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CHEMICAL AND BIOLOGICAL POTENTIALITIES OF THE ETHYL ACETATE EXTRACTS OF *DRYNARIA QUERCIFOLIA* TUBER AND LEAF

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Abstract

The ethyl acetate extracts of *Drynaria quercifolia* tubers and leaves were studied for their chemical and biological potentialities. The tuber extract was enriched with more reducing power compared to the leaf extract. But, the phenolic content was higher in the leaf extract compared to the tuber extracts. However, both the extracts were comparably cytotoxic and showed broad-spectrum antimicrobial activity. The antimicrobial compound in the tuber extract was nonpolar. The findings suggested that the ethyl acetate extracts of *D. quercifolia* may have active principles for the development of promising phyto-therapeutics.

Introduction

Herbal medicines have been used to treat different diseases since ancient times(1). Medicinal plants are a subject of research interest due to long folkloric use with minimum or no side-effects(2). A large number of different medicinal herbs and plants have been investigated for their antioxidant and antibacterial activities(3). Plants have been in focus of most researches because a number of biologically and medicinally important compounds had been isolated from different plants. With the increase in frequency of life threatening infections, researchers are exploring different plant extracts for the treatment of many diseases(4). Although a number of flowering plants have been investigated for their biological activities and chemical constituents, other medicinal plants remained almost unnoticed.

Drynaria quercifolia, commonly known as the oak-leaf fern, is an epiphyte with rhizome and deeply pinnatifid foliage fronds. This plant is native to Asian continent and grows widely in Bangladesh(5). This plant had been used ethnomedicinally to get relief from the symptoms of different diseases, and a few studies showed the antibacterial, antipyretic, anti-helminthic activities, thrombolytic potentials of the crude tuber extracts. Some compounds like naringin, epifriedelinol and friedelin have been isolated from this plant(6).

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In the present investigation, the chemical and biological activities of ethyl acetate extracts of *D. quercifolia* tuber and leaf were studied with the intention of isolating active principles.

Materials and Methods

Drynaria quercifolia plants were collected from the University of Dhaka campus between May and June, 2012 and was identified by Bangladesh National Herbarium (accession number 37592). Tuber and leaf samples were separated from the plant and were dried under shadow for 10 days. Then the samples were crushed into powder using mechanical grinder. One hundred and fifty g of dried leaf powder and 700 g of dried tuber powder was soaked in 600 ml or 2,800 ml of ethyl acetate (ACS grade, BDH, USA), respectively, for 5 days with gentle shaking. The extracts were collected as filtrate through Whatman filter paper (Grade 4) and evaporated at low temperature (45ºC) through rotary evaporator under reduced pressure[7]. Each extract was examined physically and the weight was measured. On an average, 1.8 g (0.26% w/w) of tuber extract and 4.2 g (2.8% w/w) leaf extract was obtained. Total reducing power of the extracts was determined as ascorbic acid equivalence (AAE)[8]. One hundred μg extract was dissolved in dimethyl sufoxide (DMSO) and mixed up to 2 ml with 0.2M phosphate buffer (pH 6.6). Two ml of 1% (w/v) $K_3Fe(CN)_6$ (Sigma, USA) was added and incubated at 50ºC for 40 minutes. The reaction was stopped by adding 2 ml of 10% (w/v) tricholoroacetic acid (Sigma, USA), mixed well and centrifuged at 3000 rpm for 10 minutes. Then, 2.5 ml deionized distilled water was mixed with 2 ml of the supernatant and 0.4 ml of 0.1% (w/v) aqueous solution of iron(III) chloride. After mixing well, absorbance was measured at 700 nm using spectrophotometer. Ascorbic acid of 0 - 100 μg used as standard positive control and a blank DMSO was used as negative control.

Total phenolic content of the extracts were measured as gallic acid equivalence (GAE) by Folin-Ciocalteu method with appropriate control[8]. One hundred μg extract was dissolved in DMSO and mixed with deionized distilled water up to 2 ml. The solution was treated with 200 μl Folin-Ciocalteu reagent (Sigma, USA) and was incubated for 5 minutes at room temperature. Then, 2 ml of 7% (w/v) sodium carbonate was added and mixed well. Finally 800 μl of deionized distilled water was added, incubated for 90 minutes at room temperature and then absorbance was measured at 750 nm using spectrophotometer. Each experiment was performed in triplicate.

Cytotoxic property of the extracts was evaluated on brine shrimp (*Artemia salina*) nauplii according to the Meyer protocol with some modification[7]. Simply, shrimp eggs were collected from the local market and were hatched in simulated sea water. The extract sample was dissolved in DMSO and diluted in saline to obtain final concentration ranging from 0 - 100 μg/ml during the experiment. Vincristine sulphate was used as a positive control. For each sample, minimum 10 living nauplii were taken in triplicate.

Then the nauplii were kept in dark at room temperature for 24 hours and numbers of dead and living nauplii were counted. Then the per cent mortality and correlation between concentration and mortality was analyzed to calculate the LC_{50} values. Antimicrobial activity was performed by agar-well diffusion method[7]. *Candida albicans* and five multi-drug resistant bacterial strains were used. The bacterial strains were *Staphylococcus saprophyticus* and *S. aureus*, *E. coli* DH-5α, *E. coli* pUC19, and *E. coli* pGLO. The zone of inhibition was measured in mm.

Thin-layer chromatography (TLC) was used to separate the active compound responsible for the antimicrobial activity of tuber extract [9]. Tuber extract was dissolved in 50 ml deionized distilled water. Fifty ml ethyl acetate was added to the solution and mixed well to obtain an aqueous fraction and an ethyl acetate soluble fraction and the fractions were separated using a separating funnel. The ethyl acetate soluble fraction was spotted on analytical TLC plate and compounds were separated with ethyl acetate: petroleum ether (1 : 9) solvent system. Then the plate was observed under UV light (254 nm). A single band collected from the plate was diffused overnight at 4ºC on a Muller-Hinton agar plate inoculated with bacteria. Then the agar plate was incubated at 37ºC to evaluate the antimicrobial activity of the compound found on the TLC plate. All the experiments were performed in triplicate and the data were analyzed using Microsoft Excel at 95% confidence interval.

Results and Discussion

Plant extracts and compounds obtained by direct extraction from a native producer or by semi-synthesis are now considered as biotechnology medicine[10]. Medicinal application of *Drynaria quercifolia* has been described in ethnomedicine[11]. Thereby, activities of the ethyl acetate extracts of this plant were evaluated in this study. The colour of leaf extract was dark green and the nature was light sticky or oily. On the other hand, the tuber extract was dark brown in colour and sticky in nature. Both the extracts had pungent odour and were bitter in taste.

Using ascorbic acid standard, total reducing power of each extracts were evaluated as ascorbic acid extract (AAE). AAE for tuber extract and leaf extract was 7.08 ± 0.75 and 4.84 ± 0.48 mg ascorbic acid per gram of each extracts, respectively indicating that the tuber extract had significantly more total reducing power (p value = 0.012) than that of the leaf extract. The estimated total phenolic content of the tuber extract and the leaf extract was 1.98 ± 0.62 and 10.35 ± 1.53 mg gallic acid equivalent (GAE) per gram of extract, respectively (Table 1). These results suggested that leaf extract had significantly higher phenolics ($p = 0.001$) compared to the tuber extract.

Brine shrimp lethality bioassay was performed to determine the LC_{50} value of individual extract[12]. The LC_{50} value of tuber extract was 53.33 ± 1.57 µg/ml, whereas the same value for leaf extract was 44.68 ± 1.47 µg/ml. The difference in cytotoxicity between

these extracts was not significant enough (p = 0.12). For both the extracts, there was a strong linear correlation between the concentration and the percentage of mortality (R^2 for the tuber extract = 0.996 and for the leaf extract = 0.979) at 95% confidence interval. The LC_{50} value of the positive control was similar as published before[(7)]. These data suggested that both extracts were enriched with bioactive compounds.

Table 1. Reducing power and phenolic content of the extracts of *Drynaria quercifolia*.

Type of extract	Total reducing power[a]	Phenolic content[b]
Ethyl acetate tuber extract	7.08 ± 0.75	1.98 ± 0.62
Ethyl acetate leaf extract	4.84 ± 0.48	10.35 ± 1.53
p-value	0.012	0.001

[a]mg ascorbic acid equivalence per g of extract ± Sd, and [b]gallic acid equivalence per g ± Sd.

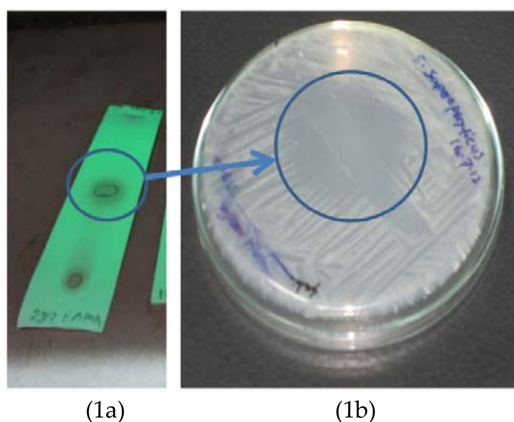
When 100 μg/μl extract was applied, both the extracts showed significant antimicrobial activity against *E. coli* (pGLO), *E. coli* (DH-5α), *S. saprophyticus* and *C. albicans* (Table 2). However, the tuber extract was also active against *E. coli* (pUC19) but the leaf extract did not possess activity against this strain. Both the extracts did not show any activity against *S. aureus* (Table 2).

Table 2. Antimicrobial activities of the extracts of *Drynaria quercifolia*.

Name of bacteria	Inhibition zone (mm ± Sd)		
	Tuber extract (μg/μl)	Leaf extract (μg/μl)	
	100	10	100
Candia albicans	23 ± 1	10 ± 1	35 ± 1
Escherichia coli (DH-5μ)	23 ± 1	15 ± 1	45 ± 1
Escherichia coli (pGLO)	15 ± 1	-	20 ± 1
Escherichia coli (pUC19)	26 ± 1	-	-
Staphylococcus saprophyticus	25 ± 1	14 ± 1	45 ± 1
Staphylococcus aureus	-	-	-

Furthermore, at 10 μg/μl concentration, tuber extract completely failed to show any antimicrobial activity but not the leaf extract. The leaf extract showed antimicrobial activity against *E. coli* (DH-5μ), *S. saprophyticus*, and *C. albicans*. Such findings indicated that the minimum inhibitory concentrations (MIC) of the leaf extract for these microorganisms were 10 μg/μl, otherwise the MIC values were 100 μg/μl for all the sensitive microorganisms. The highest diameter of zone of inhibition was observed for leaf extracts at 100 μg/μl against *E. coli* (DH-5μ) and *S. saprophyticus* (45 ± 1 mm). These results indicated that the leaf extract was more active against tested pathogenic microorganisms. However, the spectrum of the antimicrobial activity of the tuber extract

at 100 μg/μl concentration was broader compared to the leaf extract at the same concentration. Thereby, it was hypothesized that the tuber extract may contain a very low concentration of highly potent antimicrobial compound(s). Hence, the tuber extract was further fractionated and was run on a TLC plate. A single band was observed when the compounds in this fraction were separated with ethyl acetate: petroleum ether (1 : 9) solvent system (Fig. 1a)



(1a) (1b)

Fig. 1a. TLC separation pattern of tuber extract. 1b. Bioautography of ethyl acetate extract of *Drynaria quercifolia* tubers on *Staphylococcus saprophyticus* after thin-layer chromatography (TLC).

When this band was diffused and tested for antimicrobial activity, a distinct clear zone was observed in accordance with the position of the band. Also, no clear zone was observed for rest of the TLC strips (Fig. 1b). Such finding indicated that the compound observed as a band was nonpolar and also possessed antimicrobial activity. This compound will be isolated, purified and identified using ^{1}HNMR spectroscopy later. From the above studies, it can be suggested that *D. quercifolia* may be a good source of folk medicine.

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