounds that help them defend against attack from a wide variety of predators such as insects, fungi and herbivorous mammals. By chance, some of these compounds, whilst being toxic to plant predators, turn out to have beneficial effects when used to treat human diseases.

The methanolic extract of leaves of *D. filix-mas* demonstrated good antioxidant action. Naturally antioxidant present in many plants, foods and beverages offer health benefits in preventing various diseases by fighting cellular damage caused by free radicals in the body [22]. Due to the presence of flavonoids and phenol in the plant extracts, indicates having antioxidant activity [23]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching single and triplet oxygen, or decomposing peroxides [24]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [25]. The reducing properties are generally associated with the presence of reductones, which

have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom and may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals. The scavenging ability of the phenolics is mainly due to the presence of hydroxyl groups. The extract showed significantly higher inhibition percentage (stronger hydrogen-donating ability) positively correlated with total phenolic content. Therefore, phenolic components of this extract may contribute directly to antioxidant action in this study. Flavonoids are a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes [26]. Therefore flavonoids exhibit several biological effects such as antiinflammatory, antiulcer, antiallergic, anticancer activities. However, since reactive oxygen species do have useful functions in cells, such as redox signaling, the function of antioxidant systems is not to remove oxidants entirely, but instead to keep them at an optimum level [27].

As the plant extract showed potential antioxidant and cytotoxic activities in our experiment, so it may play an important role for the treatment of cancer.

CONCLUSION

From the experiment it is clear that the methanol extract of leaves of *D. filix-mas* possesses strong antioxidant and cytotoxic potential. However, these studies are preliminary in nature and there are plenty of scopes for further studies to examine underlying mechanisms of antioxidant and cytotoxic effects and to isolate the active compound (s) responsible for these pharmacological activities which can result in development of a novel compound for drug discovery.

ACKNOWLEDGEMENTS

We are grateful to the authority of International Islamic University of Chittagong (IIUC) as well as

Mohammed Aktar Sayeed, Assistant Professor and Head, Department of Pharmacy, IIUC for providing logistic support and encouragement to carry out this research.

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