Original Research Article

EMODIN ISOLATION FROM THE LEAVES OF *PTERIDIUM ACQUILINUM*

Abstract

This study was designed to isolate and characterize the active compound(s) from the leaf extract of *Pteridium acquilinum* after the aqueous and methanolic leaf extracts had been investigated on some female rats hormones One anthraquinone emodin (1, 3, 8-trihydroxy-6-methyl-anthra-quinone) was successfully purified from the methanolic extract of the medicinal plant by Chromatography (VLC, TLC and Sephadex). The initial elution was with n hexane– ethyl acetate–methanol (18:22:3, v/v/v) as the two-phase solvent system and yielded 3.4 mg of emodin. The Vacuum Liquid Chromatography (VLC), fraction (from the methanol extract) was analyzed by Nuclear magnetic resonance (NMR) and the chemical structure of the anthraquinone was confirmed by ^{1}H-NMR and ^{13}C-NMR analyses. This is the first time this anthraquinone, emodin is being reported from *P. aquilinum*.

Keywords: *P. aquilinum*, anthraquinones, Vacuum Liquid Chromatography (VLC), emodin.

Introduction

Anthraquinones constitute an important class of natural compounds with widespread distribution and a wide range of activities. Every year a large number of anthraquinones, having varied substitution patterns, are isolated from Nature (Zhang *et al.,* 2010 and Mishra *et al.*, 2010). *Rumex japonicus* Houtt., a rich source of anthraquinones, has been traditionally used for the treatment of heat phlegm, jaundice, constipation, scabies, and uterine hemorrhage in East Asian countries such as China, Korea, and Japan (Jiangsu 1977). Zhao *et al.,* (2009) isolated emodin from the *Rumex japanicus* and found to possess protective effects on hepatocytes and cholangiocytes. In the report of their research, Zhu and colleagues ~~conclusion~~concluded, that anti-inflammatory effects of emodin against collagen-induced arthritis in mice may be due to its ability to inhibit pro-inflammatory mediators and that it may be a promising potential therapeutic reagent for arthritis treatment Zhu et al., (2013) ~~In their previous research~~Similarly, Khan *et al*., (2016)

established that both the aqueous and methanolic extracts of the plant significantly ($p < 0.05$) increased the levels of follicle stimulating hormones (FSH) and luteinizing hormone (-LH) and showed a similar pattern with the standard drug, (clomiphene at 20mg/kgbwt) used thus portraying its potential to enhance fertility by increasing serum levels of FSH and LH which in turn increase the number of oocytes released at ovulation possibly through its antioxidant properties.

Pteridium aquilinum (brake, bracken or common bracken), also known as "eagle fern," is a species of African fern occurring in temperate and subtropical regions in both hemispheres. The extreme lightness of its spores has led to its global distribution, ~~Alonso *et al.*, 2001~~ (Alonso-Amelot ~~M.E.,~~and Avendano ~~M~~ (2001). The Higgi tribe in Michika Adamawa State Nigeria called it "*Kudumbula",* Hausa called it "*wuyan giwa*". The leaves of *Pteridium aquilium* are used locally in Mubi and Michika local Government Areas of Adamawa State, Nigeria, for the treatment of bacterial diseases. The young stems are used as vegetables but their consumption is linked to the highest incidence of stomach cancer (Gomes *et al.,* 2011). The leaves are traditionally given in form of decoction to women to boost their sexual drive and to improve fertility among the *Kamue (Higgi)* people (Personal Contact, 2016) of Adamawa. In particular, emodin demonstrates anti-neoplastic, anti-inflammatory, anti-angiogenesis, and toxicological potential for use in pharmacology, both *in vitro* and *in vivo.* Emodin demonstrates cytotoxic effects (e.g., cell death) through the arrest of the cell cycle and the induction of apoptosis in cancer cells. The overall molecular mechanisms of emodin include cell cycle arrest, apoptosis, and the promotion of the expression of hypoxia-inducible factor 1α, glutathione S-transferase P, N-acetyltransferase, and glutathione phase I and II detoxification enzymes while inhibiting angiogenesis, invasion, migration (shu and Jing, 2012). Emodin in vegetative organs may help protect plants against herbivores, pathogens, competitors and extrinsic abiotic factors, such metabolites also enhance plant defenses by using different molecular targets of specific enemies through a variety of mechanisms of action (Ido, 2002).

It is against this background that this research is designed to isolate, purify and characterize the active principles from the leaf extract of the plant with the available spectroscopic techniques in order to know what is responsible for the claimed activities.

MATERIALS AND METHODS

Sample Collection and Authentication

Matured green leaves of *P. acquilinum* were collected in and around Michika Local Government Area, Adamawa State on 28th July 2014. Plant species were authenticated in the state Ministry of Forestry, Mubi Adamawa State and a specimen of the plant was kept in their Herbarium. The Forestry Herbarium Index number (FHI) is 1030.

Sample Preparation

The leaves of were thoroughly washed with tap water to avoid dusts and other unwanted materials accumulated on the leaves from their natural environment. The dust free leaves were allowed to dry under shade in chemistry laboratory. The dried leaves were pulverized by using mortar and pestle. Finally, fine powder was obtained from the powdered leaves by sieving through the kitchen strainer and used for extraction.

Sequential Extraction using Microwave Assisted Extraction (MAE)

250g of the powdered sample was put into a glass (2.5 L) and 500 cm^3 of n-hexane were added to this. The bottle was put in a microwave set at defrost for 3 minutes and removed and cooled. This was repeated 10 times. After filtration, the residues were further extracted

the same way using ethyl acetate followed by methanol. The yields were as follows: N-hexane, 10g, ethyl acetate, 15g and methanol, 28g.

The Vacuum Liquid Chromatography (VLC)

15g of the ethyl acetate extract was dissolved with ethyl acetate and mixed with celite (a filter aid) and left to dry. The dried mixture was then loaded on to the VLC packed with silica gel. This was washed 20 times with 20 cm^3 each of n-hexane. The gradient of the ethyl acetate - methanol was used to elute the column and 60 fractions were collected.

Thin Layer Chromatography (TLC)

TLC was carried out on all the fractions using a solvent system of 9:1 v/v chloroform in methanol. Fractions 12- 19 were combined and allowed to dry. The dried sample was then dissolved in ethyl acetate but yellow crystals were left undissolved. This was carefully washed. A yellow component was thus purified, spotted on the TLC plate and was labeled as fraction 1 (F1).

Sephadex column

The dissolved components of fractions 12-19, were put on the sephadex column and eluted with solvent system of 1:3:3 v/v/v methanol, chloroform and ethyl acetate. 70 fractions of 20 cm^3 each were collected and spotted on the TLC plate.

Nuclear Magnetic Resonance (NMR) Analysis

Fraction 1 of the VLC, (yellow colour) purified using sephadex column was sent for NMR analyses. The NMR spectra were run at SIPBS, University of Strathclyde, Glasgow, UK. on a JEOL-LA-400 MHz FT-NMR spectrophotometer.

RESULTS AND DISCUSSION

Chromatography (VLC, TLC and Sephadex)

The yellow component was purified from fractions 12-19 since it could not readily dissolve in ethyl acetate. The TLC was carried out in the solvent system of 9:1 v/v chloroform and methanol. This yellow component gave one spot on the TLC with a retention factor (Rf) of; Rf (F1) = 2.2/3.7 = 0.59

Structural Identification, Characterization and literature Comparison of Fraction 1

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) of the spectra were recorded on NMR machine using deuterated solvents and the results are presented in **Tables 1 and 2** and their values are compared with those in literature reported by Shuying et *al*., 2011.in Table 3

Table 1: ^{1}H NMR (400 MHz, DMSO)

Position	chemical shift (δ) ppm (J in Hz)	multiplicity
1	-	-
2	7.20	1H, m
3	-	-
4	7.53 (1.57)	1H, d
5	6.62 (2.40)	1H, d
6	-	-
7	7.14 (2.39)	1H, d
8	-	-
9	-	-

10	-	-
11	-	-
12	-	-
13	-	-
14	-	-
3-CH3	2.43	3H, s
1-OH	12.06	1H, s
8-OH	12.12	1H, s
6-OH	11.30	1H,s

Table 2: ^{13}C NMR Result (DMSO)

Position	**Chemical shift (δ)**	**type of C**
1	161.8	C
2	124.6	CH
3	148.7	C
4	121.0	CH
5	108.4	CH
6	166.2	C

7	109.5	CH
8	164.9	C
9	190.7	C
10	182.2	C
11	113.9	C
12	133.4	C
13	108.5	C
14	135.7	C
3-CH3	22.0	CH_3

Table 3: Comparison of Experimental NMR signal with literature values as reported by Shuying et *al*., 2011, from *Rumex japonica.*

Position	**Experimental**		**Literature**	
	Proton δ (J/Hz)	**Carbon**	**Proton, δ (J/ Hz)**	**carbon**
1	-	161.8	-	161.1
2	7.20	124.6	7.15 (1.2)	123.9
3	-	148.7	-	148.0
4	7.53	121.0	7.48 (1.2)	120.2
5	6.62	108.4	6.59 (2.0)	107.7

6	-	166.2	-	16
7	7.14	109.5	7.11 (2.0)	108.7
8	-	164.9	-	164.1
9	-	190.7	-	189.4
10	-	182.2	-	181.0
11 (9a)	-	113.9	-	113.1
12 (4a)	-	133.4	-	132.6
13 (8a)	-	108.5	-	108.5
14 (10a)	-	135.7	-	134.9
3-CH_3	2.43	22.0	2.38	21.4
1-OH	12.06	-	12.06	-
8-OH	12.12	-	12.00	-
11-OH	11.30	-	11.35	-

From the ^{1}H NMR (**Table 1**) the sample contains; 4 sets of aromatic (7.53, 7.23, 7.12 and 6.62), two sets of phenolic (12.12 and12.06) and one set of methyl (2.38, attached to aromatic ring) protons.

The ^{13}C NMR chemical shifts (**Table 2**) revealed the presence of; 12 aromatic (166.2, 164.9, 161.8, 148.7, 135.7, 133.4, 124.6, 121.0, 113.9, 109.5, 108.5 and 108.4), 2 carbonyl (190.7 and 182.2) and a methyl (22.0, benzylic) carbons. This suggests that there are two aromatic nuclei that are joined through two carbonyl carbons. This is an anthraquinone It can be suggested again that one of the aromatic nuclei of the anthraquinone contains a methyl and a hydroxyl substituent and the other contains two, hydroxyl substituents one at position 8 and the other at position 6.

With the help of 2D NMR spectra Correlation (COSY), Heteronuclear Single Quantum Correlation (HSQC) and Heteronuclear Multiple Bonds Correlation (HMBC), the sample was identified as emodin, (1, 6, 8-trihydroxy-3-methylemodin) an anthraquinone. Both 1D and 2D NMR spectra and also a comparison of the structure of the isolated compound with some literature values of same compound isolated from *Rumex japonica,* further confirmation was attested for.

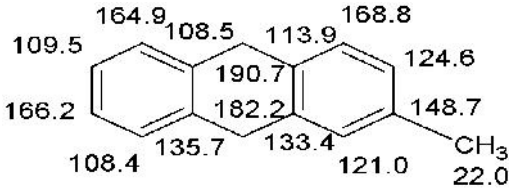

Figure 1; ^{13}C NMR Chemical Shifts values of Emodin

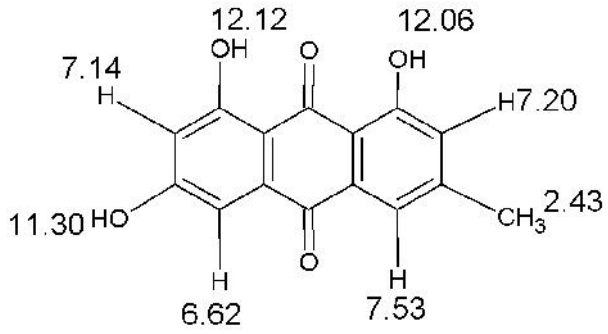

Figure 2; ^{1}H NMR Chemical Shifts of Emodin

DISCUSION

The leaves of *P. aquilinum* were extracted with ethyl acetate and methanol and quite a good yield was obtained from both solvents. The phytochemical screening of this plant showed that it

contained various classes of compounds which confirmed its traditional use. The ethyl acetate - methanol extracts were chosen for isolation because, the phytochemical screening revealed the presence of more components in them. From the vacuum liquid chromatography, a yellow sample (emodin) was obtained and sent for NMR analysis.

When the ethyl acetate solution of emodin, was incidentally mixed with a detergent solution, the colour changed to deep red. This was further tested with other kinds of detergents which contained two ingredients in common- sodium bicarbonate and optical brightener. The solution of emodin was separately mixed with solutions of NaOH and HCl to test for the acidity effect, but no colour change was observed. When mixed with a solution of sodium carbonate, the colour changed to light orange showing the unsaturated nature of the compound.

The signals / Chemical shifts of the yellow substance, emodin were recorded successfully based on 1& 2-dimensional NMR spectroscopy. The NMR experimental values of emodin are in agreement with the ones reported by (Shuying *et al.,* 2011) as compared in **Table 3**.

Conclusion

From the methanolic and ethyl acetate crude extracts of *P. aquilinum,* emodin was successfully isolated for the first time which appeared to be the dominant compound in the leaves of the plant. This could be used for bioactivity studies and the method could be used for other plant isolations

Recommendation:

Emodin (the isolated compound) should be tested for fertility- boosting effects since the plant from which it was isolated is used to treat infertility problem by some people and the deep red

colouration produced on mixing the methanol solution of emodin with solution of detergents should be investigated spectroscopically in order to reveal the structure of the red product.

REFERENCES

Alonso-Amelot M.E., Avendano M. (2001),"Possible association between gastric cancer and racken fern in Venezuela: An epidemiologic study. *"International Journal of Cancer*. 91 (2) (Pp 252-259).

Gomes J, Magalhães A, Michel V, Amado I, Aranha P, Ovesen RG, Hansen HC, Gärtner F, `` Reis CA, Touati E.(2011),"*Pteridium aquilinum* and its ptaquiloside toxin induce DNA damage response in gastric epithelial cells, a link with gastric carcinogenesis". *Toxicol Sci*.

Ido Izhaki (2002): Emodin – a secondary metabolite with multiple ecological functions in higher plants

(pages 205–217) | DOI: 10.1046/j.1469-8137.2002.00459.

Jiangsu New Medical College (1977). *Dictionary of Chinese Medicine*; Shanghai Science and Technology Press: Shanghai, China, p. 964.

Khan, M.E., E.T. Williams, A. Abel, and I. Toma (2016); Effects of the aqueous and methanolic leaf extracts of *Pteridium acquilinum* (*linnaeus*) on some female rats hormones. *Direct Research journal of public health and environmental technology* (DRJPHET), Vol. 1 (2), PP 08 – 13. http://directresearchpublisher.org/journal/drjphet

Mishra, B.B.; Kishore, N.; Tiwari, V.K.; Singh, D.D.; Tripathi, V. A (2010), Novel antifungal anthraquinone from seeds of *Aegle marmelos* Correa (family Rutaceae). *Fitoterapia 81*,104-107

Personal contact: Pa Admu M (2016): Traditional herbalist verse with medicinal plants of the Mubi and Michika regions of Adamawa State

Shu-Chun Hsu and Jing-Gung Chung Evaluation of the pharmacological effects of emodin *Biomedicine* 2012, 2(3): 108 – 116;

Shuying, G., Feng B., Ruonan Z,. Jiankang M and Wei. W (2011): Preparative isolation of three Anthraquinones from *Rumex japanicus* by high speed counter-current Chromatography. *Molecules*, 16, 1201 – 1201 www.mdpi.com/journal/molecules

Zhang, C.L.; Guan, H.; Xi, P.Z.; Deng, T.; Gao J.M. (2010), Anthraquinones from the roots of *Prismatomeris tetrandra*. *Nat*. *Prod*. *Commun*., *5*, 1251-1252.

Comment [MA1]: incomplete

Zhao, Y. L., Wang, J.B., Zhou, G.D., Shan, L. M., Xiao, X. H (2009): Investigation of free anthraquinones from rhubarb against 4 naphthylisothiocyanate – induced cholestatic live~~r~~ ~~i~~njury in rats. *Basic Clin. Pharmacol. Toxicol*, 104, 463 — 469

Zhu Xiaofeng Kai Zeng Yu Qiu Fuhua Yan Caizhu Lin (2013): Protective effects of emodin combined with danshensu on experimental severe acute pancreatitis. *Inflammation 3*6(6): 1253 – 9;