Advanced Pharmaceutical Bulletin, **2013**, 3(2), 465-467
doi: http://dx.doi.org/10.5681/apb.2013.077
http://apb.tbzmed.ac.ir/

Investigation of Phenolic Profiles, Cytotoxic Potential and Phytochemical Screening of Different Extracts of *Drynaria quercifolia* J. Smith (Leaves)

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ARTICLEINFO

Article Type:
Short Communication

Article History:
Received: 24 March 2013
Revised: 4 May 2013
Accepted: 8 May 2013
ePublished: 20 August 2013

Keywords:
Folin-Ciocalteau
Phytochemical properties
Brine shrimp

ABSTRACT

Purpose: The present study is aimed to evaluate phenolic profiles, cytotoxic activity and phytochemical screening of different extracts of *Drynaria quercifolia* leaves. ***Methods:*** The dried and powder leaves were extracted with methanol at room temperature and the concentrated methanolic extract was fractionated by the modified Kupchan partitioning method to provide pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions. Phenolic profiles were determined by using Folin-Ciocalteau reagent, which results were expressed in gallic acid equivalent (mg of GAE/g of sample). Phytochemical properties of different extractives of plant materials were tested by the method of Trease and Evans. Brine shrimp lethality bioassay was used to investigate the cytotoxic potential of *D. quercifolia*. ***Results:*** The phytochemical screening revealed the potent source of different phytochemical constituents on different extractives including alkaloid, glycosides, tannin, saponins, proteins and amino acids, flavonoids, triterpenes, phenols, phytosterols and carbohydrate. In the determination of phenolic profiles, different extractives showed a significant content of phenolic compounds ranging from 103.43 -132.23 mg of GAE/g of extractive. Compared to vincristine sulfate different extractives of plant materials demonstrated moderate cytotoxic potential (having LC_{50} of 12.45 µg/ml, 13.02 µg/ml 15.83 µg/ml, 14.95 µg/ml and 7.612 µg/ml, respectively). ***Conclusion:*** It is concluded from this study that *D. quercifolia* is an excellent source of phenolic content and phytoconstitutes as well as possesses moderate cytotoxic activity.

Introduction

From the beginning of human civilization, plants have beneficial activity in the treatment of human diseases. A survey exposes that about 80% of the world's inhabitant's problem is treated by medicinal herbal drug for their primary health care. Plants have long antiquity used in the management of malignancy. Several active constituents have been used in the management of progressive stages of numerous malignancies. There are various medicinal plants that possess anti-cancer activity in the Ayurvedic system of medicine.[1]

Auto-oxidation of lipids, as well as reactive nitrogen species (RNS) is the main cause of reactive oxygen species (ROS) in the forms of superoxide anions, hydroxyl radicals and hydrogen peroxide.[2] Generation of these excess ROS and RNS by Ultraviolet (UV) radiation, smoking and drug metabolisms are likely to damage several cellular components such as lipids, proteins, nucleic acids, and DNAs through the oxidation or nitration processes.[3] In addition, these reactive oxygen species cause inflammation or lesion on different organs and are associated with various degenerative diseases, including cancer, ageing, arteriosclerosis, and rheumatism.[4]

Drynaria quercifolia J. Smith belongs to family Polypodiaceae, locally known as Gurar, is a parasitic fern[5,6] that is usually distributed in Bangladesh, India and Thailand. The rhizomes of the plant possess antibacterial properties and are used traditionally for management of cough, tuberculosis and typhoid fever. ASEAN Centre for Biodiversity stated in their Checklist of Medicinal Plant in Southeast Asia that rhizome decoction or drink of *D. quercifolia* rhizome uses as antipyretic preparation.[7]

Materials and Methods

Collection and identification of plant material

The leaves of *D. quercifolia* were collected from Noakhali on September, 2012. After collection leaves were thoroughly washed with water and identified by expert of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. (Accession number: DACB 35489).

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Preparation, extraction and fractionation of plant material

Cold maceration technique was used for extraction. Powder of leaves (500 g) was soaked in 2500 ml of methanol for about 10 days at room temperature with occasional stirring. After 10 days the solutions were filtered using filter cloth and Whatman's filter paper which was concentrated by evaporating under ceiling fan and in a water- bath not exceeding 40 °C to have gummy concentrate of extract.

The concentrated methanol extracts were separately partitioned by the modified Kupchan method[8] using pet-ether, carbon tetrachloride, and chloroform. The aqueous methanolic fraction was preserved as aqueous fraction.

Phytochemical screening

Phytochemical properties of different extractives of plant materials were tested using the method of Trease and Evans.[9]

Determination of phenolic profiles

Phenolic profiles of *D. quercifolia* extractives were measured employing the method[10] involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard.[11] Different Gallic acid solution were prepared having a concentration ranging from 50 µg/ml to 6.25 µg/ml. 2.5 ml of Folin-Ciocalteau reagent (diluted 10 times with water) and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution was added to 0.5 ml of Gallic acid solution. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of phenolic profiles of the test samples. In 0.5 ml of extract solution (conc. 2 mg/ml), 2.5 ml of Folin-Ciocalteu reagent and 2.0 ml of Na_2CO_3 (7.5 % w/v) solution were added. The mixture was incubated for 20 minutes at room temperature. After 20 minutes the absorbance was measured at 760 nm by UV-spectrophotometer and using the standard curve prepared from gallic acid solution with different concentration, phenolic profiles of the sample was measured and results were expressed as mg of GAE (gallic acid equivalent) / gm of the extractive.

Cytotoxic activity

The brine shrimp lethality bioassay was used to predict the cytotoxic potential[12,13] of different extractives of *D. quercifolia* leaves. For the experiment, 4 mg of each of the extract was dissolved in dimethylsulfoxide (DMSO) and solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 µg/ ml) were obtained by the serial dilution technique using simulated sea water. The solutions were then added to the pre-marked vials containing 10 live brine shrimp nauplii in 5 ml simulated seawater. After 24 hour 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 lethality of the brine shrimp nauplii for each concentration and control was calculated. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted and the values of LC_{50} were calculated using Microsoft Excel 2010. Vincristine Sulphate was used as positive control.

Results

Phytochemical screening

The preliminary phytochemical investigation showed the presence of phytochemical constituents such as alkaloid, glycosides, tannin, saponins, proteins and amino acids, flavonoids, triterpenes, phenols, phytosterols and carbohydrate but absence of fats & fixed oils and gum and mucilages in different extractives (Table 1). Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of com-pounds that act as primary antioxidants or free radical scavengers.[14]

Table 1. Results of phytochemical screening of different extractives of *D. quercifolia.*

Group of phytoconstituents	MEF	CTSF	CSF	PTSF	AQSF
Alkaloids	+	+	+	+	+
Carbohydrates	+	+	+	+	+
Glycosides	-	+	-	-	-
Saponins	+	-	-	+	+
Phytosterols	-	+	+	+	-
Phenols	+	+	+	+	+
Tannins	-	+	+	+	+
Flavonoids	+	-	+	+	+
Proteins and amino acids	-	+	+	-	-
Fats & fixed oils	-	-	-	-	-
Gum and mucilages	-	-	-	-	-
Triterpenes	-	+	+	+	+

Here, MEF= Methanolic extract fraction, CTSF= Carbon tetrachloride soluble fraction, CSF=Chloroform soluble fraction, PTSF= Petroleum ether soluble fraction, AQSF= Aqueous soluble fraction

Determination of phenolic profiles

Phenolic profiles of different extractives of *Drynaria quercifolia* leaves were found to contain the significant amount of phenols (Table 2). Different extractive possesses phenolic content ranging from 103.43-132.23 mg of GAE /g of extractive.

Table 2. Phenolic profiles of different extractives of *Drynaria quercifolia.*

sample	Total phenol content (mg/g, in GAE)
MEF	125.84 ± 0.23
CTSF	103.43 ± 0.33
CSF	129.41± 0.56
PTSF	116.57 ± 0.43
AQSF	132.23 ± 0.63

Cytotoxic activity

Compared to vincristine sulfate (with LC_{50} of 0.839 µg/ml), the LC_{50} values of crude methanolic extract, chloroform, carbon tetrachloride, pet-ether and aqueous soluble fractions of *D. quercifolia* leaves were found to be 12.45, 14.95, 13.02, 15.83and 7.612 µg/ml, respectively (Table 3). Therefore, the obtained result tends to suggest that plant extracts of *D. quercifolia* leaves possesses moderate cytotoxic activity.

Table 3. Cytotoxic potential of different extractives of *Drynaria quercifolia* along with Vincristine Sulphate.

Sample	LC_{50} (µg/ml)	Regression Equation	R^2
VS	0.839	y = 34.02x + 52.58	R^2 = 0.9521
MEF	12.45	y = 39.46x+6.776	R^2 =0.921
CSF	14.95	y = 41.27x+1.515	R^2 = 0.922
CTSF	13.02	y = 37.64x+8.036	R^2 =0.909
PTSF	15.83	y = 40.26x+1.771	R^2 = 0.935
AQSF	7.612	y = 19.126 +33.141	R^2 = 0.973

Here, VS= Vincristine Sulphate

Discussion

There is a growing interest in the investigation of natural antioxidant compounds from plants, since they contain secondary metabolites with structural diversity.[15] Literature revealed that antioxidant activity of plant extract is mainly due to presence of phenolic compounds.[14] Different extract of *D. quercifolia* leaves were found to contain the significant amount of phenols that suggest our investigated plant may be a potent source of antioxidant activity.

In case of cytotoxicity, the degree of lethality shown by the extractives was found to be directly proportional to the concentration of the extractives ranging from the lowest concentration (0.781 µg/ml) to the highest concentration (400 µg/ml). This concentration dependent increment in percent mortality of brine shrimp nauplii produced by the *D. quercifolia* leaves extracts indicate the presence of moderate cytotoxic principles in this extractives.

Conclusion

Through our study it can be concluded that, different extracts of *D. quercifolia* leaves possesses good phenolic profiles and moderate cytotoxic activity. Phytochemical screening of different extracts is also good source of different phytoconstitutes which suggest investigating other biological activities.

Conflict of Interest

No conflict of interest has been declared.

Acknowledgements

Authors are thankful to Bangladesh National Herbarium to identify the plant. Authors are also grateful to all the teachers and staffs of the Department of Pharmacy, Noakhali Science and Technology University for their support and co-operation.

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