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Full Length Research Paper

Antibacterial activity and cytotoxicity of rhizomes of *Dryneria quercifolia*

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The different organic solvent extracts of roots of *Dryneria quercifolia* J. Smith were tested for their antibacterial activity and cytotoxicity. Chloroform and ethyl acetate extracts (150 μg/disc) showed good antibacterial activity as determined by disc-diffusion method against four Gram-positive and six Gram-negative bacteria than petroleum ether extract. The minimum inhibitory concentration (MIC) of each extract was determined by serial dilution technique against *Escherichia coli, Shigella dysenteriae, Salmonella typhi, Bacillus subtilis* and *Streptococcus-β-haemolyticus*. The MIC values ranged from 16 to 64 μg/ml. The cytotoxicity of each extract was assessed by brine shrimp lethality bioassay and LC_{50} values were determined. The LC_{50} values for petroleum, chloroform and ethyl acetate extracts were 22.0, 16.5 and 16.5 μg/ml, respectively. The positive results of these bioactive extracts should encourage the further investigation of the active principles responsible for these activities.

Key words: *Dryneria quercifolia,* antibacterial activity, minimum inhibitory concentration, cytotoxicity.

INTRODUCTION

Drynaria quercifolia J. Smith (Polypodiaceae) is a fern, it has highly differentiated normal and basal fronds (foliage and nest leaves). The nest leaves are up to 40 cm long, very broad, lobed, and narrowed at the base. The normal, fertile leaves are very large, to about 100 cm long, and deeply lobed almost to the central vein. The sori are in a regular row on each side of a main secondary vein (Kirtikar and Basu, 1980). It is locally known as Garur and is widely distributed in tropical and subtropical countries including Bangladesh, India, Pakistan, North America and Africa (Kirtikar and Basu, 1980; Hasan and Hoque, 1993). The rhizomes of this plant used as a folk medicine in the treatment of tuberculosis, loss of appetite, cough, alopecia and scarlet fever (Kirtikar and Basu, 1980). However, phytochemical studies on the rhizomes of *D quercifolia* revealed that it contained a number of compounds as friedlin, epifriedelion, β-amyrin, β-sitosterol-3-β-D-glucopyranoside and naringin (Ramesh et al., 2001; Anujaa et al., 2010). Preliminary screening on the rhizome of this plant also shows the positive test for sterol and polyphenol type compounds. To determine the potential medicinal use of plants it is important to screen them for activity against a wide range of pathogenic bacteria. The reputed medicinal properties of this plant prompted us to screen the root extracts for activity against a wide range of pathogenic bacteria as well as to determine their toxicity level. Bioactive extracts and compounds are almost toxic in high doses, thus *in vivo* lethality in a simple zoologic organism can be used as a convenient monitor for screening of bioactive botanicals (Mclaughlin et al., 1998). Brine shrimp lethality bioassay is a recent development in the toxicity study for natural products. The cytotoxicity, which is related with a number of pharmacological activity such as anticancer, antiviral, insecticidal, pesticidal etc (Mclaughlin et al., 1998). Therefore the present work was carried out to evaluate the antibacterial properties of different solvent extracts of rhizomes *D. quercifolia* and to determine their toxic effect on brine shrimp nauplii. To date no such studies on the rhizomes of this plant appear in the literature.

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MATERIALS AND METHODS

Plant materials

The fresh rhizomes of *D. quercifolia* are collected in the month of October to November 2008 was taxonomically identified by professor A.T.M. Naderuzzaman, Department of Botany, University of Rajshahi and its herbarium voucher specimen (H-136) has been deposited in the Botany Unit of the University of Rajshahi, Bangladesh.

Extractions

The dried rhizomes were first extracted with ethanol (95%) and the extract was evaporated to dryness. the yield was 6.5%. The dried extract were treated with pet-ether, followed by chloroform, ethyl acetate and finally with methanol. All the extracts were evaporated to dryness and subjected to cytotoxicity assay and antibacterial activity. The yield was 2, 1, 1.2 and 1.6% on dried basis for pet-ether, chloroform, ethyl acetate and methanol extracts, respectively.

Antibacterial screening

The microorganisms used in this study included four Gram-positive bacteria for example, *Bacillus subtilis* (QL-40), *Bacillus megaterium* (QL-38), *Staphylococcus aureus* (ATCC-259233), and *Staphylococcus-β-haemolyticus* (CRL); six Gram-negative bacteria for example. *Escherichia coli* (FPFC-1407), *Shigella dysenteriae* (AL-35587), *Shigella sonneii* (AJ-8992), *Shigella flexneri* (AL-30372), *Pseudomonus aeruginosa* (CRL) and *Salmonella typhi*. All these cultures were obtained from the stock cultures of the Microbiology Laboratory of the Department of Microbiology, University Of Dhaka, Bangladesh. All bacterial strains were grown on nutrient agar. Subculturing was done once weekly. The microbial growth inhibitory potential of the extracts was determined by the standard disc-diffusion method (Baeur et al., 1966; Murray et al., 1995). Inocula was prepared by mixing few colonies with sterile 0.8% nutrient broth solution and comparing the turbidity with that of standard 0.5 McFarland solution, which is equivalent to 10^6 to 10^8 CFU/ml. The inoculum was added to molten agar and the media was thoroughly shaken to disperse the microorganisms. Extracts were dissolved in chloroform or methanol in such a manner so that each 25 µl solution contains 150 µg of plant extract. Filter paper discs (5 mm in diameter) for the disc diffusion method were prepared in-house were taken in a blank petridish and sterilized in oven at 110°C and 25 µl solution of plant extracts was placed on discs. All the discs are air-dried. For positive control, standard kanamycin (Sigma, USA) discs containing 50 µg/disc was used. All plates were left undisturbed, at room temperature for 2 h before incubation, to allow the diffusion process to take place. Then the plates were incubated at 37°C for 24 h for the growth of bacteria. All determinations were performed in triplicate. Zones of inhibition were measured in millimeter (mm).

Determination of minimal inhibitory concentration (MIC)

The MIC of the antibacterial agents against two Gram positive bacteria for example *B. subtilis* (QL-40) and *Staphylococcus-β-haemolyticus* (CRL); three Gram-negative bacteria for example *E. coli* (FPFC-1407), *S. dysenteriae* (AL-35587, *and S. typhi* was determined by serial dilution technique (Hammond and Lambert, 1987). The inocula prepared for antibacterial screening used for this study. Extracts were dissolved in 10% DMSO to make a concentration of 1.02 mg/ml for extracts. Twelve test tubes were taken for each experimental sample was marked as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. 1 ml of nutrient broth medium was poured to each of the twelve test tubes. 1 ml of sample solution was added to the first test tube and mixed well and then 1 ml of this content was transferred to the second test tube. This process of serial dilution was continued up to ninth test tube. 10 µl of properly diluted inoculum was added to each of the nine test tubes and mixed well. Rest of three test tubes (10, 11 and 12) were used as control for medium, medium + test samples and medium + inoculums respectively to confirm the clarity and sterility of the medium. All the test tubes were incubated at 37°C for 24 h for the growth of bacteria. All determinations were performed in triplicate. Finally, all the test tubes were checked for turbidity.

Brine shrimp lethality bioassay

This is a general bioassay that detects a variety of toxic substances and has been applied to plant extracts since 1982 (Meyer et al., 1982; McLaughlin, 1991; Solis et al., 1993). Usually, the results of this test correlate with cytotoxicity and pesticidal activity. The eggs of brine shrimp *Artemia salina* Leach was used in this bioassay. The protocol described by McLaughlin et al. (1998) was followed in our study. Brine shrimp eggs were acquired in a pet shop and hatched in seawater at room temperature. After 48 h matured shrimp as nauplii was collected and used for the experiment (McLaughlin et al., 1998). For sample preparation, extracts were dissolved in 10% DMSO in seawater to make a concentration of 5 µg/µl of extracts. The experiment was carried out into five groups for each sample. Each group consisted of three vials with 10 nauplii in 5 ml of seawater. A suspension with 10 shrimp in 5 ml was added to each vial containing test solution at different concentrations and the covered vial was placed under white light for 24 h at room temperature. The same assay procedure was carried out for the standard ampicillin trihydrate (Sigma, USA). Three vials contained 10 brine shrimp nauplii in 5 ml seawater with 20 µl DMSO were used for control group. After 24 h, the dead nauplii were counted using magnifying glass and recorded. The percent of mortality of brine shrimp nauplii was calculated at each concentration of sample. The LC_{50} (Median lethal concentration) values were determined in g/ml.

RESULTS AND DISCUSSION

Antibacterial activity

Antibacterial activities of pet-ether, chloroform and ethyl acetate extracts of the rhizomes of *D. quercifolia* are shown in Table 1. The results are expressed as mean ± standard deviation. The data were subjected to a one-way analysis of variance (ANOVA) and Tukey's test ($p < 0.05$) was performed to determine the significance of the difference between means. The extracts (150 µg/disc) showed comparatively lower activity ($p < 0.05$) than the standard kanamycin (50 µg/disc). When the results were taken into general consideration it was found that all the extracts showed wider range of activities against Gram-positive as well as Gram-negative bacteria. Chloroform and ethyl acetate extracts showed comparatively higher activity than petroleum ether extract. The MIC of the antibacterial extracts against two Gram positive bacteria for example, *B. subtilis* and *Staphylococcus-β-haemolyticus*; three Gram-negative bacteria for example, *E. coli, Shigella dysenteria,* and *S. typhi* were determined by serial dilution technique. The MIC for the chloroform

Table 1. Antibacterial activity of different organic solvent extracts of rhizomes of *D. quercifolia*.

Microorganisms	[a]Zone of Inhibition (mm ± S.D) (n = 3)			
	Pet-ether extract (150 µg/disc)	Chloroform extract (150 µg/disc)	Ethyl acetate extract (150 µg/disc)	Kanamycin (50 µg/disc)
Bacillus subtilis QL-40	8.7 ± 0.2	7.3 ± 0.6	19.3 ± 0.5	30. 6 ± 0.3
Bacillus megaterium QL-38	8.3 ± 0.3	20.1 ± 0.2	21.6 ± 0.5	29.1 ± 0.1
Staphylococcus aureus ATCC-259233	10.1 ± 0.3	24.2 ± 0.3	24.7 ± 0.2	37.1 ± 0.1
Streptococcus-β-haemolyticus CRL	7.2 ± 0.2	7.1 ± 0.3	24.5 ± 0.4	35.1 ± 0. 1
Escherichia coli FPFC-1407	8.2 ± 0.6	12.4 ± 0.5	19.8 ± 0.8	24.2 ± 0.2
Shigella dysenteriae AL-35587	12.2 ± 0.3	16.1 ± 0.2	21.6 ± 0.5	41.0 ± 0.1
Shigella sonneii AJ-8992	10 ± 0.4	11.1 ± 0.7	12.5 ± 0.5	40.1 ± 0.1
Shigella flexneri AL-30372	5.2 ± 0.7	16.5 ± 0.5	17.3 ± 0.4	35.1 ± 0.2
Salmonella typhi	0	11.1 ± 0.8	10.7 ± 0.4	35.2 ± 0.2
Pseudomonas aeruginosa CRL	7.2 ± 0.2	6.1 ± 0.7	28.5 ± 0.7	31.1 ± 0.2

Table 2. The minimal inhibitory concentration (MIC) of different organic solvent extracts of rhizomes of *D. quercifolia*.

Test organisms	Concentration of test sample (µg/ml)								
	Chloroform extract								
	512	256	128	64	32	16	8	4	2
Bacillus subtilis QL-40	-	-	-	-	-	+	+	+	+
Streptococcus-β-haemolyticus CRL	-	-	-	-	-	-	+	+	+
Escherichia coli FPFC-1407	-	-	-	-	-	+	+	+	+
Shigella dysenteriae AL-35587	-	-	-	-	-	-	+	+	+
Salmonella typhi	-	-	-	-	+	+	+	+	+
	Ethyl acetate extract								
Bacillus subtilis QL-40	-	-	-	-	-	+	+	+	+
Streptococcus-□-haemolyticus RL	-	-	-	-	-	-	+	+	+
Escherichia coli FPFC-1407	-	-	-	-	-	-	+	+	+
Shigella dysenteriae AL-35587	-	-	-	-	-	-	+	+	+
Salmonella typhi	-	-	-	-	+	+	+	+	+

extract were 32, 16, 64, 32 and 16 µg/ml; 16, 16, 64, 32 and 16 µg/ml were for ethyl acetate extracts against *E. coli, S. dysenteriae, S. typhi*, *B. subtilis* and *Staphylococcus-β-haemolyticus,* respectively are shown in Table 2. It appears that the different chemical fingerprints of the extracts

Table 3. LC_{50} values of extracts of rhizomes of *D. quercifolia* and standard ampicillin trihydrate on brine shrimp lethality bioassay.

Test sample	Concentration (µg/ml)	No. of applied shrimp in each vial	No. of nauplii dead in each vial			Mean mortality (%) (n =3)	*LC_{50} (µg/ml)	*b*	*r*
			1	2	3				
	5	10	0	1	0	3.3			
	10	10	2	3	1	20			
Pet-ether extract	20	10	3	5	4	40	22.0	57.06	0.98
	40	10	7	8	7	73.3			
	80	10	10	10	9	96.0			
	5	10	2	1	0	10			
	10	10	3	4	3	33.3			
Chloroform extract	20	10	6	6	6	60	16.5	39.082	0.99
	40	10	7	9	8	80			
	80	10	10	10	9	96.6			
	5	10	0	0	0	0			
	10	10	3	5	4	40			
Ethyl acetate extract	20	10	5	6	6	56.6	16.5	48.5	0.95
	40	10	9	10	8	90			
	80	10	9	10	10	96.6			
	5	10	3	2	3	26.6			
	10	10	5	5	4	46.6			
Ampicillin trihydrate	20	10	8	7	7	73.3	11.17	8.03	0.96
	40	10	9	8	7	80.0			
	80	10	9	9	10	93.3			

*LC_{50} (Median lethal concentration) values were calculated from regression lines where: x was log of tested sample concentration and y was percent mortality of nauplii; *b* = slope of the regression line; *r* = correlation coefficient.

contributed to their wide ranges of antibacterial activity.

These values are very encouraging to support subsequent studies on the identification of the antibacterial compounds that can be used as antimicrobial agents in new drugs for therapy of infectious diseases in human beings.

Brine shrimp lethality bioassay

The results of toxicity of experimental samples and standard ampicillin trihydrate on brine shrimp nauplii are shown in Table 3. The experimental and standard samples showed that different mortality rates at different concentrations and it was found that the mortality rate of brine shrimp nauplii was increased with the increase of the concentration of samples (Figure 1). So it was evident that extracts were lethal to the brine shrimp nauplii as well as biologically active. The LC_{50} values were calculated by regression analysis is presented in Table 3 (the slope and

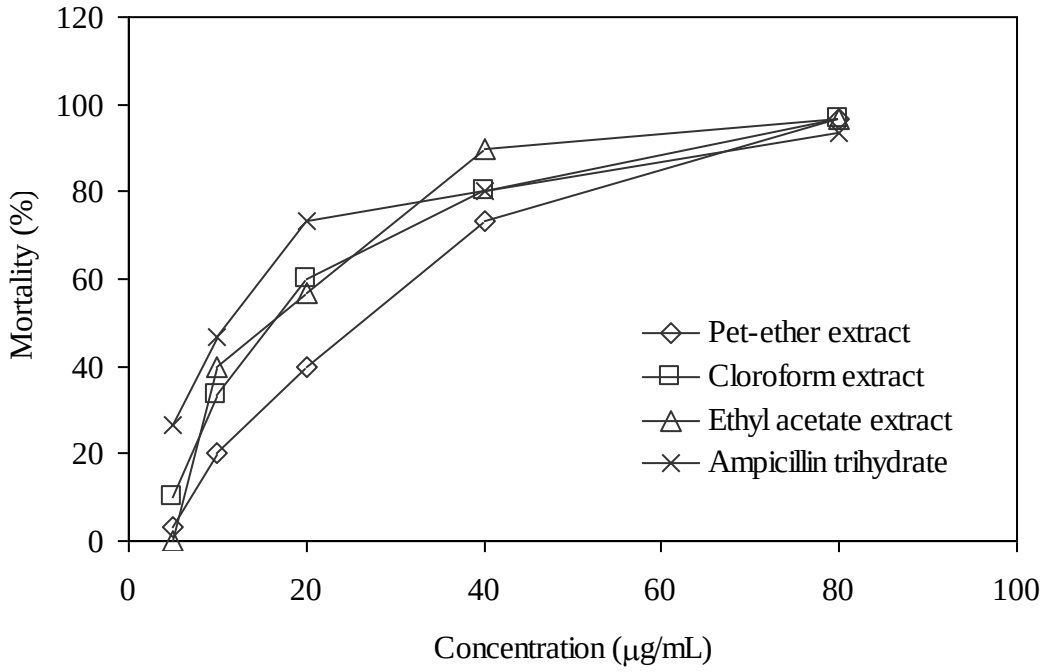

Figure 1. The effect of extracts of rhizomes of *D. quercifolia* and standard ampicillin trihydrate on the mortality of nauplii in brine shrimp lethality bioassay.

correlation of coefficient of the regression lines are also presented in the table). The LC_{50} values for pet-ether, chloroform and ethyl acetate extracts of the rhizomes of *D. quercifolia* and ampicillin trihydrate were found to be 22.0, 16.5, 16.5 and 11.7 µg/ml, respectively. The extracts were less toxic with higher LC_{50} values than ampicillin trihydrate. Amongst the extracts, chloroform and ethyl acetate extracts were more cytotoxic with lower LC_{50} value than pet ether extract. Preliminary phytochemical screening on these extracts revealed the presence of sterol and alkaloid type compound present in the pet ether and chloroform extracts and sterol, alkaloid and polyphenolic type compounds present in the ethyl acetate extracts. According to these results, there is a good probability that metabolites of these plants may have anticancer, antiviral, insecticidal or pesticidal activities.

This work has confirmed biological activities of different organic solvent extracts of roots of this plant. It is expected that these positive results will serve as a motivation for further examination of the active principles.

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