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Antimicrobial, Antipyretic, and Anti-Inflammatory Activities of Selected Philippine Medicinal Pteridophytes

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ABSTRACT

Pteridophytes are some of the herbal plants used to cure ailments. The present study scientifically finds out the folkloric therapeutic claims of 15 species of Philippine medicinal pteridophytes. Phytochemical components namely alkaloids, flavonoids, saponins, tannins, and anthraquinones of each plant samples were qualitatively determined. Antimicrobial activity of ethanolic extract

was tested on *Staphylococcus aureus, Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa*, and *Candida albicans*. The antipyretic activity using yeast-induced pyrexia and the anti-inflammatory activity using egg-albumin-induced paw edema were done on white rats. The results revealed the presence of alkaloids, saponins, flavonoids, and tannins in most of the extracts. Anthraquinones were absent. Extract form three species of pteridophytes namely: *Equisetum ramosissimum, Pyrrosia piloselloides,* and *Selaginella usterii* did not manifest any antibacterial activity. *Blechnum orientale* has no antifungal activity. All extracts showed significant effect in reducing yeast-induced pyrexia when compared with the negative control (NSS). Fourteen (14) out of the fifteen (15) plant extracts in 400 mg/kg Body Weight dosage inhibited anti-inflammatory activities in egg-albumin induced rat paw edema except for *Pyrossia piloselloides.* However, all plant extracts in 800 mg/kg Body Weight dosage showed significant rat paw reduction. A dose-dependent anti-inflammatory effect was observed within 6 hours of paw thickness observation.

Keywords - Phytochemicals, Ferns, Bioassay, Philippines

INTRODUCTION

Natural products, such as plant extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched chemical diversity they can provide (Cos et al. 2006). These compounds are significant in therapeutic application against pathogens, including bacteria, fungi and viruses (Khan et al. 2003). Some of these medicinal plants with reported medicinal value belong to the family of pteridophytes or ferns and their allies.

Pteridophytes are a group of plants commonly used as a source of medicine, food, ornamentals, as sources of fiber, bioremediation, and as organic fertilizer. Studies on the medicinal potentials of these plants have been very few as reflected from scarce literature. Indeed, out of 1,100 species only more than 50 species of these plants were reported to have medicinal values throughout the Philippines and 41 species of which can be found in Mindanao (Amoroso 2013).

In this study, selected pteridophytes with medicinal value were gathered, identified, screened for their phytochemical components and were evaluated for their antimicrobial, antipyretic, and anti-Inflammatory activities.

OBJECTIVES OF THE STUDY

This study was conducted to evaluate the folkloric therapeutic claims of some Philippine medicinal pteridophytes by determining their phytochemical and pharmacologic properties.

Specifically, it aimed to (1). find out qualitatively the presence of the phytochemicals on the ethanolic plant extracts; (2). determine if the ethanolic plant extracts have antifungal activity on *Candida albicans;* (3). determine if the ethanolic plant extracts have antibacterial activity on the Gram positive *Staphylococcus aureus* and *Bacillus subtilis* and on Gram negative *Escherichia coli* and *Pseudomonas aeruginosa;* and, (4) assess whether the ethanolic plant extracts of the pteridophytes have antipyretic and anti-inflammatory activities.

MATERIALS AND METHODS

A. Collection and Processing of Specimens

Fifteen (15) species of pteridophytes were collected in the campus of Notre Dame of Marbel University (NDMU), City of Koronadal, Mt. Salumay, Marilog District, Davao City and Mt. Kiamo in Malaybalay, Bukidnon (Plate I). These were wrapped individually in clean plastic bags and were brought to Central Mindanao University, Musuan, Bukidnon for identification, confirmation, and preparation of herbarium specimens. Fresh, free from insect bites, and intact pteridophyte fronds were collected and brought to NDMU for the biological assay.

Ethanol Extraction:

Fronds of the plants were cleaned and two hundred grams 200 g fresh weights of each plant were weighed separately. These were then separately chopped into small pieces and were placed in conical flasks. Ninety-five percent ethanol was added to each flask until the plant parts were completely submerged. The plant materials were soaked in ethanol for 24 hours. Filtration was done on each sample and the filtrates were concentrated in a rotary evaporator at a temperature of 45°C. The concentrates of each fern fronds were measured and stored in properly labeled vials. The concentration of the extract was computed and expressed as

grams fern frond per mL of extract. The concentrated ethanolic crude extracts were then stored in the refrigerator to prevent the growth of microorganisms.

B. Phytochemical Analysis

Phytochemical screening of the plant extracts was carried out as per the methods described by Guevarra (2005).

Test for Alkaloids

An equivalent of 20 g plant material from the stock plant extract was placed in an evaporating dish. This was evaporated to a syrupy consistency over a steam bath. Five (5) mL of 2M hydrochloric acid (HCl) was added and was stirred for about 5 minutes and was allowed to cool. One mL of the filtrate was tested with 2 to 3 drops of Dragendorff's reagent. Another 1 mL of the filtrate was tested with 2 to 3 drops of Meyer's Reagent. The relative amount of precipitation was observed as follows: (+) slight turbidity; (++) definite turbidity; and (+++) heavy precipitation. Aside from the above test, the following were conducted:

a. Test for quarternary bases and/or amine oxide
b. Test for Saponins (Froth Test)
c. Test for Flavonoids
d. Test for leucoanthocyanins by Bate-Smith and Metcalf method
e. Test for γ-benzopyrone nucleus: Wilstatter "cyanidin" test
f. Test for Tannins
g. Gelatin Test
h. Ferric Chloride Test
i. Test for Anthraquinones (Borntrager's Test)

C. Antimicrobial Assay by Agar Well Diffusion Method

The antimicrobial testing was carried out using the methods described by Guevarra (2005).

a. Preparation of Liquid Media for the Inoculum
b. Sterilization Techniques
c. Inoculation of Test organisms

d. Preparation of Base Plate and Top Agar
e. Preparation of Treatments
f. Delivery of the extract into the Agar wells and determination of equivalent antibiotic concentration

D. *In-vivo* Antipyretic and Anti-inflammatory Screening

D1. Preparation of Test Animals

Mature male albino rats weighing 80-115 g body weight (BW) were used. The animals were housed in steel cages with wooden frames under standard and hygienic conditions as they were acclimatized to laboratory conditions 7 weeks prior to experimentation. Prior to treatment administration, they were made to fast for 16 hours with water *ad libitum*. Rats were weighed using a digital balance and were numbered in comparable groups according to the treatment they were subjected to. The animal assays were carried out following the principles of good laboratory practice and animal handling (National Institutes of Health Guide for the Care and use for Laboratory animals; Publication No. 85-23, revised 1985).

D2. Test for Anti-pyretic Activity

The anti-pyretic activity of the ethanolic frond extract of the selected pteridophytes was carried out using the methods modified from Al-Ghamdi (2001).

One hundred fifty male albino rats were individually weighed and the baseline body temperature of each rat was taken by inserting a flexible digital thermometer into the rectum for about 2 minutes. The steady temperature readings obtained were recorded as the pre-treatment or baseline body temperatures. Pyrexia was induced in rats by the administration of 1mL/kg BW of 15% baker's yeast suspension intraperitoneally (i.p.). Nineteen hrs after yeast administration, the rectal temperatures were remeasured. Rats that did not show a minimum increase of 0.5^0C were eliminated from the study. Eighty-five rats with fever were divided into 17 groups of 5 rats each. Each of the 15 groups received immediately 800 mg/kg of the ethanolic frond extract dissolved in Normal Saline Solution (NSS). One group received NSS and the last group received 150 mg/kg paracetamol. All treatments were administered intraperitoneally.

D3. Test for Anti-inflammatory Activity (Egg albumin-induced rat paw edema)

The method used in screening for the anti-inflammatory potential of the extracts is a modification of the protocol employed by Winter et al. (1962).

One hundred seventy male albino rats (80-110 grams) were divided into groups of 10 rats. Four hundred mg/kg BW and 800 mg/kg BW of each ethanolic frond extract were injected intraperitoneally to rats. Diclofenac sodium 50mg/kg BW was used as standard anti-inflammatory drug for extract comparison. NSS served as the negative control. Ethanolic frond extracts were injected one hour prior to egg-albumin injection.

Egg-albumin edema was induced by injecting subcutaneously (s.c.) 0.1 mL of egg albumin (Salawu et al. 2008) into the plantar region of the right hind paws of the rats. Two hours after albumin administration, the thickness of the right paw was measured with a Vernier caliper. This was repeated after 4 hours and 6 hours.

The percentage increase in paw thickness every 2 hours was also calculated. The percentage inhibition of edema was calculated for each extract using the formula by Perez (1986).

% Inhibition = 100 (1- (a-x/b-y)).

Where: a = mean paw volume of treated animals after egg albumin injection
x = mean paw volume of treated animals before egg albumin injection
b = mean paw volume of control animals after egg albumin injection
y = mean paw volume of control animals before egg albumin injection

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventing properties for the plant. Fifteen (15) Philippine medicinal pteridophytes viz., *Adiantum philippense* Linn., *Asplenium nidus* Linn., *Blechnum orientale* Linn., *Cyathea contaminans* (Hook.) Copel., *Dicranopteris linearis* (Burm.) Underw., *Diplazium esculentum* (Retz) Sw., *Drynaria quercifolia* (Linn.) J. Sm., *Equisetum ramosissimum* (Roxb.) Hauke, *Lygodium microphyllum* (Cav.) R. Br., *Microsorum punctatum* (Linn.) Copel., *Nephrolepis cordifolia* (Linn.) Presl., *Oleandra pistillaris* (Sw.) C. Chr., *Pteridium aquilinum* (Linn.) Kuhn,

Pyrrosia piloselloides (Linn.) Price, and *Selaginella usterii* Linn. were subjected to phytochemical screening following the procedure of Guevarra (2005). Five phytochemicals were qualitatively evaluated namely: alkaloids, saponins, flavonoids, tannins, and anthraquinones (Appendix 1).

Alkaloids

The results (Table 1) showed that except for *Selaginella usterii,* fourteen (14) pteridophyte ethanolic extracts have detectable alkaloids of either primary, secondary, and/or quarternary and/or amine oxides when tested with Meyer's and Dragendorff's reagent and only *Selaginella usterii* does not have detectable alkaloids by the method. Alkaloids generally have been noted for their antimalarial and antibacterial activities although it seems their mechanism of action on microbes remains unclear (Raghavendra et al. 2008).

Saponins

Saponins are present in the ehtanolic extracts of *Blechnum orientale, Cyathea contaminans, Dicranopteris linearis, Diplazium esculentum, Drynaria quercifolia, Equisetum ramosissimum, Nephrolepis cordifolia, Oleandra pistillaris* and *Pyrrosia piloselloides.* The relevant properties include membranolytic effects, toxic and fungitoxic effects, adverse effects on animal growth and performance, and the important hypocholesterolemic effect (Price et al. 1997). Saponins are also claimed to possess immunostimulatory and anticarcinogenic properties (Rao and Koratkar 1997).

Flavonoids

Flavonoids are present in all the extracts both in the form of leucoanthocyanins and/or y-benzopyrone. They possess important pharmacological properties (Porter 1989) and been recognized to have anti-inflammatory, anti-coagulant and aphrodisiac properties (Zabri et al. 2008). In the study of Mutalik et al. (2003), the dry residue of fresh juice of *Solanum melongena* produced significant antipyretic effect in a dose dependent manner. The antipyretic activity they observed was attributed to the presence of flavonoids. In many studies, flavonoids have been reported to exhibit antipyretic claims by traditional medicine practitioners (Vimala et al. 1997).

Plate I. The 15 Species of Medicinal Pteridophytes used in the Study

Tannins

Tannins in the form of hydrolysable and condensed tannins are present in most of the extracts except for *Microsorum punctatum*, *Pyrrosia piloselloides*, and *Selaginella usterii*. Hydrolyzable tannins have been shown to be effective antagonists against viruses, bacteria, (Funatogawa et al. 2004) and antiparasites (Kolodziej and Kiderlen 2005). In the past few years tannins have also been studied for their potential effects against cancer through different mechanisms

Anthraquinones

Anthraquinones were not detected in all fifteen (15) ethanolic extracts of pteridophyte fronds. Anthraquinones are the largest group of naturally occurring quinones. Both natural and synthetic anthraquinones have been widely used as colorants in food, drugs, cosmetics, hair dyes and textiles (Mori et al. 1990). Herbs containing anthraquinone derivatives are used as laxatives (Sonnenberg and Muller 1993). Based on this information, the absence of anthraquinones in the pteridophyte extract in this study may prevent the occurrence of diarrhea when the plant extracts are administered orally as herbal remedy.

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, and precursors for the synthesis of complex chemical substances. In addition, knowledge of the chemical constituents of plants would further be valuable in discovering the economic and health benefits of folkloric remedies (Mojab et al. 2003).

Table 1. Phytochemical analysis of the pteridophytes ethanolic extract

Phytochemicals	Tests		Results														
			AP	AN	BO	CC	DL	DE	DQ	ER	LM	MP	NC	OP	PA	PP	SU
Alkaloids	Mayer's Test		+	+	+++	+	++	-	++	-	+	-	+	+	++	+	-
	Dragendorff's Test		+	+	+++	+	++	+	+++	++	+	++	+	+	+	+	-
	Confirmatory Test	Mayer's Test	++	++	++	++	+	++	+	+	+	+	+	+	+	++	-
		Dragendorff's Test	-	-	-	-	+	-	+	+	-	-	+	+	+	++	-
	Quarternary Bases and/or amine oxides	Mayer's Test	+	+	-	+	+	+	+	-	++	-	-	++	+++	+	-
		Dragendorff's Test	+	+	-	++	+	++	+	-	++	-	-	++	+++	+	-
Saponins	Froth Test		-	- (with fatty acid)	+	+	+	+	+	+	- (with fatty acid)	-	+	+	- (with fatty acid)	+	-
Flavonoids	Leucoanthocyanins "Bate-Smith and Metcalf Method		-	-	+	+	+	-	-	-	+	-	+	+	+	-	-
	y-benzopyrone nucleus Wilstatter "cyanidin" Test		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	Gelatin Test		+	+	+	+	+	+	+	+	+	-	+	+	+	-	-
	Ferric Chloride Test		+	+	+	+	+	+	+	+	+	-	+	+	+	+	-
Anthraquinones	Borntrager's Test		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Legend: AP (*Adiantum philippense* Linn.); AN (*Asplenium nidus* Linn.); BO (*Blechnum orientale* Linn.); CC (*Cyathea contaminans* (Hook.) Copel.); DL (*Dicranopteris linearis* (Burm.) Underw.); DE (*Diplazium esculentum* (Retz) Sw.); DQ (*Drynaria quercifolia* (Linn.) J. Sm.); ER (*Equisitum ramosissimum* (Roxb.) Hauke); LM (*Lygodium microphyllum* (Cav.) R. Br.); MP (*Microsorum punctatum* (Linn.) Copel.); NC (*Nephrolepis cordifolia* (Linn.) Presl.); OP (*Oleandra pistillaris* (Sw.) C. Chr.); PA (*Pteridium aquilinum* (Linn.) Kuhn); PP (*Pyrrosia piloselloides* (Linn.) Price); and SU (*Selaginella usterii* Linn.)

Alkaloid Mayer's and Dragendorff's Test: (+) slight turbidity;
(++) definite turbidity; (+++) heavy precipitation
Confirmatory Test: (+) primary alkaloid; (++) secondary alkaloid;
(+++) tertiary alkaloid
Test for quarternary bases and/or amine oxide: (+) absent; (++)
or (+++) present
Test for Saponins, Tannins, and Anthraquinones: (+) present; (-) absent

Antimicrobial Assay

The search for antimicrobial potential among medicinal pteridophytes was performed on the ethanolic extract of their fronds (Plate 2). Among the 15 pteridophytes evaluated, the following were attained: 1) Twelve (12) plant extracts inhibited the growth of *Bacillus subtilis 2)* Eight (8) inhibited the growth of *Escherichia coli,* 3) Eleven (11) plant extracts inhibited the growth of *Pseudomonas aeruginosa,* 4) Six (6) plant extracts inhibited the growth of *Staphylococcus aureus*, and 5) Fourteen (14) plant extracts inhibited the growth of *Candida albicans*. Among the fifteen pteridophytes plant extracts, *Equisetum ramosissimum, Pyrrosia piloselloides,* and *Selaginella usterii* extract did not show any antibacterial activity on all the four (4) bacterial isolates (Table 2).

The antibiotic equivalents were consistently and relatively high in *Asplenium nidus, Blechnum orientale, Dicranopteris linearis, Oleandra pistillaris, and Pteridium aquilinum* extracts when compared with the three antibiotics Amikacin, Ampicillin, and Streptomycin (Figures 1-3).

Table 2. Zones of inhibition (mm) of the ethanolic plant extract on the five (5) microbial isolates

Plant Extract	Mean Zones of Inhibition (mm)				
	B. subtilis	*S. aureus*	*E.coli*	*P. aeruginosa*	*C. albicans*
Adiantum philippense	$10.33^{e,f}$	8.00^{e}	8.00^{e}	12.67^{e}	16.00^{d}
Asplenium nidus	14.33^{c}	14.00^{c}	14.33^{d}	22.33^{a}	18.00^{c}
Blechnum orientale	20.67^{b}	20.00^{a}	19.67^{b}	17.67^{b}	8.00^{g}
Cyathea contaminans	12.67^{d}	11.33^{d}	12.67^{e}	$13.67^{d,e}$	$15.33^{d,e}$
Dicranopteris linearis	21.67^{a}	16.67^{b}	17.33^{c}	15.33^{c}	14.00^{e}
Diplazium esculentum	13.00^{d}	8.00^{e}	17.67^{c}	$14.33^{c,d}$	$19.67^{a,b}$
Drynaria quercifolia	11.00^{e}	8.00^{e}	8.00^{e}	9.33^{f}	18.00^{c}
Equisetum ramosissimum	8.00^{g}	8.00^{e}	8.00^{e}	8.00^{g}	16.33^{d}
Lygodium microphyllum	10.00^{f}	8.00^{e}	8.00^{e}	$8.67^{f,g}$	15.67^{d}
Microsorum punctatum	8.33^{g}	8.00^{e}	8.00^{e}	8.00^{g}	13.00^{f}
Nephrolepis cordifolia	14.33^{c}	8.00^{e}	14.33^{d}	16.67^{b}	15.00^{e}
Oleandra pistillaris	21.67^{a}	20.00^{a}	22.67^{a}	22.00^{a}	18.67^{c}
Pteridium aquilinum	20.33^{b}	17.67^{b}	22.00^{a}	22.33^{a}	20.33^{a}
Pyrrosia piloselloides	8.00^{g}	8.00^{e}	8.00^{e}	8.00^{g}	13.67^{f}
Selaginella usterii	8.00^{g}	8.00^{e}	8.00^{e}	8.00^{g}	$19.67^{a,b}$

Legend: Superscript letters indicate means having similar letter have no significant difference at 0.5 level of significance

The antifungal equivalents of *Diplazium esculentum, Peridium aquilinum, and Selaginella usterii* were significantly high when compared to ketokonazole (Figure 4).

The differences in the antimicrobial effects of pteridophyte extracts are more likely due to the differences in their phytochemical components. The results obtained from the phytochemical analysis and the antimicrobial activity of these plants need further investigations that may lead to the development of antibiotics. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs (Iniaghe et al. 2009).

The five (5) microbial isolates used in the study

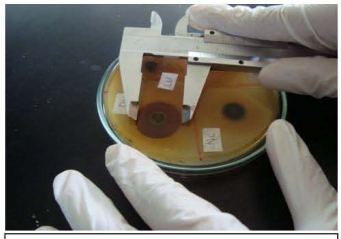
Pouring of sterile MHA for the base agar

Inoculation of top agar with specific microorganism

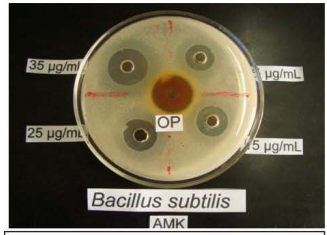
Boring of 8 mm holes on the seeded agar

Delivery of plant extracts and antibiotics on the agar wells

Incubation of cultures for 24 hours at 35-37^{0}C



Measuring of the Zone of Inhibition (mm) after 24 hours of Incubation



Zones of Inhibition showing microbial sensitivity

Plate 2. Protocols in Antimicrobial Screening

Antimicrobial resistance continues to grow rapidly among key microbial pathogens such as *Staphylococcus aureus*, *Pseudomonas* spp, *Streptococcus* spp, and *Enterobacteriaceae* all around the world (Bax et al. 2003). Development of new antimicrobial agents is therefore imperative. The increased prevalence of antibiotic-resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control some diseases caused by bacteria (Cowan 1999). Bax et al. (2003) have reported that global antibacterial resistance is becoming an increasing public health concern. Bacterial resistance to almost all available antibacterial agents has been reported. Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are combing the earth for phytochemicals and "leads" which could be developed for the treatment of infectious diseases.

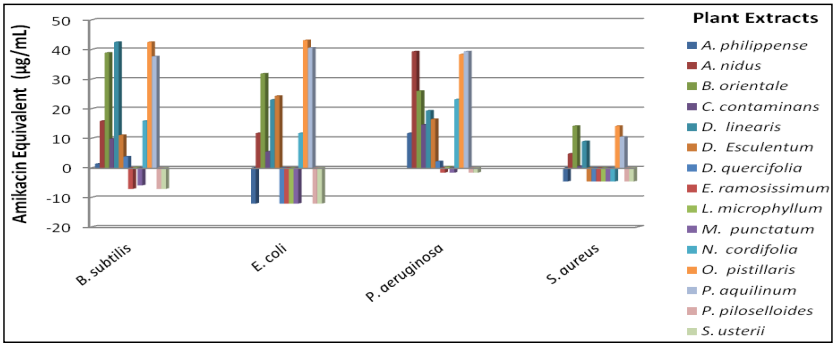


Figure 1. Antibiotic equivalents (µg/mL) of the ethanolic extracts when compared with Amikacin

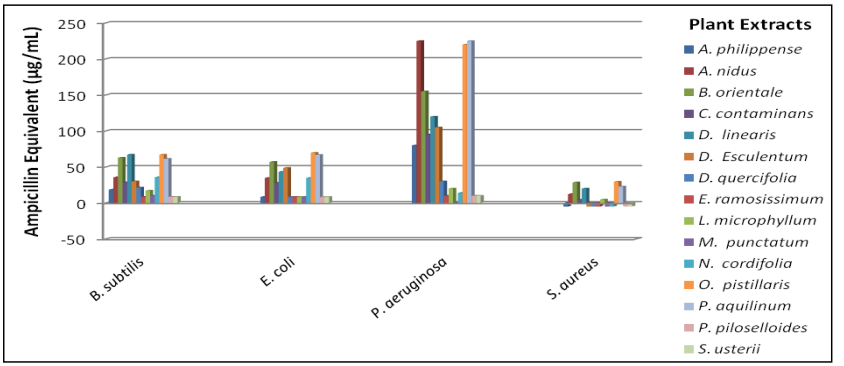


Figure 2. Antibiotic equivalents (µg/mL) of the ethanolic extracts when compared with Ampicillin

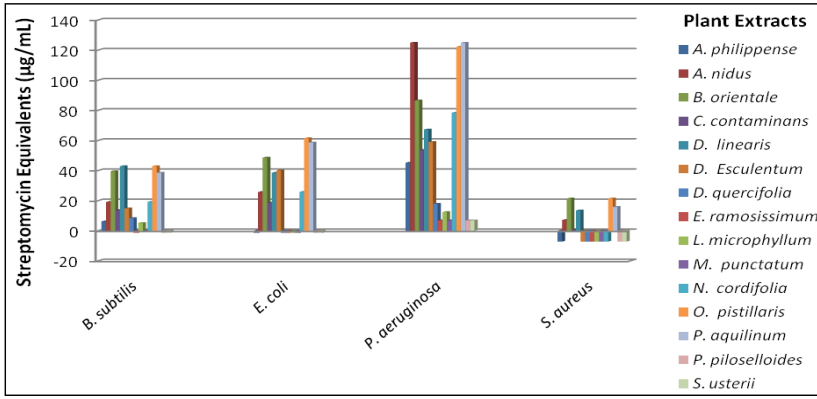


Figure 3. Antibiotic equivalents (µg/mL) of the ethanolic extracts when compared with Streptomycin

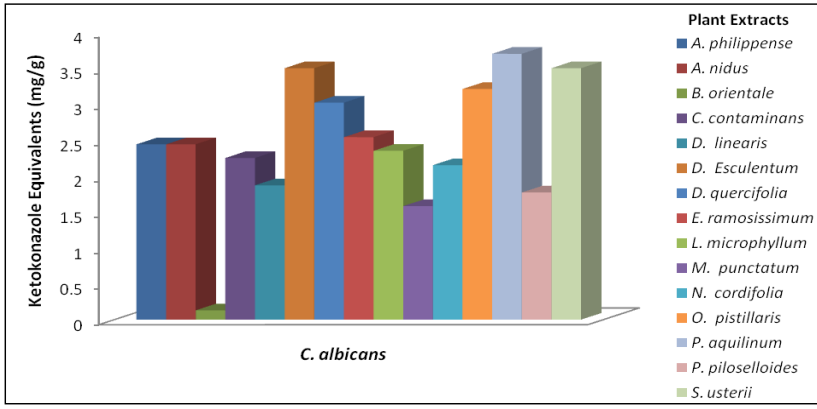


Figure 4. Antifungal equivalents (mg/g) of the ethanolic extracts when compared with Ketokonazole

Antipyretic Screening

Pyrexia was induced in white rats with 1mL/kg BW of 15% baker's yeast administered intraperitoneally. The ethanolic pteridophyte extracts were evaluated for their antipyretic potential when compared with the control (NSS).

The fifteen (15) pteridophyte extracts screened for their antipyretic potential showed varying significant reduction in yeast-induced pyrexia in rats when compared with the control (NSS) (Table 3). Although there was a delay on the onset of lowering of rectal temperatures, the 800 mg/kg BW pteridophyte extracts are still comparable in their antipyretic effect with the standard 150 mg/kg BW Paracetamol used in this study. In many studies, flavonoids have been reported to exhibit antipyretic effect (Mutalik et al. 2003). This confirms the results of the antipyretic activity of all the pteridophyte extracts in this study, which were all tested positive to flavonoids.

Table 3. Mean rectal temperature (°C) readings of albino rats as affected by the plant extracts on taken every hour

Plant Extract	Rectal Temperature (°C)					
		Temperature before Baker's yeast injection	The Initial Temperature (19 hr)	20 hr	21 hr	22 hr
Adiantum philippense	Mean	36.88	37.90	38.46	38.16	37.8
	Calculated t (T-Test)			3.62**	4.97**	2.57*
Asplenium nidus	Mean	36.88	37.88	37.76	37.92	38.32
	Calculated t (T-Test)			6.46**	6.27**	4.41**
Blechnum orientale	Mean	36.50	37.66	38.52	38.32	39.32
	Calculated t (T-Test)			5.95**	5.26**	0.241^{ns}
Cyathea contaminans	Mean	37.34	38.04	38.50	38.96	38.46
	Calculated t (T-Test)			3.45**	2.23^{ns}	2.75*
Dicranopteris linearis	Mean	36.36	37.94	38.26	38.22	38.46
	Calculated t (T-Test)			7.02**	2.90*	3.85**
Diplazium esculentum	Mean	36.88	38.08	37.84	37.94	37.70
	Calculated t (T-Test)			6.60**	5.70**	5.82**
Drynaria quercifolia	Mean	37.40	38.32	38.86	38.78	38.92
	Calculated t (T-Test)			3.78**	2.95*	3.85**
Equisetum ramosissimum	Mean	36.42	38.24	38.36	37.78	38.14
	Calculated t (T-Test)			7.94**	4.84**	2.79*

Lygodium microphyllum	Mean	36.44	37.88	38.24	38.14	38.70
	Calculated t (T-Test)			9.90**	6.14**	3.16*
Microsorum punctatum	Mean	36.74	37.84	38.48	38.36	38.38
	Calculated t (T-Test)			3.97**	4.21**	2.80*
Nephrolepis cordifolia	Mean	36.28	37.82	37.92	38.42	38.40
	Calculated t (T-Test)			6.96**	5.78**	4.09**
Oleandra pistillaris	Mean	36.38	38.16	38.22	38.12	39.22
	Calculated t (T-Test)			5.78**	5.54**	0.601^{ns}
Pteridium aquilinum	Mean	37.26	38.32	38.78	38.84	38.72
	Calculated t (T-Test)			3.21*	2.97*	2.55*
Pyrrosia piloselloides	Mean	37.48	38.24	38.72	39.22	38.88
	Calculated t (T-Test)			5.37**	1.74^{ns}	2.01^{ns}
Selaginella usterii	Mean	36.50	37.46	38.84	38.88	39.42
	Calculated t (T-Test)			4.26**	1.93^{ns}	0.145^{ns}
Paracetamol 150mg/kg BW	Mean	37.40	38.70	38.02	37.9	37.44
	Calculated t (T-Test)			11.00**	5.40**	4.82**
NSS	Mean	37.64	38.94	39.46	39.66	39.38

$t_{.05} = 2.31$ $t_{.01} = 3.36$

Anti-Inflammatory Screening

The 15 species of pteridophyte extracts were screened for their anti-inflammatory activity. As observed, all extracts significantly reduced inflammation among the male albino rats within six (6) hours of extract administration except for the 400 mg/kg BW of *Pyrossia piloselloides*. The two doses, 400 mg/kg BW and 800mg/kg BW of the extracts produced a dose-dependent effect on the inflamed paw of rats (Table 4).

Table 4. Paw thickness reading (mm) of albino rats over a period of 6 hours in anti-inflammatory screening of pteridophyte ethanol extracts using egg albumin-induced rat paw edema

Plant Extracts	Treatments	Thickness of Paw (mm) before egg-albumin injection	Paw thickness reading over a period of 6 hours in mm					
			2hrs	% paw reduction	4 hrs	% paw reduction	6 hrs	% paw reduction
A. philippense	400 mg / Kg BW	2.78	6.52*	23.05	5.44*	30.37	4.06**	54.93
	800 mg / Kg BW	2.46	5.86**	30.04	5.24**	27.23	4.00**	45.77
A. nidus	400 mg / Kg BW	2.34	6.04*	23.87	4.88**	33.51	3.16**	71.13
	800 mg / Kg BW	2.64	6.00**	30.86	4.68**	46.60	3.12**	83.10
B. orientale	400 mg / Kg BW	2.64	6.22*	26.33	5.48^{ns}	25.65	4.20*	45.07
	800 mg / Kg BW	2.76	5.90**	35.39	4.18**	62.83	3.74**	65.49
C. contaminans	400 mg / Kg BW	2.58	6.88*	11.52	5.40**	26.18	4.22**	42.25
	800 mg / Kg BW	2.56	6.34**	22.22	5.38**	26.18	3.96**	50.70
D. linearis	400 mg / Kg BW	2.54	6.90^{ns}	10.29	5.66^{ns}	18.32	4.38*	35.21
	800 mg / Kg BW	2.48	5.82**	31.28	4.78**	39.79	3.98*	47.18
D. esculentum	400 mg / Kg BW	2.66	6.46^{ns}	21.81	5.50*	25.65	4.18**	46.48
	800 mg / Kg BW	2.26	6.40**	14.81	4.80**	33.51	3.62**	52.11
D. quercifolia	400 mg / Kg BW	2.52	6.66**	14.81	5.26**	28.27	4.14**	42.96
	800 mg / Kg BW	2.32	5.96**	25.10	4.94**	31.42	3.60**	54.93
E. ramosissimum	400 mg / Kg BW	2.50	6.62*	15.23	5.80^{ns}	13.61	4.32**	35.92
	800 mg / Kg BW	2.54	6.16**	25.51	4.92*	37.70	3.96**	50.00
L. microphyllum	400 mg / Kg BW	2.32	6.68*	9.92	5.46*	17.80	4.20**	33.80
	800 mg / Kg BW	2.28	6.54*	12.35	5.22**	23.04	4.14**	34.51
M. punctatum	400 mg / Kg BW	2.46	6.54*	16.05	5.90^{ns}	26.18	4.68*	21.83
	800 mg / Kg BW	2.46	6.06**	25.93	5.24**	27.23	4.12**	41.55
N. cordifolia	400 mg / Kg BW	2.74	7.10^{ns}	10.29	5.94^{n}s	16.23	4.60*	34.51
	800 mg / Kg BW	2.42	6.68^{ns}	12.35	4.94**	34.03	4.18**	38.03
O. pistillaris	400 mg / Kg BW	2.44	7.52^{ns}	-4.53	6.26^{ns}	0.00	4.48**	28.17
	800 mg / Kg BW	2.46	7.20^{ns}	2.47	5.52*	19.90	4.14**	40.85
P. aquilinum	400 mg / Kg BW	2.42	7.06^{ns}	4.53	5.82^{ns}	10.99	4.64*	24.65
	800 mg / Kg BW	2.22	6.66*	37.04	5.34**	18.32	4.28*	27.46
P. piloselloides	400 mg / Kg BW	2.62	7.30^{ns}	3.7	6.30^{ns}	3.66	4.84^{ns}	21.83
	800 mg / Kg BW	2.52	6.60*	16.05	5.70*	16.75	4.34**	35.91
S. usterii	400 mg / Kg BW	2.54	6.44*	19.75	5.52*	22.00	4.54*	29.58
	800 mg / Kg BW	2.52	6.14**	25.51	5.34**	26.18	3.94**	50.00
Control	50 mg / Kg Diclofenac	2.64	5.56**	39.92	4.10**	61.78	2.82**	93.66
	NSS	2.50	7.36		6.32		5.34	

* significant at P<0.05, ** significant at P<0.01, ^{ns} - not significant

There were slight delays on the effect of some pteridophyte extracts in inhibiting paw edema. This may be due to the slow absorption of the herbal preparations or metabolites in the rats as a response of living tissues to injury. Inflammation involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Elias and Rao 1988). The early phase of inflammation is mainly mediated by histamine and serotonin. Histamine and other mediators of inflammation increases vascular permeability at various times after injury (Whaley and Burt 1996).

Several investigations have repeatedly proven that different flavonoid molecules exhibit anti-inflammatory functions. Thus, the anti-inflammatory activities of flavonols (quercetin, rutin and morin) and flavanones (hesperetin and hesperidin) were investigated in acute and chronic inflammation in animal models. The most important compound in reducing paw edema induced by carrageenan is quercetin (Havsteen 2002). On the other hand Paradkar et al. (2004) demonstrated that an isoflavone-containing diet with daidzin, glycitin, genistein and their glucosides, can modulate the inflammatory reaction in the intestine and liver of mice after lipopolysaccharide injection. Thus, flavaonoids in the form of flavonols, flavanones or isofalvon can exhibit anti-inflammatory activities.

CONCLUSIONS

Based on the findings of the present study, the following conclusions are drawn:

1. The phytochemical components namely alkaloids, saponins, flavonoids, and tannins were present in frond ethanolic extract of the selected pteridophytes. Anthraquinones were not detected in the ethanolic extract of all pteridophytes evaluated.
2. The ethanolic frond extracts of the pteridophytes exhibited antifungal activity on *Candida albicans* except in *Blechnum orientale* extract.
3. Some of the ethanolic frond extract of the pteridophytes exhibited antibacterial activity on Gram positive *Bacillus subtilis* and *Staphylococcus aureus and on* Gram negative *Escherichia coli* and *Pseudomonas aeruginosa*. Among the fifteen (15) pteridophyte ethanolic extracts, *Oleandra pistillaris* and *Pteridium aquilinum* manifested the greatest antibacterial and antifungal activities.

4. The ethanolic frond extract of all the pteridophytes exhibited antipyretic and anti-inflammatory properties.

RECOMMENDATIONS

The present experimental findings suggest that the fifteen (15) pteridophytes have varying antimycotic, antibacterial, antipyretic, and anti-inflammatory potentials. To further maximize the medicinal uses of these plants, we recommend the following to further evaluate these pteridophyte potentials:

1. to perform quantitative phytochemical identification and isolation on their ethanolic extracts
2. to further investigate the biochemical pathways using purified chemical constituents of the pteridophyte extracts which may result in the development of potent antipyretic and anti-inflammatory agent with low toxicity and better therapeutic index
3. to design and perform pharmacodynamic studies to establish the specific mechanism of antipyretic and anti-inflammatory action of the different plant extracts

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