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Phytochemical and Chemotaxonomic Studies on *Pteris* Species from Southwestern Nigeria

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ABSTRACT

Ferns and Lycophytes are composed of primitive vascular ornamental, medicinal and very valuable plants. In the present study, seven *Pteris* from Southwestern Nigerian were screened using standard phytochemical screening methods, and Thin Layer Chromatography (TLC). This was with the view to identifying their various bioactive compounds that are medicinally and taxonomically important. Results showed the presence of alkaloids in all the species except *P.acanthoneura.* Saponins, flavonoids, cardiac glycosides, triterpenes and steroids also tested positive in all the species studied while tannins was positive only in *P.togoensis* and *P. vittata*.TLC studies displayed spots with retardation factor (R_f)values which indicated similarities and variations among the *Pteris* species. The information on the phytoconstituents of *Pteris* species provided by this study is valuable for further pharmacognostic and chemotaxonomical investigations.

Keywords: Chemotaxonomy, Medicinal values, Phytochemicals, Pteris, TLC.

Introduction

Plants contain secondary metabolites with high economically valuable products which also function as defense mechanism against plant pathogenic invasions.[1] Phytochemical studies of ferns especially in Nigeria are limited. Ferns are rich in a wide variety of secondary metabolites such as alkaloids, tannins and flavonoids which are known to possess *in-vitro* antimicrobial properties. These products are used as high value chemicals such as drugs, flavors, insecticides, etc.[2] Apart from these medicinal values, the science of chemotaxonomy which is based on use of phytochemical constituents for plant classification is increasing rapidly nowadays.[3]The source of any economically useful material could be determined through phytochemical studies. Phytochemical screening of flowering plants has been done on a larger scale than the non-flowering plants except for the Indian ferns and fern allies.[4]

According to Wallace(1991),[5] ferns and fern allies are relatively not attacked by diseases and this is probably one of the reasons believed to have made them better survived with many more diverse changes of environment than the other primitive vascular plants that survived from Paleozoic times. Phytochemicals such as flavonoids, cardiac glycosides, phlobatannin, tannins, alkaloids, saponins, tannins, phenols, triterpenoid compounds, were reported in *Nephrolepis* species.[6] Methanol extracts of four *Cheilanthes* fern species from Northern Ghats of India also contained steroids, phenolic compounds, reducing sugars, anthraquinones and amino acids.[7] Several antibiotics, anticancer and chemotherapeutic agents were extracted from various species of *Ophioglossum* by Khandewal(1989).[8] Fazli *et al*. (2015)[9] identified flavonoids through LC–MS in the extracts of *Pteris vittata*. Similarly, Meenakshi *et al.* (2008)[10] confirmed antimicrobial activity of *P. vittata* against certain gastro-intestinal (GI) pathogens. Wu *et al*. (2005)[11] reported the anti-inflammatory properties of *P.ensiformis* in Palang where the juice of tender young fronds of *P. ensiformis* was used as an astringent preparation for cleansing unhealthy tongues of children.

Chemotaxonomy is a newly expanding area in plant taxonomy which relies on chemical information to improve on plant classification. To complement plant morphological characters, phytochemical constituents, molecular markers have been developed with analytical techniques to identify, differentiate and compare plant species.[12,3] Flavonoids, for instance are chemically complex, distinct structural variation, widespread in distribution, relatively stable and taxonomically significant. The use of flavonoids in evaluating contemporary classificatory systems has been mainly based on their distribution patterns and ample exhibited correlations. According to Ekeke and Nduku (2013),[13] the biosynthesis and accumulation of secondary metabolites in plants are affected by genetic, morphological and environmental factors.

Owing to the fact that the study of ferns phytoconstituents is very scanty in this part of the world, despite their underlying recent pharmaceutical and taxonomic importance, this investigation becomes necessary. It is envisaged that the study will provide useful information on the phytochemical relationships as well as medicinal values of the studied *Pteris* species.

Materials and Methods

Collection of Materials

Mature leaflets of each of the species were collected (July - November, 2017) from various locations from Lagos, Ogun, Oyo, Osun, Ondo and Ekiti States in Southwestern Nigeria within Latitude 6º-9º N and Longitude 3º- 9ºE. Plant samples were identified and authenticated using IFE Herbarium specimens at Botany Department,

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Obafemi Awolowo University, Ile-Ife, Nigeria. The vouchers specimens were deposited in same Herbarium.

Preparation of ethanolic extract

Plant sample of each species was air-dried and ground into powder with a blender. Each of the ground samples was soaked with 200mL of ethanol for 48 hours in conical flask. Samples were filtered, concentrated with rotary evaporator and stored in vials at room temperature for further analysis.

Phytochemical screening

Using standard procedures, ethanol extract of each studied *Pteris* species was subjected to phytochemical screening for: alkaloids, saponins, steroids, triterpenoids, flavonoids, phlobatannins, saponins, cardiac glycosides, tannins and anthraquinones using the procedures earlier established by Sofowora (2008).[14] Also, Thin Layer Chromatography (TLC) was carried out to obtain chromatogram which showed detailed distribution of the phytochemical constituents in different species in the genus. Further quantitative analytical test was done for some phytoconstituents detected.

Estimation of cardiac glycosides

To 8 mL of plant extract was added 60 mL of water and 8 mL of 12.5% lead acetate, mixed and filtered. 50 mL of the filtrate and 8 mL of 4.7%Na_2HPO_4 was added to precipitate excess Pb ion. Mixture was filtered to remove excess lead phosphate. Ten milliliters (10 mL) of purified filtrate was treated with 10 mL Baljet reagent. A blank titre was carried out using 10 mL distilled water and 10 mL Baljet reagent which was allowed to stand for one hour for total colour development. The colour intensity was calculated colorimetrically at 495 nm. The percentage of flavonoids in the sample was obtained using the formular below:

$$\frac{A \times 100}{17}\ (g\%)$$

Where A is Absorbance

Estimations of Flavonoids concentrations

The flavonoids concentration in the aqueous extract was obtained spectrophotometrically using the method described by Sun *et al*. (1999).[15] Ethanol (20 mL of 80% (v/v) was added to aqueous extract (0.1g) and filtered. To diluted filtrate (5.5 mL) was added 0.1 mL of w/v $NaNO_3$, 0.1 mL of 10% (w/v) $AlCl_3$, and 1.3 mL of 4% (w/w) NaOH. The mixture was incubated at room temperature for 15 minutes and the absorbance was read at 500 nm against the blank. The concentration of flavonoids in the sample was calculated from standard calibration curve expressed in rutin equivalent per g of extract (mg RE/g extract).

Estimation of saponin content

Saponin content was estimated by using the methods described by Wagner(1984).[16] 40 g of the ethanol extract was washed in chloroform (50 mL x 2) and ethyl acetate (50 mL x 2). After dissolving residue in 20% (v/v) ethanol and extraction with butanol (100 mL x 3), the obtained residue was absorbed and dissolved in 50 mL methanol (50% v/v) and precipitated in diethyl ether (100 mL). Precipitate was decanted and dried in the oven until steady weight.

Thin layer chromatography

Commercially obtainable standard TLC plate with typical particle dimension range to achieve better reproducibility was used. Plates were activated for three minutes at 120^oC, spotted and placed into the chromatographic tank saturated with solvent system (mobile phase) as described in Table 2. Each chemical constituent moves up at different rates depending on its solubility in the mobile phase and the strength of its absorption to the stationary phase. The retardation factor (R_f) which is the distance travelled by the solute divided by the distance travelled by the solvent was obtained from the equation below:

$$R_f = \frac{\text{distance travelled by the solute}}{\text{distance travelled by the solvent}}$$

Results and Discussion

The qualitative phytochemical screening of *P. acanthoneura*, *P. atrovirens*, *P. ensiformis*, *P.milbraedii*, *P. similis*, *P.togoensis* and *P. vittata* secondary metabolites were presented in Table 1.Table 3 showed TLC profiles of each of the phytochemical constituents in the *Pteris* species [Figure1 (A-G)] and the R_f values of their spots.

In the present study, the secondary metabolites and the TLC profiles (Table 3) showed variations among the studied *Pteris* species. Alkaloids were present in all the species except in *P. acanthoneura*, tannins occurred only in *P. togoensis* and *P. vittata* while saponins, flavonoids, triterpenes, steroids and cardiac glycosides occurred in all the seven *Pteris* species.

The presence of flavonoids, alkaloids, saponins, tannins and phenolic compounds in ferns extracts have been reported in previous studies.[17-19] There were no documentation on phytochemical constituents of the studied *Pteris* species except in *P. vittata*, In this study, *P. vittata* extract tested positive for flavonoids, tannins, cardiac glycosides, saponins, triterpenes, alkaloids and steroids similar to the findings of (Jaishee and Chakraborty).[20] In contrast, alkaloids and steroids detected in *P. vittata* in this study was reported absence in Indian specimens[20] and the difference may probably be attributed to variation in ecological factors. Generally, sources of variations in plant secondary metabolites include; age of the plant, time and situation of harvest, part of plant used, solvent, extraction procedures, the amount of sample used, percentage humidity of the harvested material and plant substrate.[14]

The TLC profiles of each of the phytochemical constituents in the studied *Pteris* species [Figure 1 (A-G)] and their R_f values (Table 3) showed highest number of bands for flavonoids (6) in *P. similis* and *P. togoensis*, steroids (5)in *P. similis* and *P. togoensis*, triterpenes(5) in *P. togoensis*, alkaloids (4) in *P ensiformis*, saponinns(3) *in P. atrivirens*, cardiac glycoside(2)in *P. similis*, *P. togoensis* and *P. vittata* while tannin had a single band in *P. togoensis* and *P.vittata*. Also, each class of phytochemical of the studied *Pteris* species showed identical (e.g alkaloid with R_f 0.7 is common in all with the exception of *P. acantthoneura*) and diverse R_f values (e.g flavonoids had 24 varied R_f). The similarity and variations in the R_f values displayed by the TLC profiles indicates generic relationships and specific variations among the species according to Oladipo *et al.* (2017).[21] The occurrence and distribution of various types of chemical substances present in plants have proved to be of taxonomic significance.[22]

Flavonoids are the major group of plant phenols and the foremost studied one.[23] Flavonoids have gained recent attention because of their broad biological and pharmacological activities which include cytotoxic, antioxidant, antiinflamatory and anti-tumour activities.[24,25]In addition, flavonoid compounds have proven to be of chemotaxonomic importance by many authors.[26-28] For instance, the flavonoids identified in 14 species of *Salvia* were flavones, flavonols, flavanones, isoflavones, dihydroflavonoids and chalcones. Kharazin (2014) affirmed that flavonoids are appropriate indicators to determine the taxonomic position of *Salvia* species.

Also, alkaloids were present in all the studied *Pteris* species except *P. acanthoneura*. Therefore, absence of alkaloids in only *P. acanthoneura* could be taken as diagnostic of the species. Alkaloids have been reported to be significant for the survival of plant by providing protection against micro-organisms, insects as well as herbivores.[30] Likewise, anti-cancerous, anti-malarial, anti-inflammatory, antimicrobial, etc. activities of alkaloids have been reported.[31,32] Alkaloids contents have been considered as sources of taxonomic evidence as alkaloids characterizing species of a particular taxon are frequently of the same chemical or biogenic group suggesting that related plants share the same pathways of alkaloid synthesis.[33]

All the studied *Pteris* species are rich in saponins (Table 4) with the exception of *P. togoensis*. Various documented pharmacological properties of saponin include *anti*-inflammatory, emetics, antiviral, antifungal, insecticidal, molluscicidal, piscidal and anti-bacterial activities.[34]

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Cardiac glycosides and steroids occurred in all the studied *Pteris* species. Cardiac glycosides are group of plant metabolites that comprise the most drug-like molecules proved to be fruitful in developing potential drugs for the treatment of congestive heart failure. They are streroids possessing the ability to exert specific action on the cardiac muscle.[35] Steroids perform many key biological functions which includes hypocholesterolemic, anticancer, antihelminthic and antimutagenic activies.[36]

Similarly, all the studied *Pteris* species tested positive for terpenoids. Recently, triterpenoids have arisen as a major group of secondary metabolites with important spectrum of pharmacological activities, including anti-inflammatory, anti-oxidant, antibacterial, antiviral, hepatoprotective, gastroprotective, cardioprotective, hypolipidemic, anti-atherosclerotic, immunoregulatory and anticancer activities.[31]

The use of triterpenes as a chemotaxonomic tool in investigating botanical affinities have been confirmed.[37]

In the present study, tannins were found in *P. togoensis* and *P. vittata* only. Theurapeutic properties like antiviral, antibacterial, antiparasitic, anticarcinogenic, and antimutagenic activities of tannins have been reported.[20]

The quantitative estimations of some phytoconstituents of the studied *Pteris* species (Table 4) showed that they are rich in saponins as well as cardiac glycosides and contained moderate flavonoids. In contrast, high flavonoids, low saponins and tannins were observed in some *Pteris* species.[18]

Conclusion

This study revealed pertinent information on phytochemical constituents and various chemotypes that could enhance systematic and pharmaceutical values of the species. Further research on isolation and characterization of the secondary metabolites will be taxonomically useful for unveiling generic affinities among the *Pteris* species.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Table 1: Phytochemical constituents of seven *Pteris* species.

	P.acanthoneura	***P.atrovirens***	***P.ensiformis***	***P.miilbraedii***	***P.similis***	***P.togoensis***	***P.vittata***
Saponins	+	+	+	+	+	+	+
Flavonoids	+	-	-	-	-	-	-
Flavonoids	+		-	-	-	-	-
Tannins	-	-	-	-	-	-	-
Xanthoproteins	-	-	-	-	-	-	-
Alkaloids	-	+	+	+	+	+	+
(Dragendorf)							
Alkaloids (Mayer)		-	-	-	-	-	-
Phlobatanins	-	-	-	-	-	-	-
Triterpenes	+	+	+	+	+	+	+
Cardiac glycosides	+	+	+	+	+	+	+
Steroids	+	+	+	+	-	+	+
Anthraquinones	-	-	-	-	-	-	-

Legends: + (Present); - (Absent)

Table 2: TLC mobile phase of leaf extracts of *Pteris*species.

S/N	Secondary Metabolites	Solvent System	Solvent Ratio	Spraying Reagent
1	Alkaloids	Ethyl acetate & methanol water	10:1.4:1	Dragendorff
2	Flavonoids	Toluene:Acetone: Formic acid	4.5:4.5:1.0	1% EthanolicAluminium chloride
3	Tannins	Ethylacetate: Formic acid: Methanol	3:3:0.8:0.2	5% Iron chloride
4	Saponins	Chloroform : Methanol	1.2:0.2	Iodine crystal
5	Steroids	Hexane :Ethylacetate	7.2:2.9	70% H_2SO_4-Acetic acid in ethanol
6	Cardiac glycosides	Ethylacetate: Methanol : H_2O	81:11:8	
7	Triterpenes	Dichloromethane		

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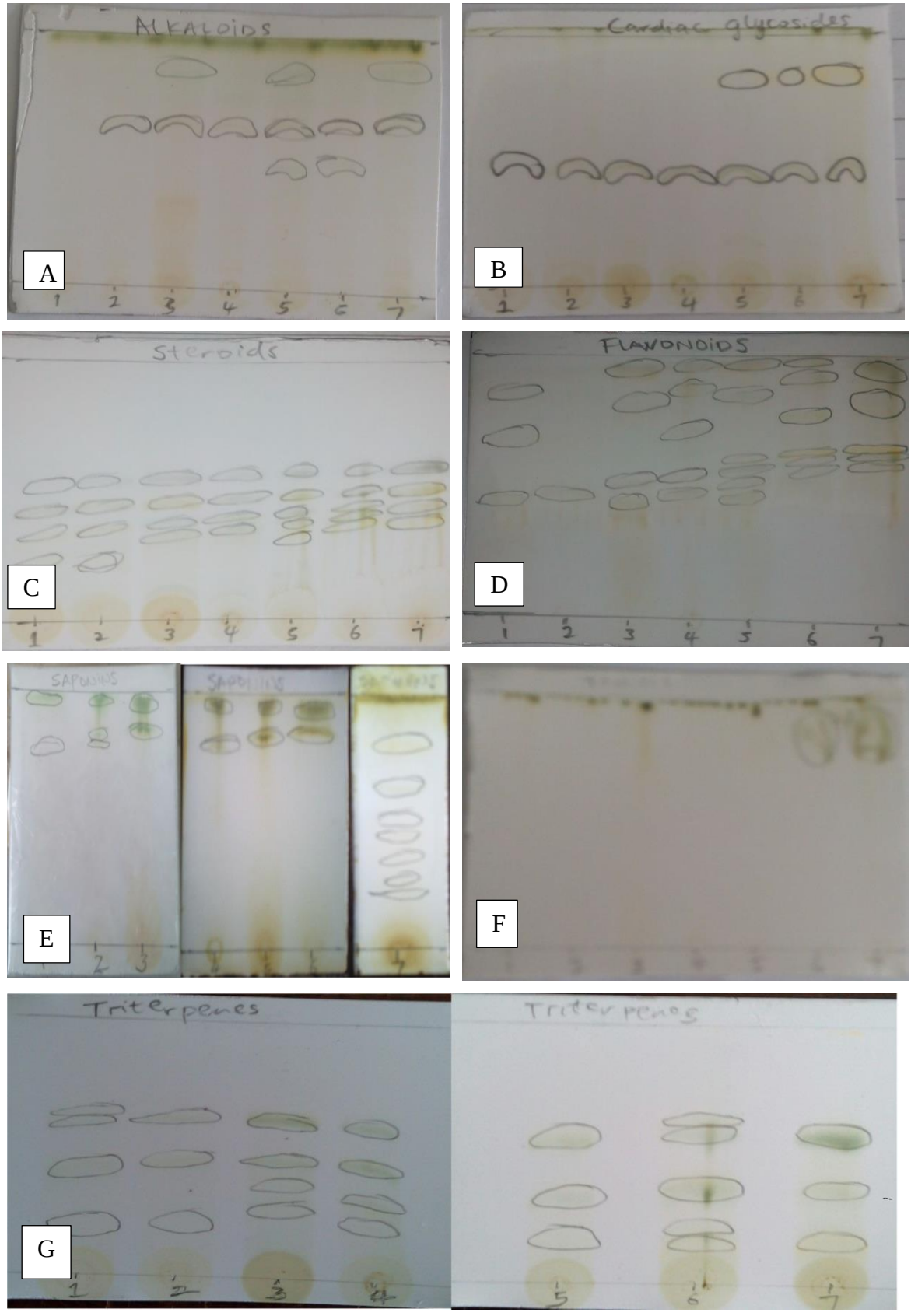

Figure 1: TLC profiles of the *Pteris* species
Legend: A-Alkaloids, B- Cardiac-glycosides, C- Steroids, D- Flavonoids, E- Saponins, F- Taninns, G-Triterpens. 1-*P. acanthoneura*, 2- *P atrovirens*, 3- *P. ensiformis*, 4- *P. mildbraedii*, 5-*P.similis* , 6- *P.togoensis.* 7- *P.vittata.*

Table 3: The TLC profile of the phytoconstituents in the leaf extracts of *Pteris* species.

	Phytochemical						
	Alkaloids	**Cardiac Glycoside**	**Flavonoids**	**Saponins**	**Steroid**	**Tannins**	**Triterpenes**
Plant species	Rf values	Rf values	Rf values	R_f values	Rf values	Rf values	R_f values
P.aacnthoneura	-	0.52	0.47,0.71,0.86	0.81,1.00	0.26,0.34,0.44,0.54	-	0.26,0.46,0.64,0.68
Patrivirens	0.7	0.48	0.49	0.81,0.86,1.00	0.26,0.36,0.46,0.56	-	0.26,0.52,0.70
P ensiformis	0.12,0.38,0.7,0.92	0.46	0.48,0.56,0.86,0.97	0.88,1.00	0.34,0.40,0.48,0.58	-	0.30,.40,0.50,0.66
P.miilbraedii	0.7	0.46,0.88	0.51,0.57,0.75,0.91,0.99	0.78,0.81	0.36,0.40,0.48,0.58	-	0.24,0.34,0.48,0.64
Psimilis	0.52,0.7,0.92	0.48,0.86	0.48,0.54,0.59,0.62,0.88,0.99	0.86,0.89	0.32,0.38,0.42,0.50,0.58	-	0.22,0,38,0.62
P.togoensis	0.52,0.7	0.48,0.86	0.60,0.63,0.67,0.77,0.95,0.99	0.86,0.91	0.36,0.40,0.46,0.50,0.60	0.96	0.20,0.26,0.42,0.62,0.68
P.vittata	0.7,0.92	0.50,0.86	0.62,0.64,0.67,0.89,0.98	0.24,0.30,0.39,0.47,0.54,0.67,0.84	0.40,0.44, ,0.50,0.60	0.96	0.22,0.42,0.62

Table 4: Flavonoids, saponins and cardiac glycosides concentrations(mg/g) in seven *Pteris*species

Phytochemical	**Pac**	**Pat**	**Pen**	**Pmi**	**Psi**	**Pto**	**Pvi**
Flavonoids	0.27 ± 0.03	0.56 ± 0.01	0.60 ± 0.10	0.40 ± 0.21	0.45 ± 0.10	0.46 ± 0.31	0.66 ± 0.22
Saponins	125 ± 0.33	162.5 ± 0.80	15 ± 0.32	135 ± 2.17	167.5 ± 1.31	125 ± 2.0	222.5 ± 2.05
C. glycosides	14.89 ± 0.18	17.53 ± 1.0	33.35 ± 1.23	7.35 ± 0.48	49.0 ± 1.33	5.17 ± 1.26	28.18 ± 0.88

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