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ORIGINAL ARTICLE **OPEN ACCESS**

Phytochemical Screening, Antioxidant and Anti-inflammatory Activities of the Three Fern (Polypodiaceae) Species in Bukidnon, Philippines

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ABSTRACT

The phytochemical constituents, antioxidant activity and anti-inflammatory activities of the rhizome and frond methanolic extracts of Drynaria quercifolia, Microsorum punctatum and Pyrrosia adnascens collected in Bukidnon, Philippines were determined using spectrophotometric methods. The presence of phytochemicals such as alkaloids, anthraquinones, phenolics, saponins, tannins and terpenoids in the fern samples were determined using thin layer chromatography. The total phenolic content ranged from 37.44 ± 0.91 to 130.44 ± 0.87 mg gallic acid equivalents (GAE) / gram sample. The total flavonoid concentrations varied from not detectable to 36.74 ± 2.17 µg quercetin equivalents (QE)/gram sample. The results of DPPH radical scavenging activity, expressed as percentage DPPH inhibition relative to ascorbic acid, and the egg albumin denaturation assay showed highest activity for the M. punctatum rhizome extracts with recorded values of 56.58 ± 2.35 % and 64.80 ± 5.79, respectively. The high antioxidant and anti-inflammatory activity of M. punctatum rhizome suggest that it can be regarded as a promising natural source of antioxidant and anti-inflammatory compounds.

Keywords: *ferns, antioxidant, anti-inflammatory, polypods, phytochemical*

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INTRODUCTION

The medicinal importance of pteridophytes (ferns and lycopods) has long been established, particularly by the Chinese who have been using plants in traditional medicine for more than 2000 years [1]. This traditional claim prompted many researchers to investigate on its pharmacological values that include among others, its phytochemical composition and bioactivities [2]. With more specific reports on antioxidant [3], antitumor [4], cytotoxic [5] and anticancer [6] activities in certain species, the pharmacological value of these plants becomes even more apparent.

In the Philippines, about 1,100 species of pteridophytes belonging to 144 genera and 39 families have been reported [7]. Several of these have been used in the traditional treatment of several illnesses [8] . However, scientific data to show the pharmacological significance of these Philippine species are wanting in the literature.

This initial report on the pharmacological potential of Philippine ferns is focused on three species of Family Polypodiaceae: 1) *Drynaria quercifolia* is noted by key informants from the Talaandig indigenous peoples of Bukidnon (personal communications) in the treatment of asthma, injuries and other inflammations. 2) *P. adnascens* is common and can be easily collected 3) *M. punctatum* is rather rare and thus, might easily become vulnerable.

In this paper, the methanolic extracts of these polypods were screened for flavonoids and phenolic content. This has been in line with reports indicating that secondary metabolites minimize oxidative damage by inactivating harmful free radicals particularly the reactive oxygen species thereby playing important protective role in the cells as antioxidants and anti-inflammatory molecules [9]. This initial

report on antioxidant and anti-inflammatory activities of selected Philippine ferns using DPPH radical scavenging activity and egg albumin assay is therefore intended to draw attention to the importance of these species.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant Material

Three species of ferns, namely, *D. quercifolia* (L.) J. Sm., *M. punctatum* (L.) Copel. and *P. adnascens* (Sw.) Ching under the family Polypodiaceae were collected from Mt. Kitanglad, Bukidnon and forested areas in Central Mindanao University, Musuan, Bukidnon (Plate 1). Plant samples were identified and voucher specimens were prepared and deposited at Central Mindanao University (CMU) Museum, Musuan, Bukidnon, Philippines. The plant materials were then cleaned and air-dried. The fronds and rhizomes of each plant species were ground separately and sieved to produce homogenous samples for extraction.

Plate 1. Left: *Drynaria quercifolia* (L.) J. Sm., (N 07°52.89 E 125°03.41), Center: *Microsorum punctatum* (L.) Copel. (N 07°52.93 E 125°03.29), Right: *Pyrrosia adnascens* (Sw.) Ching (N 07°52.93 E 125°03.29).

Extraction and Phytochemical Studies

A. Methanolic Extraction

Ground samples were exhaustively soaked in methanol for 3-5 days. Occasional stirring was done during the soaking. Samples were then filtered. The crude methanolic extracts were concentrated using rotary evaporator at 40°C and the concentrates were stored at -4°C.

B. Qualitative Phytochemical Analysis

Preliminary phytochemical screening for the presence of alkaloids, anthraquinones, phenolics, saponins, tannins and terpenoids were carried out via thin-layer chromatography (TLC) as described by [10].

C. Quantitative Phytochemical Analysis

C.1. Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteau method using a 96-well microtiter configuration was used for the total phenolic content determination. A 2000 mg/L test solutions in DMSO: methanol: water (15:5:2) were prepared [11]. Twenty (20) µL of each test solution was added with 100 µL 0.2 N Folin-Ciocalteau (Sigma) reagent and allowed to stand for 30 minutes. After 30 minutes, 80 µL 5% Na_2CO_3 was added as modification of the procedure in [10]. The mixtures were then incubated for 2 hours at room temperature before the absorbance was read at 750 nm using SpectraMAX 250. The obtained data were used to estimate the total phenolic content (mg gallic acid equivalent/g sample, mg GAE/g) of the samples using a standard calibration curve obtained from various concentrations (50, 100, 150, 250, 500) of gallic acid.

C.2. Determination of Total Flavonoid Content (TFC)

The total flavonoid content was determined using Aluminum Chloride Method in a 96-well microtiter configuration. A standard calibration curve was prepared from various concentrations (50, 100, 150, 250, 500) of quercetin. For the analysis of the samples, thirty (30) µL of 2000 mg/mL test solutions in DMSO: methanol: water (15:5:2) was added with 30 µL of 10% $AlCl_3$, 30 µL of 1M sodium acetate, and 110 µL of water. The mixture was then incubated at room temperature for 30 minutes and absorbance was measured against the blank at 415 nm using SpectraMAX 250. The total flavonoid content was expressed as µg quercetin equivalents per gram sample (µg QE/g).

D. Determination of Antioxidant Activity

The antioxidant activity of the samples was determined employing a DPPH Radical Scavenging Assay in a 96-well microtiter configuration. An aliquot (50 µl) of 2000 mg/mL test solutions in 15 DMSO: 5 methanol: 2 water were added with 150 µl 0.1mM DPPH. After 30 minutes of incubation, absorbance was read at 517 nm using SpectraMAX50. Ascorbic acid was used as reference while 15 DMSO: 5 methanol: 2 water was used as control. DPPH radical scavenging activities of the samples were computed in percentage relative to that of ascorbic acid using the formula below:

% DPPH radical scavenging activity = $[(A_0-A_1)/A_0]/ [(A_0-A_{AA})/A_0] \times 100$

where: A_0, A_1, and A_{AA} represent the absorbance of the control, sample and ascorbic acid, respectively.

E. Determination of Anti-inflammatory Property using Albumin Denaturation Assay

Fresh chicken egg albumin (50 µL) in 750µL 1x Phosphate - buffered saline (pH 6.4) was added with 30 µL of extract (1 mg/mL) or standard (diclofenac sodium). The reaction mixture was then incubated at 37°C for 15 min and then at 72°C in a heating block for 3 min. After cooling, turbidity was read at 660 nm with SpectraMAX50. All tests were carried out in triplicates. Percent inhibition was calculated [12] as follows: Percent (%) Inhibition = $(Absorbance_{control} - Absorbance_{sample})/ Absorbance_{control} \times 100$.

RESULTS AND DISCUSSION

A. Qualitative Phytochemical Analysis of Crude Methanolic Extracts

Qualitative phytochemical screening of plant extracts is an important preliminary step leading to the discovery of novel drugs. In this study, six phytochemicals were observed in the methanolic extracts of the frond and/or rhizome of the three fern species: alkaloids, anthraquinones, phenolics, saponins, tannins and terpenoids (Table 1). Among them, phenolics and saponins were detected in all extracts. The presence of these compounds may support ethno-medicinal reports on *D. quercifolia, P. adnascens*, and *M. punctatum* [8]. Furthermore, phytochemical compounds mentioned above are known to support varied biological activities such as antimicrobial, antioxidant, anticancer, and anti-inflammatory among others [2-6, 9, 12-14].

Table 1. Phytochemicals in the methanolic extracts of the three fern species in Bukidnon, Philippines

Fern species		Phytochemicals					
		Alkaloids	**anthraquinones**	**phenolics**	**saponins**	**Tannins**	**terpenoids**
***Drynaria quercifolia* (L.) J. Sm.**	**frond**	+	-	+	+	+	-
	rhizome	-	+	+	+	-	+
***Microsorum punctatum* (L.) Copel**	**frond**	+	+	+	+	-	-
	rhizome	-	+	+	+	-	+
***Pyrrosia adnascens* (Sw.) Ching**	**frond**	+	+	+	+	+	+
	rhizome	-	+	+	+	-	+

+ presence - absence

B. Quantitative Phytochemical Analysis of Crude Methanolic Extracts

Total Phenolic Content

The total phenolic content (TPC) of the crude methanolic extracts of the three fern species (Figure 2) ranged from 37.44 ± 0.91 to 130.44 ± 0.87 mg GAE / g sample with rhizomes showing higher levels than the fronds except for *P. adnascens* (Sw.) Ching. However, the obtained data are contrary to some reported results. Tan and Lim [13] have reported higher TPC in the fronds of *D. quercifolia* (2939 ± 469 mg GAE/ 100g sample) than in the rhizome (1732 ± 437 mg GAE/ 100g sample). Furthermore, TPC levels of *D. quercifolia* and *M. punctatum* from Bukidnon were higher compared to previously reported data [13,14]. Accordingly, regions where plants grow influence its chemical constituents [15].

Total Flavonoid Content (TFC)

Flavonoids are phenolic substances that exhibit a wide range of biological activities that includes antioxidant, radical scavenger, anti-cancer, antibacterial, and anti-ageing [16].

As shown in Figure 3, flavonoids were detected in the methanolic extracts of the frond (6.69± 2.60 µg QE/g sample) and rhizome (17.38 ± 7.82 µg QE/g sample) of *M. punctatum* (L.) Copel. The highest level (36.74 ± 2.17 µg QE/g sample) was observed in the methanolic extracts of *D. quercifolia* (L.) J. Sm. rhizomes while its fronds showed no detectable levels. Neither was the methanolic extracts of *P. adnascens* registered detectable levels of flavonoids.

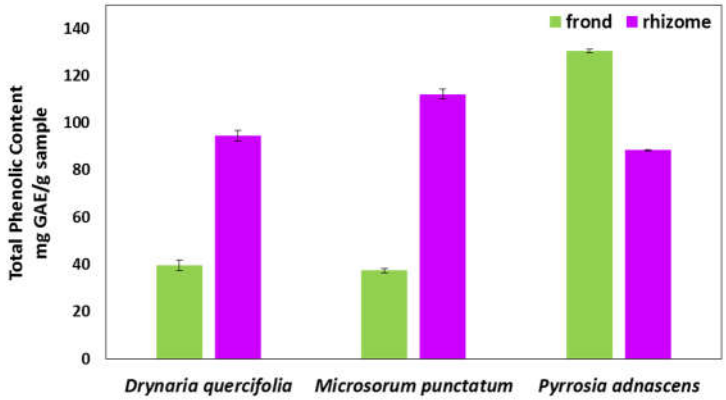

Figure 2. Total phenolic content (mg GAE)/g sample) of crude methanolic extracts.

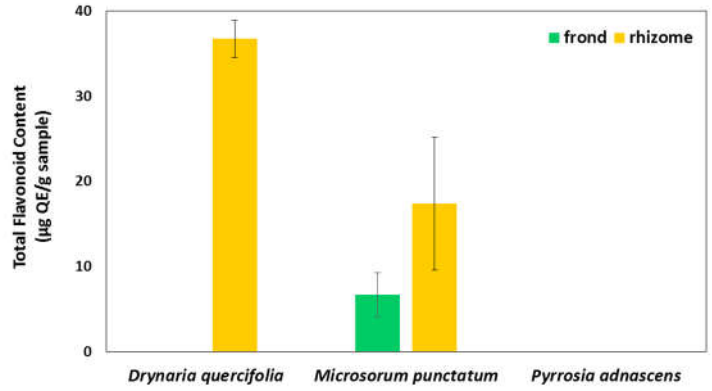

Figure 3. Total flavonoid content (µg QE/g sample) of the three species of polypods.

DPPH Radical Scavenging Assay

The antioxidant activities of the frond and rhizome methanolic extracts of *D. quercifolia* , *M. punctatum* and *P. adnascens* were evaluated for their DPPH radical scavenging activity.

Results show that all frond and rhizome methanolic extracts of the three species showed DPPH scavenging activities ranging from 21.94% (*D. quercifolia* fronds) to 56.28% (*M. punctatum* rhizomes). Generally. the rhizome methanolic extracts exhibit higher scavenging activity than the fronds in all species (Table 2).

These observations imply that the methanolic extracts of the fern samples may contain antioxidant molecules that quenched DPPH radicals. Further, the three fern species studied may have potential protective agents capable of scavenging free radicals that may damage proteins, lipids and nucleic acids *via* oxidation [9].

Table 2. DPPH radical scavenging activity (%) of fern methanolic extracts.

FERN SPECIES	% DPPH Radical Scavenging Activity Relative to Ascorbic Acid*	
	Frond	rhizome
Drynaria quercifolia (L.) J. Sm.	21.94±1.49	26.19±2.20
Microsorum punctatum (L.) Copel.	23.30±0.60	56.28±2.35
Pyrrosia adnascens (Sw.) Ching	28.12±1.31	43.55±0.58

*values are expressed as means ± SE (n=3)

Although the frond methanolic extracts of *P. adnascens* showed the highest TPC (130.44 mg GAE/g sample) among the three fern species, its DPPH radical scavenging activity (28.12%) was found lower than the rhizome extracts of *M. punctatum.* Furthermore, *D. quercifolia*, with the highest TFC (36 mg QE/g sample) exhibited the lowest DPPH radical scavenging activity of 26.19%. This suggests no correlation between high levels of TPC and TFC and DPPH radical scavenging activity for the fern species under study. Presence of other antioxidant compounds maybe involved. Thus, further studies are needed.

Anti-inflammatory Assay using Albumin Denaturation Assay

When albumin is heated, it undergoes denaturation and expresses antigens associated with hypersensitive reaction related to diseases such as serum sickness, glomerulonephritis, rheumatoid arthritis and systemic lupus erythematosus [12].

The results on the potential of *D. quercifolia* , *M. punctatum* and *P. adnascens* to inhibit denaturation of albumin in vitro (Table 3) show that only the methanolic rhizome extract of *M. punctatum* had greater than 50% inhibition (64.80±5.79%). The methanolic extracts of *M. punctatum* frond and *P. adnascens rhizomes* gave a value of 39.00±11.31% and 36.29±4.83%, respectively. *D. quercifolia,* on the other hand, exhibited a very low inhibition of albumin denaturation.

Table 3. Percentage inhibition of albumin denaturation for anti-inflammatory assay of three polypod species from Bukidnon, Philippines.

Species	Percent Inhibition of Albumin Denaturation (%)	
	Frond	Rhizome
Drynaria quercifolia (L.) J. Sm	n.d.	6.56 ± 6.62
Microsorum punctatum (L.) Copel.	39.00 ± 11.31	64.80 ± 5.79
Pyrrosia adnascens (Sw.) Ching	22.59 ± 2.72	36.29 ± 4.83

CONCLUSION AND RECOMMENDATIONS

The present investigation provides useful information on the DPPH radical scavenging and anti-inflammatory activities and total phenolic and flavonoid contents of the three Polypodiaceae species. Phytochemical analysis revealed the presence of medicinally important constituents in the plants studied. The extracts from these three plant species exhibited antioxidative and anti-inflammatory activities, indicating that they can be potential natural sources of antioxidants and anti-inflammatory compounds.

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REFERENCES

1. Ma, X.Y., Xie, C.Y., Liu, C., Song, J.Y., Yao, H., Lou, K., Zhu, Y.J., Gao, T., Pang, X.H., Qian,J., & Chen, S.L.. (2010). Species identification of medicinal pteridophytes by a DNA barcode marker, the chloroplast psbA-trnH intergenic region. *Biol. Pharm. Bull*. 33(11):1919-1924.
2. Shin, S.L., & Lee, C.H. (2010). Antioxidant effects of the methanol extracts obtained from aerial part and rhizomes of ferns native to Korea. *Korean J. Pant Res*. 23:38–46.
3. Dion, C., Haug, C., Guan, H., Ripoll, C., Spiteller, P., Coussaert, A., Boulet, E., Schmidt, D., Wei, J., Zhou, Y., & Lamottke, K. (2015). Evaluation of the anti-inflammatory and antioxidative potential of four fern species from China intended for use as food supplements. *Nat Prod Commun*. 10(4):597-603.
4. Huang, X.H., Xiong, P.C., Xiong, C.M., Cai, Y.L., Wei, A.H., Wang, J.P., Liang, X.F., & Ruan, J.L. (2010). In vitro and in vivo antitumor activity of Macrothelypteris torresiana and its acute/subacute oral toxicity. *Phytomedicine.* 2010 Oct;17(12):930-4. doi: 10.1016/j.phymed.2010.03.006. Epub 2010 Apr 9.
5. Uddin, S.J., Grice, D., & Tiralongo, E. (2012). Evaluation of cytotoxic activity of patriscabratine, tetracosane and various flavonoids isolated from the Bangladeshi medicinal plant Acrostichum aureum. *Pharmaceutical Biology*. 2012 Oct; 50(10):1276-80. doi:10.3109/13880209.2012.673628.
6. Wu, M.J, Weng, C.Y., Wang, L., & Lian, W. T. (2005). Immunomodulatory mechanism of the aqueous extract of sword brake fern (Pteris ensiformis Burm.). Journal of Ethnopharmacology. Volume 98, Issues 1–2, 8 April 2005, Pages 73–81.
7. Delos Angeles, M. & Buot, I. (2012). Orders and Families of Philippine Pteridophytes. *Journal of Nature Studies*. 11 (1&2): 19-33.
8. Amoroso, V.B. (2013). Medicinal Ferns and fern allies of Mindanao, Bukidnon, Philippines 2nd edition. *CMU Instructional and Materials Development Center*.
9. De Beer, D., Joubert, E., Gelderblom, WCA & Manley, M. (2002). Phenolic Compounds: A Review of Their Possible Role as In Vivo Antioxidants of Wine. S. *Afr. J. Enol. Vitic*. 23(2): 50-61.
10. Gracelin, D.H.S., De Britto, A.J., Kumar, P.B.J.R. (2013). Qualitative and quantitative analysis of phytochemicals in five Pteris species. *International Journal of Pharmacy and Pharmaceutical Species* 5(1): 105-107.
11. Amoroso, V.B., Lagumbay, A.J., Mendez, R.A., Dela Cruz, R.Y., & Villalobos, A.P. (2014). Bioactives in three Philippine edible ferns. *Asia Life Sciences* 23(2): 445-454.
12. Hossain, H., Jaran, I.A., Nimmi, I., Hossain, A. & Kawsar, H. (2011). Anti-Inflammatory Activity of the Ethanolic Extract of *Acrostichum aureum* (Linn.) root. *Bangladesh Pharmaceutical Journal*,14(2), 2011.
13. Tan, J. B. L. & Lim, Y. Y. (2015). Antioxidant and tyrosinase inhibition activity of the fertile fronds and rhizomes of three different Drynaria species. *BMC research notes*, *8*(1): 1.

14. Lai, H.Y. & Lim, Y.Y. (2011) Evaluation of Antioxidant Activities of the Methanolic Extracts of Selected Ferns in Malaysia. *International Journal of Environmental Science and Development*, 2(6)
15. Bayili, R.G., Abduol-Latif , Kone, O.H., Diao, M., Bassole, I.H.N. & Dicko, M.H. (2011). Phenolic compounds and antioxidant activities in some fruits and vegetables from Burkina Faso. *African Journal of Biotechnology* 10(62): 13543-13547.
16. Jayanthy, A., Prakash Kumar, U. & Remashree, A.B. (2013). Seasonal and Geographical Variation in Cellular Characters and Chemical Contents in Desmodium gangeticum (L.) DC – An Ayurvedic Medicinal Plant. *International Journal of Herbal Medicine* 2(3):34-37
17. Sharma, D.K. (2006). Pharmacological properties of flavonoids including flavonolignans – Integration of petrocrops with drug development from plants. *Journal of Scientific and Industrial Research* 65: 477-484.

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