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Effect of Extraction Solvents on Phytochemical Composition of Selected Medicinal Ferns in Ekiti State, Nigeria

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Original Research Article

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Abstract: The effect of extraction solvents (water, ethanol and hexane) on the content of phytochemicals such as alkaloids, saponins, total phenols, tannins and flavonoids in three selected medicinal ferns (*Nephrolepis cordifolia* (L.) Presl, *Nephrolepis biserrata* (Sw.) Schott and *Pneumatopteris afra* (Christ) Holttum) in Ekiti State, Nigeria was investigated. Matured leaflets were collected from healthy plants in Ekiti State University, Ado Ekiti, Nigeria. Extracts from shade dried and powdered leaflets of each of the ferns were subjected to phytochemical screening using standard methods. The results showed the presence of total phenols, tannins and flavonoids in all the extracts of the ferns while saponin was present only in water extracts. Hexane was not able to extract alkaloids and saponins in the three ferns. The results showed that extraction solvents significantly affected the phytochemical composition of the ferns. The water and ethanol extracts had higher content of the phytochemicals than the hexane extracts. The diversity of phytochemicals found present suggests that the ferns could serve as sources of useful drugs.

Keywords: phytochemicals, extraction solvents, leaflets, ferns, Ekiti State.

INTRODUCTION

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, food and supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. The medicinal values of plants lie in some chemical active substances that produce definite physiological action on human body [2]. Moreover pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds.

These bioactive substances are normally accumulated as secondary metabolites in all plant cells. Phytochemicals are bioactive chemicals of plant origin. They are regarded as secondary metabolites because the plants that manufacture them may have little need for them. Phytochemicals have been recognized as the basis for traditional herbal medicine practiced in the past and currently en vogue in parts of the world [3]. Researchers sometimes follow leads provided by local healers in a region in the search for phytochemicals that may be of benefit to the pharmaceutical industry [4]. Following such leads, plants are usually screened for phytochemicals that may be present. The presence of a phytochemical of interest may lead to its further isolation, purification and characterization. Then it can be used as the basis for a new pharmaceutical product.

Use of antibiotics is often associated with adverse effects such as hypersensitivity, depletion of beneficial gut and mucosal microorganisms, immune-suppression and allergic reactions in the user. These apart, the indiscriminate use of antimicrobial drugs have created immense clinical problems in the treatment of infectious diseases [5], as the microorganisms develop resistance to often used antibiotics. This necessitates development of alternative antimicrobial drugs, for the treatment of infectious diseases from the medicinal herbs which are a rich source of novel antibacterial and antifungal chemotherapeutics [6]. Though angiosperms are an immense source of therapeutics, lower plants are attracting more attention in recent times for the search of new and effective molecules. The medicinal value of the pteridophytes has been known for several years. [7, 8] reported that antimicrobial properties of ferns are remarkable compared to the higher plants probably due to the presence of a large number of defensive biochemical compounds.

Pteridophytes are not infected by microbial pathogens, which may be one of the important factors for their evolutionally success as they survived for more than 350 million years [9]. The synergistic interaction among crude extracts or the active compounds may be useful in the preparation of improved herbal or drug formulations. Traditionally people used pteridophytes as medicine and antibacterial agents.

N. biserrata and *N. cordifolia* belong to the family Nephrolepidaceae and have been reported to be of immense ethnobotanical importance. *N. biserrata* is widely distributed and having escaped cultivation has become naturalized in many countries. [10] reported its ethnomedicinal importance in boils, abscesses and blisters. Incidentally, boils [11] and abscess [12] are due to bacterial infections and blisters are caused by fungal infections [13]. Thus, *N. biserrata* can be a potential fern to fight against pathogenic microbes. *N. cordifolia* is a pantropical species growing from tropical to temperate areas [14] and ethnomedically used in general disorders of renal and liver systems, skin diseases and as a contraceptive [10]. [15] reported the antimicrobial activity aqueous extracts of *N. cordifolia. Pneumatopteris afra* belongs to the family Thelypteridaceae. It is a water-side popular fern used for various diseases like gastroenteritis and skin problems, ascaris disease, cold and diarrhoea [16, 17].

It has been reported that successful determination of biologically active compounds from plant materials is largely dependent on the type of solvent used in the extraction procedure [18].

The main objective of this research work is to analyse the presence of secondary metabolites as the promising agent responsible for the medicinal activities of the ferns (*N. biserrata*, *N. cordifolia* and *P. afra*) by carrying out the phytochemical screening of the plants using different solvents.

MATERIALS AND METHODS

Collection of plant materials

Matured, healthy and disease free leaflets of three ferns: *N. cordifolia*, *N. biserrata* and *P. afra* were collected from Ekiti State University, Ado Ekiti, Ekiti State, Nigeria. The plants were identified at the herbarium of the Department of Plant Science and Biotechnology, Ekiti State University, Ado Ekiti, Nigeria. Voucher specimens were deposited in the herbarium.

Preparation of plant extracts

The plant samples were thoroughly washed, shade dried and then converted to powder using a blender. The powder was further passed through a 2mm sieve to obtain finer particles. 50g of the powder of each plant was extracted successively with 250ml of distilled water, ethanol and hexane using a soxhlet extractor for 8 h at a temperature that did not exceed the boiling point of the solvent. The extracts were concentrated and preserved in air tight bottles until needed for analysis.

Qualitative phytochemical screening

The phytochemical constituents of the plant extracts such as alkaloids, saponins, phenols, flavonoids and tannins were identified using standard methods [19, 20].

Test for Alkaloids

0.5 g of the extract was stirred with 5 ml 1% aqueous hydrochloric acid on a steam bath and filtered. Then, 1 ml of the filtrate was treated with few drops of Mayer's reagent and another 1 ml portion of the substrate was treated with Dragendroff's reagent. Turbidity or precipitation with either of these reagents was taken as an evidence for the the presence of alkaloids.

Test for Saponins

This was determined by double extraction gravimetric method described by [21] in which 0.5 g from each of the plant extracts was shaken with water in a test tube. Frothing which persisted on warming was taken as an evidence for the presence of saponins.

Test for Phenols

Ferric chloride test: A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium Ferro cyanide were added. Formation of blue or green color showed the presence of polyphenols.

Tests for Flavonoids

0.5 g of the extract was added to 1% Aluminium chloride and dissolved in methanol. Few drops of concentrated HCL, magnesium turnings and potassium hydroxide solutions were added. Appearance of orange or pink colour was taken as an evidence for the presence of flavonoids.

Tests for Tannins

0.5 g of the extract was stirred with 10 ml of distilled water, filtered and ferric chloride salt was added. A blue-black, green or blue-green precipitate was taken as an evidence for the presence of tannins.

Quantitative phytochemical Screening

The quantitative amounts of the phytochemicals which were found in the plant extracts were determined using standard procedures as described by [22, 20].

RESULTS AND DISCUSSION

The result of the qualitative phytochemical analysis of the selected ferns is shown in Table-1. Among the nine extracts of the ferns, each extract contained a minimum of three phytochemicals. Water extracts showed maximum number (five) of the phytochemicals. Total phenols, tannins and flavonoids were present in all the nine extracts. Alkaloids occurred in the ethanol extracts of all the ferns and aqueous extracts of *N. biserrata* and *P. afra*. Saponins were present only in the aqueous extracts of the ferns. The present study revealed that phytochemicals such as alkaloids, saponins, phenols, tannins and flavonoids

were either present or absent in the plant extracts using different solvents. Results from the study showed that water and ethanol were able to extract more of the phytochemicals than hexane. This tends to agree with the findings of [23] who worked on the preliminary phytochemical analysis of different solvent extracts of *Scoparia dulcis* and reported that aqueous extract was the most effective, followed by ethanol and that phytochemicals present were solvent dependent. In previous research studies on the effect of extraction solvent on the phytochemical composition of walnut green husk extracts, it was revealed that hexane had the lowest quantities of the phytochemicals [24]. The result of the present research is in agreement with this. It was reported in the preliminary phytochemical screening of different solvent extracts of the stem, bark and root of *Dannetia tripetala* that the presence of phytochemicals was also solvent dependent [25]. This is also in consonance with the results of the present investigation. In the extraction and preliminary phytochemical screening of active compounds in *Morinda citrifolia* fruit, it was reported that the three solvents used (water, ethanol and methanol) produced similar results [26]. The results of the present investigation also agree with this. It was also reported that water and ethanol revealed more phytochemicals than the other solvents when some pteridophytes were screened for phytochemicals using different solvents [27]. The finding in this study is also in consonance with this.

As earlier reported, the extraction of phytochemicals is dependent on the dissolution of each compound in the plant material matrix and their diffusion into the external solvent [28]. Hence, the choice of extraction solvent is one of the most important factors to consider for solid-liquid extraction. Factors such as safety of the solvent, potential for formation or extraction of undesirable compounds and solubility of the target compound should be considered in the choice of solvents or solvent system for extraction of phytochemicals [29]. Other factors that need to be considered when choosing the solvent for the extraction of phytochemicals include quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process and the potential health hazard of the extractant [18].

Table-2 reveals the result of the quantitative phytochemical analysis of the *N. biserrata*. The contents of alkaloids (0.14mg/g), tannins (3.24 mg TAE/g) and flavonoids (2.67 mg QE/g) in ethanol extracts were significantly higher than that of the water and hexane extracts while the total phenol content (8.18 mg GAE/g) in water extract was significantly higher than that of the other two extracts. Similar trends were observed in the results of the quantitative phytochemical analysis of the other two ferns except that the contents of tannins (2.82 mg TAE/ g in *N. cordifolia* and 1.87 mg TAE/ g in *P. afra*) in water extracts were significantly higher than that of the ethanol and hexane extracts (Tables 3 and 4). The study revealed the presence of phytochemicals considered as active medicinal chemical constituents in varying quantities in different solvents. The results revealed that water and ethanol are better solvents for the extraction of the phytochemicals from the ferns. With regard to alkaloids and flavonoids contents, solvents could be sequenced in the following decreasing order: ethanol > water > hexane. However, with regards to the contents of saponins, total phenols and tannins, the order was water > ethanol > hexane. As previously reported, the results obtained in the present study indicated that differences in the polarity, dispersibility and penetrability of the solvents could be responsible for their selective extraction of different phytochemicals [30]. Besides, factors such as dielectric constant, chemical structure of organic solvents as well as chemical properties of plant phytochemicals could possibly influence the levels of the phytochemicals in the solvents [31].

The result of the present study indicates that the matured leaflets of these ferns hold promises as sources of pharmaceutically important phytochemicals. From ancient times, many pteridophytes are being used medicinally in various communities, ethnic groups and folklore throughout the world. Plant parts such as rhizomes, stems, fronds, pinnae and spores are being used to treat various ailments since ancient time [32]. The medicinal values of the ferns may be due to the presence of some phytochemicals. Alkaloids have been shown to be analgesic, antispasmodic and antibacterial [33]. Tannins exhibit potential antiviral, antibacterial and free radical scavenging properties [27]. Phenolic compounds possess a variety of biological properties such as antioxidant, anti-carcinogenic, anti-inflammation, anti-aging, cardiovascular protection and inhibition of proliferation activity [34]. Flavonoids have also exhibited a wide range of biological activities such as antimicrobial, antioxidant, anti-inflammatory, anti-allergic and cytostatic properties [35]. Saponins are among various secondary plant metabolites with potent antifungal, anti-bacterial, anti-inflammatory and phytoprotectant properties which form barriers to microbial attack and in plant defence against herbivores [36].

Table-1: Qualitative phytochemical composition of the ferns

Phytochemicals	Water			Ethanol			Hexane		
	1	2	3	1	2	3	1	2	3
Alkaloids	+	-	+	+	+	+	-	-	-
Saponins	+	+	+	-	-	-	-	-	-
Total phenols	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+

1: *N. biserrata*, 2: *N. cordifolia*, 3: *P. afra*
+ present, - absent

Table-2: Quantitative phytochemical composition of *N. biserrata*

Solvents	Alkaloids (mg/g)	Saponins (mg/g)	Total Phenols (mg GAE/g)	Tannins (mgTAE/g)	Flavonoids (mg QE/g)
Water	0.11^{b}	0.11	8.18^{a}	3.11^{b}	2.41^{b}
Ethanol	0.14^{a}	ND	6.17^{b}	3.24^{a}	2.67^{a}
Hexane	ND	ND	1.44^{c}	0.61^{c}	0.41^{c}

Means with the same letter within columns are not significantly different at $p<0.05$

Table-3: Quantitative phytochemical composition of *N. cordifolia*

Solvents	Alkaloids (mg/g)	Saponins (mg/g)	Total Phenols (mg GAE/g)	Tannins (mgTAE/g)	Flavonoids (mg QE/g)
Water	ND	0.06	7.43^{a}	2.82^{a}	2.94^{b}
Ethanol	0.06	ND	5.82^{b}	2.63^{b}	3.21^{a}
Hexane	ND	ND	1.24^{c}	0.21^{c}	0.16^{c}

Means with the same letter within columns are not significantly different at $p<0.05$

Table-4: Quantitative phytochemical composition of *P. afra*

Solvents	Alkaloids (mg/g)	Saponins (mg/g)	Total Phenols (mg GAE/g)	Tannins (mgTAE/g)	Flavonoids (mg QE/g)
Water	0.14^{b}	0.07	5.45^{a}	1.87^{a}	4.22^{b}
Ethanol	0.17^{a}	ND	4.94^{b}	1.73^{b}	5.23^{a}
Hexane	ND	ND	1.32^{c}	0.24^{c}	0.13^{c}

Means with the same letter within columns are not significantly different at $p<0.05$

CONCLUSION

The phytochemicals found present in the matured leaflets of the three ferns indicate their potential as a source of active principles that may supply novel medicines. This study also leads to further research in the way of isolation, purification and characterization of the active compounds from the selected ferns. The occurrence and quantities of the phytochemicals in the selected ferns are however solvent dependent.

REFERENCES

1. Hammer, K. A., Carson, C. F., & Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. *Journal of applied microbiology*, *86*(6), 985-990.
2. Aiyelaagbe, O. O., & Osamudiamen, P. M. (2009). Phytochemical screening for active compounds in Mangifera indica leaves from Ibadan, Oyo State. *Plant Sci Res*, *2*(1), 11-13.
3. Thamaraiselvi, P., & Jayanthi, P. (2012). Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.) Solms. *Asian Journal of Plant Science and Research*, *2*(2), 115-122.
4. Das, K., Tiwari, R. K. S., & Shrivastava, D. K. (2010). Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. *Journal of medicinal plants research*, *4*(2), 104-111.
5. Davis, M. A., Hancock, D. D., Besser, T. E., Rice, D. H., Gay, J. M., Gay, C., ... & DiGiacomo, R. (1999). Changes in antimicrobial resistance among Salmonella enterica serovar Typhimurium isolates from humans and cattle in the Northwestern United States, 1982–1997. *Emerging infectious diseases*, *5*(6), 802.
6. Jones, C. G., & Firn, R. D. (1978). The role of phytoecdysteroids in bracken fern, Pteridium aquilinum (L.) Kuhn as a defense against phytophagous insect attack. *Journal of Chemical Ecology*, *4*(2), 117-138.
7. Hansel, C. G., & Lagare, V. B. (2005). Antimicrobial Screening of Maranao Medicinal Plants.

8. Bir, S. S. (1987). Pteridophytic flora of India: rare and endangered elements and their conservation. *Indian Fern Journal*.
9. Sharma, B. D., & Vyas, M. S. (1985). Ethnobotanical studies on the ferns and fern allies of Rajasthan. *Nelumbo*, *27*(1-4), 90-91.
10. Baltrushes, N. (2006). Medical ethnobotany, phytochemistry and bioactivity of the fern of Moorea, French Polynesia. Moorea digital flora project.www. ucjeps.berkeley.edu/moorea/baltrushes2006pdf.
11. Lindenmayer, J. M., Schoenfeld, S., O'grady, R., & Carney, J. K. (1998). Methicillin-resistant Staphylococcus aureus in a high school wrestling team and the surrounding community. *Archives of Internal Medicine*, *158*(8), 895-899.
12. Smit, L. H. M., Leemans, R., & Overbeek, B. P. (2003). Nocardia farcinica as the causative agent in a primary psoas abscess in a previously healthy cattle inspector. *Clinical microbiology and infection*, *9*(5), 445-448.
13. The MERCK Manuals. Online Medical library. www.merck.com/mmhe/sec18/ch203/ch203c.html.
14. Dixit, R. D. (1984). Botanical survey of India: A census of India Pteridophytes. Howrah, India.
15. Bernejee, R. D., & Sen, S. P. (1980). Antibiotic activity of pteridophytes. Eco Bot., 34: 284-298.
16. Omotayo, F. O., & Borokini, T. I. (2010). Ethnobotanical survey and chemotaxonomy of some tropical pteridophytes in Ekiti and Ondo States of Nigeria. *Journal of Applied and Environmental Sciences*, 6(1): 20-24.
17. Yapar, N., Erdenizmenli, M., Oğuz, V. A., Kuruüzüm, Z., Senger, S. S., Çakır, N., & Yüce, A. (2006). Infectious disease consultations and antibiotic usage in a Turkish university hospital. *International journal of infectious diseases*, *10*(1), 61-65.
18. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale pharmaceutica sciencia*, *1*(1), 98-106.
19. Sofowora, E. A. (1982). Medicinal Plants and Traditional Medicine in Africa. John Wiley and sons, U.S.A. 10-40.
20. Trease, G. E., & Evans, W. C. (2002). Pharmacognosy. 15th Edn. Saunders, pp. 214-393.
21. Harborne, J. B. (1973). Methods of plant analysis. In: Phytochemical Methods. Chapman and Hall, London.
22. Obadoni, B. O., & Ochuko, P. O. (2002). Phytochemical studies and comparative efficacy of the crude extracts of some haemostatic plants in Edo and Delta States of Nigeria. *Global Journal of pure and applied sciences*, *8*(2), 203-208.
23. Phan, Y., & Nguyen, A. (2014). Preliminary phytochemical analysis of different solvent extracts of *Scoparia dulcis* L. *International Journal of Scientific and Research Publications*, 4 (12): 1-3.
24. Zhang, Q. (2015). Effects of extraction solvents on phytochemicals and antioxidant activities of walnut green husk extracts. *European Journal of Food Science and Technology*, 3(5): 15-21.
25. Solomon, C. U., Arukwe, U. I., & Onuoha, I. (2013). Preliminary phytochemical screening of different solvent extracts of stem, bark and roots of *Dannetia tripetala* G. Baker. *Asian Journal of Plant Science and Research*, 3(3): 10-13.
26. Sridevi, N., Changam, S. S., & Kotturathu, M. C. (2012). Extraction and preliminary phytochemical screening of active compounds in *Morinda citrifolia* fruit. *Asian Journal of Pharmaceutical and Clinical Research*, 5: 974-979.
27. Mir, S. A., Mishra, A. K., Reshi, Z. A., & Sharma, M. P. (2013). Preliminary phytochemical screening of some pteridophytes from district Shopian. *International Journal of Pharmacy and Pharmaceutical Scienc*es, 5: 975-979.
28. Shi, J., Nawaz, H., Pohorly, J., Mittal, G., Kakuda, Y., & Jiang, Y. (2005). Extraction of polyphenolics from plant material for functional foods—Engineering and technology. *Food reviews international*, *21*(1), 139-166.
29. Seidel, V. (2012). Initial and bulk extraction of natural products isolation. In *Natural Products Isolation* (pp. 27-41). Humana Press.
30. Rebey, I. B., Zakhama, N., Karoui, I. J., & Marzouk, B. (2012). Polyphenol composition and antioxidant activity of cumin (Cuminum cyminum L.) seed extract under drought. *Journal of food science*, *77*(6), C734-C739.
31. Cheok, C. Y., Chin, N. L., Yusof, Y. A., & Law, C. L. (2012). Extraction of total phenolic content from garcinia mangostana linn. hull. I. Effects of solvents and UV–vis spectrophotometer absorbance method. *Food and Bioprocess Technology*, *5*(7), 2928-2933.
32. Kumar, A., & Kaushik, P. (1999). Antibacterial effect of Adiantum capillus veneris Linn. *Indian Fern J*, *16*, 72-74.
33. Okwu, D. E., & Okwu, M. E. (2004). Chemical composition of Spondias mombin Linn plant parts. *J Sustain Agric Environ*, *6*(2), 140-147.
34. Han, X., Shen, T., & Lou, H. (2007). Dietary polyphenols and their biological significance. *International Journal of Molecular Science*, 8: 950-958.
35. Tsuchiya, H., Sat, M., Miyazaki, T., Fujiwara, S. Tanigaki, S., & Ohyama, M. (1996). Comparative study on the antibacterial activity of phytochemical flavonones against methicillin resistant Staphylococcus aureus. *Journal of Ethnopharmacology*, 50: 27-34.
36. Papadopoulou, K., Melton, R. E., Leggett, M., Daniels, M. J., & Osbourn, A. E. (1995). Compromised disease resistance in saponin-deficient plants. *Proceedings of the National Academy of Sciences*, *96*(22), 12923-12928.