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Antibacterial, Phytochemical and Toxicity Studies of *Pteridium aquilinum* L. (Dennstaedtiaceae) in Rabbits

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Abstract: The aqueous and organic solvent leaves extracts of *Pteridium aquilinum* were screened for antibacterial activity by hole-in-plate bioassay procedure. The effect of aqueous leaves extract of the plant on hepatorenal functions in rabbits was also studied. Hexane (HX) fraction at 10 to 120 mg mL^{-1} did not show activity against *Escherichia coli* and *Pseudomonas aeruginosa*. However, it was significantly ($p<0.05$) active against *Staphylococcus aureus* at 90 and 120 mg mL^{-1}. The chloroform (CHL) extract fraction at concentrations of 50-120 mg mL^{-1} was significantly ($p<0.05$) active on all the bacterial species. Petroleum ether (PE) at 10-120 mg mL^{-1} showed significant ($p<0.05$) inhibition of *S. aureus* and *E. coli*. Aqueous (W) extract exhibited significant inhibitory activity at 50-120 mg mL^{-1} on *S. aureus* and *P. aeruginosa*. Tannins, anthraquinone glycosides, cardiac glycosides, cyanogenic glycosides and volatile oils were detected in the extracts. The lethal dose (LD_{50}) of the aqueous leaves extracts was found to be greater than 3000 mg kg^{-1} (p.o.) in rabbits. Non significant ($p>0.05$) and significant ($p<0.05$) changes in renal and liver indices, respectively were observed. Aqueous leaves extract of *Pteridium aquilinum* is toxic to the liver of rabbits only at 1500-3000 mg kg^{-1}. These results have provided scientific evidence to justify the indigenous use of the plant against infectious diseases.

Key words: *Pteridium aquilinum*, hepatorenal function, antibacterial activity, phytochemical analysis

INTRODUCTION

The growing interest in herbal medicine demands toxicity risk assessment of the various indigenous preparations used in the treatment of diseases (Yakubu *et al*., 2005). Moreover, the increasing use of plant extracts in the food, cosmetic and pharmaceutical industries suggest that, in order to find active compounds, a systematic study of medicinal plants is very important (Nostro *et al*., 2000). The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Lis-Balchin and Deans, 1996; Maoz and Neeman, 1998; Hammer *et al*., 1999). *Pteridium aquilinum* belongs to a species of fern with a cosmopolitan distribution, occurring in temperate and subtropical regions throughout much of the world. The leaves are bipinnate with conspicuously divided pinnules (Wikipedia Contributions, 2006). In northern Nigeria, the leaves of *Pteridium aquilinum* are used for the treatment of bacterial diseases. The young stems are used as vegetables but their consumption is linked to the highest incidence of stomach cancer rates in the world (Wikipedia Contributions, 2006).

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Bacterial organisms used in this research cause wound infection, diarrhea, pneumonia and chest pain. Alkaloids, glycosides, tannins, flavonoids, volatile oils, saponins and other phenolics have been reported to have antimicrobial activities (Hostettmann and Nakanishi, 1979; Hostettman *et al*., 1995; Isaac and Chinwe, 2001). The research was aimed at evaluating the antibacterial activity, phytochemical content and risk assessments of the plant extract.

MATERIALS AND METHODS

Chemicals

All the chemicals used were of analytical grade.

Collection of Plant and Authentication

The leaves of *Pteridium aquilinum* were collected from within Usmanu Danfodiyo University Campus, Sokoto, Nigeria. The leaves were botanically authenticated at the herbarium, Botany Unit of the same Institution where voucher specimens were kept. The leaves were room-dried and pulverized in to powder and the powdered parts were subjected to aqueous and organic solvent extraction (Matawalli *et al*., 2004).

Organisms

The species of bacterial organisms were *S. aureus*, *P. aeruginosa* and *E. coli*. They were clinical isolates obtained from Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. The cultures were maintained on nutrient agar slants at 4°C, re-identified by biochemical test (Cheesbrough, 1982) and sub-cultured in to nutrient broth for 24 h prior to testing.

Animals

Male rabbits weighing 1423.40 to 1503.20 g were purchased from Sokoto Central Market and certified healthy by a Veterinary Doctor. The animals were kept at the animal house in wire mesh cages, Department of Biological Science, Usmanu Danfodiyo University, Sokoto, Nigeria. They were maintained under veterinary supervision and were fed with pellet diet, seasonal vegetables and tap water *ad libitum* for one week to acclimatize them before starting the experiment.

Extraction and Fractionation Procedure

Fractionation of the extract was done by activity-guided fractionation using ethanol-water (1:1) and different (hexane, petroleum ether and chloroform) organic solvents. The powdered extracts of the leaves (40 g) were extracted with ethanol-water (1:1,500 mL) separately at room temperature overnight (Moris and Aziz, 1976; Springfield and Weitz, 2006). The extract was filtered and partitioned in hexane separately (250 mL) and clarified by further filtration. Evaporation of hexane fraction to dryness in an oven at 45°C yielded residue of 0.5% (w/w). The aqueous filtrate (ethanol-water) of the extract fraction was further partitioned (to obtain fractions of different polarities) with petroleum ether (250 mL) and chloroform separately (250 mL). Evaporation of the petroleum ether, chloroform (CHL) and the last remaining aqueous filtrate of leaves yielded residues of 0.75, 2.83 and 12.03% (w/w), respectively. A separate portion of powdered leaves (40 g) was extracted with 500mL distilled water at room temperature overnight and filtered. The filtrate was then evaporated to dryness yielding residue of 7.65% (w/w). All the residues obtained were reconstituted in sterilized distilled water and screened for antibacterial activity, phytochemical properties and toxicity studies. The above procedure enabled us obliterate the possible contributory antibacterial effect of the organic solvents.

Phytochemical Screening

This was carried out using standard procedure as described by Wall *et al*. (1954), Persinos and Quimby (1967), Harbone (1973), Trease and Evans (1978) and El-Olemyl *et al*. (1994).

Antibacterial Activity

The antibacterial activity was done by utilizing the hole-in-plate bioassay procedure as reported by Hugo and Russell (1983) and Vlietinck *et al*. (1995). Pure culture of the organisms were inoculated into Muller-Hinton nutrient broth (Oxoid, England), incubated for 24 h at 37°C, diluted with sterile nutrient broth to a density of 9×10^8 cfu mL^{-1} equivalent to MC-Farland test tube number 3. The suspension was used to streak for confluent growth on the surface of Muller-Hinton agar plates with sterile swab. Using a sterile cork-borer of 6 mm diameter, four holes were made in to the set agar in Petri dishes containing the bacterial culture. Concentrations of 10 to 120 mg mL^{-1} of the extracts were poured in to the wells. Tetracycline (10 mg mL^{-1}) a product of Greenfield Pharmaceutical (Jiang su) Co., Ltd. China, was used as reference or positive control. The plates were placed in the incubator at 37°C overnight. Antibacterial activity was recorded if the zone of inhibition was greater than 12 mm. The significance of the difference of the antibacterial activities of the extracts was tested by one-way analysis of variance (ANOVA).

Acute Toxicity Studies (Determination of LD50)

Water extract of *Pteridium aquilinum* (3000 mg kg^{-1} body weight) was administered to five groups of rats (one after the other at a grace observation period of 48 h) in a single oral dose by using a feeding needle. The control group received distilled water. Observations of toxic symptoms were made and recorded systematically at one, two, four and six hours after administration. Finally, the number of survivors was noted after 48 h for each animal. The toxicological effect was assessed on the basis of mortality, which was expressed as LD50 and was calculated by using the limit test dose, up and down procedure of Organization for Economic and Cultural Development (OECD) (2001).

Subacute Toxicity

A total of twenty-five rabbits, divided into the following groups: Group I (n = 4) was control group and received distilled water. Group II, III and IV (each of 5 animals) were orally administered (1 mL of 600, 1500 and 3000 mg kg^{-1}) aqueous leaves extract of *Pteridium aquilinum* once daily for 28 days, respectively.

Parameters of the Study

The following parameters were analyzed in all the animals during pretreatment and treatment phases.

Body Weight

The body weight of all the animals before and after 28 days of treatment was recorded.

Blood Samples and Clinical Chemistry

Animals were sacrificed and blood samples were collected, allowed to clot and centrifuged to obtain sera. The biochemical parameters, serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined using Randox assay kit by standard methods of Reitman and Frankel (1957). Alkaline phosphatase activity was estimated by the Randox kit (colorimetric) of Rec, (1972). Total bilirubin (Randox assay kit) was determined by the methods of Jendrassik and Grof (1938) and Sherlock (1951). Albumin (Bromocresol green) and urea (Diacetyl monoxime) were determined by the methods of Cheesbrough (1991) and Wybenga *et al*. (1971), respectively.

Electrolytes and creatinine (Colorimetric with deproteinization) were estimated by the methods of Uriyo and Singh (1974) and Henry (1974), respectively. Uric acid was by the method of Collins and Diehl (1959) and Morin and Prox (1973).

Statistical Analysis

Results are expressed as mean±standard error. The data collected in the study was subjected to one-way analysis of variance (ANOVA), Post Hoc test (multiple comparison) using least significant difference (LSD) and t-test. The level of $p<0.05$ was considered significant.

RESULTS

Antibacterial Activity of Aqueous Extract

The amount of residues obtained after extraction are presented in Table 1. Table 2 presents the antibacterial activity of aqueous (W), HX, PE and CHL extracts of *Pteridium aquilinum*. There was no activity against *E. coli* and *P. aeruginosa* with HX extract at 10 to 120 mg mL^{-1} but significant ($p<0.05$) activity on *S. aureus* at 90 to 120 mg mL^{-1}. Extract of CHL (at 50 to 120 mg mL^{-1}) was significantly ($p<0.05$) active on all the organisms. The PE extract inhibited *S.aureus* and *E. coli* significantly at 10 to 120 mg mL^{-1}. Crude water extract at 50 to 120 mg mL^{-1} showed significant ($p<0.05$) inhibitions of *S. aureus* and *P. aeruginosa*.

Table 1: Amount of residue obtained after extraction (40 g)

Leaves fractions	Amount recovered (%)
HX	0.5
PE	0.75
CHL	2.83
LR	12.03
W	7.65

HX = Hexane, PE = Petroleum ether, CHL = Chloroform, W = Water and LR= Last remaining Water -ethanol fractions

Table 2: Antibacterial activity of aqueous and organic solvent leaves extracts of *Pteridium aquilinum*

Fractions	Concentration (mg mL^{-1})	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonasaeruginosa*
HX	10. 00	-	-	-
	50.00	-	-	-
	90.00	14.25±0.28	-	-
	120.00	18.45±0.23*	-	-
PE	10.00	13.43±0.16	17.10±0.08*	-
	50.00	19.35±0.13*	23.55±0.21*	-
	90.00	23.00±0.82*	24.25±0.55*	-
	120.00	26.10±0.47*	25.30±0.13*	-
CHL	10.00	-	15.18±0.32	-
	50.00	13.24±0.18	17.00±0.47*	16.50±0.32*
	90.00	26.25±1.71*	21.22±0.09*	17.37±0.22*
	120.00	29.40±0.26*	23.25±0.98*	17.67±0.54*
LR	10.00	-	-	-
	50.00	-	-	-
	90.00	-	-	-
	120.00	13.25±0.29	13.75±0.29	-
W	10.00	-	-	-
	50.00	22.67± 0.53*	-	18.00±0.94*
	90.00	22.93±0.05*	-	19.37±0.13*
	120.00	23.75±0.55*	-	20.75±0.55*
Tetracycline	10	20.00±0.47*	25.5±1.20*	-
Water		-	-	-

HX = Hexane, PE = Petroleum ether, CHL = Chloroform, W = Water and LR= Last remaining Water -ethanol fractions, - = No activity, values greater than 12 mm indicated some activity. Zone of inhibitions is in mm, * = Significantly different from the control ($p<0.05$) by using analysis of variance ($n = 4$), Values are mean±standard error
Post Hoc test multiple comparison using least significant difference (LSD) and t-test

Phytochemical Screening

Tannins, glycosides, cyanogenic glycosides, anthraquinone glycosides, cardiac glycosides and volatile oils were detected in organic and aqueous extracts (Table 3).

Acute Toxicity Study and Behavioural Effects

Acute toxicity test at 3000 mg kg^{-1} of *Pteridium aquilinum* produced no mortality after 2 days of observation. The median lethal dosage (LD_{50}) of the aqueous leaves extract was greater than 3000 mg kg^{-1} body weight. Oral administrations of low and moderate doses (600 to 1500 mg kg^{-1}) produced no obvious toxicity. However, higher dose of 3000 mg kg^{-1} caused slow movement and clumping of the animal together at the corners of the cages.

Body Weight

There were no significant ($p<0.05$) changes in the body weights of the animals that tolerated fair amounts of *Pteridium aquilinum* extract compared with the control group (Table 4).

Subacute Toxicity (Biochemical Indices of Kidney and Liver Functions)

There was no significant ($p>0.05$) differences in all the indices of kidney function in the experimental rabbits compared with control group (Table 5). Indices of liver function (AST, ALT, TB, ALP and ALB) were significantly different ($p<0.05$) in animals administered 1500 and 3000 mg kg^{-1} of the aqueous extract compared with control (Table 6).

Table 3: Phytochemical screening of aqueous and organic solvent leaves extracts of *Pteridium aquilinum*

Fractions	ALK	SAP	TA	FL	VLO	GLY	CYG	CG	SAG	ATG	FG
HX	-	-	+	-	+	+	+	+	-	+	-
PE	-	-	+	-	+	+	+	+	-	+	-
CHL	-	-	+	-	+	+	+	+	-	+	-
LR	-	-	+	-	+	+	+	+	-	+	-
W	-	-	+	-	+	+	+	+	-	+	-

- = Absence, + = Presence, HX = Hexane, PE = Petroleum ether, CHL = Chloroform, LR = Last remaining Water -Ethanol extract, W = water, ALK = Alkaloids, SAP = Saponins, TA = Tannins, FL= Flavonoids, VLO = Volatile oil, GLY = Glycosides, CyG = Cyanogenic glycosides, CG = Cardiac glycosides, SAG = Saponin glycosides, ATG = Anthraquinone glycosides, FG =Flavonoid glycosides

Table 4: Body weight of rabbits before and after administration (28 days) of aqueous extract of *Pteridium aquilinum*

Dose (mg kg^{-1})	Weight before administration	Weight after 28 days of administration
600	1433.90±3.20	1431.98±3.120
1500	1514.50±16.32	1512.22±16.05
3000	1933.48±11.96	1930.48±11.61
Control	1529.00±9.6	1535.08±27.05

Values are mean± standard deviation. The values are not significantly different ($p>0.05$) by using student t-test

Table 5: Serum kidney function indices in rats administered aqueous leaves extract of *Pteridium aquilinum*

Dose (mg kg^{-1})	Sodium (mmol L^{-1})	Potassium (mmol L^{-1})	Bicarbonate (mmol L^{-1})	Urea (mmol L^{-1})	Uric acid (µmol L^{-1})	Creatinine (µmol L^{-1})
0 (Control)	157.60±4.00	4.40±0.37	25.11±2.08	5.56±1.15	4.93±0.89	1.26±0.38
600	141.00±5.00	4.70±0.20	23.50±0.50	4.70±0.28	5.90±2.40	0.90±0.20
1500	133.50±7.50	4.40±0.20	24.00±1.00	5.30±0.88	7.50±0.30	1.13±0.23
3000	144.50±2.50	5.00±0.15	23.50±3.50	5.25±1.55	7.40±1.50	1.19±0.29

Values are mean± standard error. All the values are not significantly ($p>0.05$) different from the control using analysis of variance Post Hoc test multiple comparison using least significant difference and t-test. (n = 4)

Table 6: Serum liver function indices in rats administered aqueous leaves extracts of *Pteridium aquilinum*

Concentration (mg kg^{-1})	AST (U L^{-1})	ALT (U L^{-1})	ALP (U L^{-1})	TB (µmol L^{-1})	ALB (g dL^{-1})
Control	17.00±1.000	35.30±0.67	153.10±0.76	7.00±0.43	4.83±0.07
600	20.50±0.41	36.50±0.75	155.00±1.240	8.50±0.29	4.40±0.21
1500	24.00±0.82*	47.00±0.82*	185.90±3.10*	13.35±0.21*	2.16±0.08*
3000	31.00±0.82*	54.00±1.06*	280.00±3.24*	17.90±0.490*	1.34±0.02

*Values are mean±standard error. * = Significantly ($p<0.05$) different from the control using analysis of variance. Post Hoc test multiple comparison using least significant difference and t-test. (n=4). ALT = Alanine transaminase, AST = Aspartate transaminase ALP = Alkaline phosphatase, ALB = Albumin and TB = Total bilirubin

DISCUSSION

In recent years, naturally occurring bioactive compounds from medicinal plants have been used as chemo-preventive agents to treat bacterial diseases without risk assessments. The findings of anti bacterial activity in the study present an easy *in vitro* system that can be used for assessing the antibacterial activities of plants. In this study, application of HX, PE, CHL and aqueous extracts of *Pteridium aquilinum* showed significant ($p<0.05$) inhibitory activity on some isolates used at 10 to 120 mg mL^{-1} (Table 2). The *in vitro* activities of crude plant extracts provide evidence to support the use of such plants (Wurochekke and Nok, 2004). The CHL extract was the most active at concentration of 50 to 120 mg mL^{-1}. Thus, different solvent extracts of some plant may have different pharmacological properties (Freiburghans *et al*., 1996). The results obtained suggest that water is not the most effective solvent for extracting the bioactive constituents from *Pteridium aquilinum*. However, water was the commonly used solvent by traditional healers to extract pharmacologically active compounds because of its easy availability (Shale *et al*., 1999). The result of CHL extract as the most active against the isolates contradicts this assertion. The non-significant activity of the last ethanol extract could be due to the bioassay-guided fractionation. The antibacterial potential of the leaves extract of *Pteridium aquilinum* has been elucidated by the result of this study. The antimicrobial properties of this plant may probably explain its traditional usage for treating bacterial diseases.

The presence of tannins, volatile oils, cardiac glycosides and anthraquinone glycosides, in the extracts has earlier been associated with antimicrobial activity (Hostettman and Nakanishi, 1979; Okwute and Hann, 1999). It is probable that the antibacterial agents in the extract of *Pteridium aquilinum* act by inhibition of nucleic acid, protein and membrane phospholipids biosynthesis (Franklin *et al*., 1989).

In the present investigation, the aqueous leaves extract of *Pteridium aquilinum* did not induce any significant ($p>0.005$) changes in kidney parameters. Thus, this finding provides evidence for the clinical safety of the plant. But significant increase in the liver indices implies possible necrotic injury of the liver and cholestatic disease (Speech and Liehr, 1983; Panteghini *et al*., 1984; Lott and Wolf, 1986). Elevation of ALP is an indication of cholestasis (Birkett *et al*., 1986; Van Hoof and De Broe, 1994). The significant ($p<0.05$) increase in TB and decrease in albumin indicates the altered liver excretory function (Weiss *et al*., 1983; Cheesbrough, 1991) and impaired synthetic function of the liver (Harold *et al*., 1980; Corless and Middleton, 1983; Cheesbrough, 1991). On the basis of this, *Pteridium aquilinum* may not be clinically safe and may be toxic to the liver as seen from the results. It is highly probable that the presence of tannins, cardiac glycosides, cyanogenic glycosides and anthraquinone glycoside in the leaves of *Pteridium aquilinum* may be responsible for the altered serum liver indices. The present research has shown the efficacy of the leaves extract of the plant used for the treatment of bacterial diseases. A chronic toxicity study would give a final answer on the safety of *Pteridium aquilinum* as an antibacterial agent. Further studies on isolation of the active antibacterial component (s) are worthwhile.

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