



β -Sitosterol and ferulic acid from the hexane extract of *Bryophyllum pinnatum* stems

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Abstract

This study aims at the isolation and characterization of bioactive constituents from the stem of *Bryophyllum pinnatum* in n-hexane extract. Spectroscopic and chromatographic methods were used. The isolation was conducted through vacuum Liquid Chromatography (VLC) in the polarity gradient of solvent systems, Thin Layer Chromatography (TLC) was used to confirm their purity, and purification was carried out using gel filtration chromatography (GFC). Characterization of the isolated compounds was carried out using Nuclear Magnetic Resonance Spectroscopy. Experimental data of active compounds was in agreement with NMR data of β -Sitosterol and ferulic acid available in the literature, Elemental analysis showed that percentages of C, H and O in the isolated compounds conformed with calculated values of those in β -Sitosterol and ferulic acid. Isolation and characterization of β -Sitosterol and ferulic acid was reported for the first time, from the stem of *Bryophyllum pinnatum* n-hexane extract.

Keywords: *Bryophyllum pinnatum*, Microwave, NMR, VLC, GFC, TLC plate.

Introduction

Bryophyllum pinnatum popularly known as “Resurrection plant” is a perennial herb used in folkloric medicine in tropical Africa (Amabe et al., 2014). It contains bioactive substances such as saponins, terpenoids, phenolic compounds, steroids, alkaloids, and flavonoids (Edewor et al., 2015). The plant exhibits a wide range of biological and pharmacological activities such as anti-cancer, anti-inflammatory, diuretic, oxytoxic, laxative, antispasmodic, antihypertensive, anti-diabetic and antimicrobial activities (Hussan et al., 2023). *Bryophyllum pinnatum* is an erect, succulent, perennial shrub that grows to about 1.5m tall and reproduces through seeds (Edewor et al., 2015). Thus it can be propagated through its stems or leaf cutting. It is an ornamental plant and also grows as weeds around plantations (Edewor et al., 2015; Okwu et al., 2006). *Bryophyllum pinnatum* is used in ethnomedicine to treat earache, burns, abscesses, ulcers, insect bites, whitlow, diarrhea and cithiasis (Okwu et al., 2006). The Ethyl acetate extracts of the stem were active against *S. aureus*, *E. coli*, *P. aeruginosa* and *K. Pneumonia* (MIC 5.0 mg/mL) and *S. dysenteriae*, *C. albicans*, *Microsporumspp*, and *T. rubrum* (MIC 10 mg/mL). The methanol extract was also found to be

active against *P. aeruginosa*, *C. albicans* and *A. niger*. *S. aureus* with an MIC of 6.29 mg/ml (Dikko et al., 2016). This study is aimed at isolating the constituents of the plant’s stem responsible for its reported bioactivity.

Methods and material

Stems of *Bryophyllum pinnatum* were collected from Aguluezechuwu in Aguata L.G.A of Anambra State Nigeria in August 2015. It was authenticated by Dr B. A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria. A herbarium specimen with voucher number FHI 107762 was deposited with the Forest Research Institute of Nigeria. The stems were cleaned, rinsed with tap water and air-dried at ambient temperatures to a constant weight over four weeks. They were then powdered using a wooden mortar.

Microwave-assisted extraction

This was carried out according to methods described by (Dikko et al., 2016). Hexane 1.3 L was added to the plant material (1kg) and was heated for three minutes in a modified domestic kitchen microwave (Mio-star, Model 7173.295,

Germany) set at 70 Watts/Defrost Function. This was repeated ten times with about 15 minutes cooling intervals ensuring that the temperature does not rise above 70°C and any pressure build up was vented after every successive heating. The extract was then allowed to cool at ambient temperature, filtered, air-dried, and stored until required.

Isolation of compounds

The hexane extract (6.8 g) was adsorbed unto Celite and subjected to VLC using 30g of silica gel (GF 254 MERCK). The column was eluted 20 times with hexane (40 mL), then with increasing amounts of ethyl acetate in hexane until 100% ethyl acetate (Dikko et al., 2016). The fractions were examined by TLC and similar fractions were combined based on their TLC profiles. Combined fractions BPH 02 (10 – 14)

showed better separation and were subjected to Sephadex (GFC) for purification. Cotton wool was inserted down into an empty column and Sephadex in methanol was poured into the column. The column was allowed to settle and was equilibrated with a solvent mixture of Methanol: ethyl acetate: dichloromethane (4:4:1). The combined fractions were loaded on the top of Sephadex and eluted isocratically collecting 50 fractions (Table 1). The column fractions were monitored by TLC and similar ones were combined (Cyprian et al., 2019). Combined fractions SBPH 25–39 were re-chromatographed on Sephadex to obtain further sub-fractions. From these, sub-fractions SBPH²-03 and SBPH²-04 were confirmed to be pure by TLC with ethyl acetate/diethyl ether (50:50) as they gave single spots. These fractions were allowed to dry and labeled SO-11 and SI-01 respectively and were analyzed using NMR spectroscopy.

Table 1. Compound isolation and purification

VLC		GFC			
		BPH 10 – 14		SBPH 25-39	
Fraction code	Combined fractions	Fraction code	Combined fractions	Fraction code	Combined fractions
BPH 01	01 -- 09	SBPH 01	1 – 24	SBPH ² 01	1 – 16
BPH 02	10 – 14	SBPH 02	25 – 39	SBPH ² 02	18 – 23
BPH 03	15 – 22	SBPH 03	30 – 39	SBPH ² 03	24
BPH 04	23 – 31	SBPH 04	40 – 50	SBPH ² 04	25 – 35
BPH 05	32 – 33			SBPH ² 05	36 – 50
BPH 06	34 – 39				
BPH 07	40 – 45				

BPH: *Bryophyllum pinnatum* hexane, SBPH: Sephadex *Bryophyllum pinnatum* hexane

Results and discussion

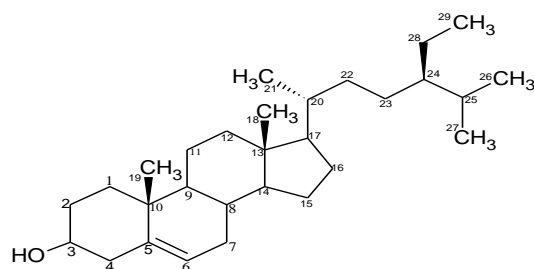
Characterization of SBPH²-04 as β -Sitosterol (1)

The compound was obtained as colourless needles with melting point 134-137°C and on TLC gave an R_f of 0.78 using the solvent system of ethyl acetate/diethyl ether (50:50). The ¹H NMR spectrum revealed one proton multiplet at δ_H 3.54 ppm the position and multiplicity of which was indicative of the H-3 of a C-3 hydroxy substituted triterpenoid. An olefinic proton signal at δ_H 5.34 integrated for one proton was typical of a sitosterol/stigmasterol type steroid. The spectrum also showed doublets at δ_H 0.82 and 0.87 which were attributed to methyl groups at C-26 and C-27 and other methyl groups could be assigned to C-21 and C-29 at δ_H 0.93 and δ_H 0.89. The ¹³C spectrum showed characteristic signals at 140.7 and 121.7 ppm, which were assigned to C-5 and C-6 olefinic carbons. The Spectrum showed other twenty-seven carbon signals including six methyls and three quaternary carbons. The

chemical shift values were similar to those of the stigmasterol except that two olefinic protons were absent while a methylene signal at δ_H 1.06 and δ_H 1.18 were present for H-22 and H-23 respectively. The above spectral features are in agreement to those observed for β -Sitosterol (1) and was confirmed by comparison with literature Tables 2 and 3.

Characterization of SBPH²- 03 as ferulic acid

The compound gave an R_f of 0.82 on TLC using the solvent system ethyl acetate/diethyl ether (50:50). The ¹H-NMR spectrum showed two olefinic trans coupled protons at δ_H (ppm) 7.57 (1H, d, J=15.9 Hz, H-7), 6.25 (1H, d, J= 15.9 Hz, H-8), three aromatic ring protons at 7.05 (1H, dd, J= 8.5, 2.3 Hz, H-2), 6.76 (1H, d, J= 8.5 Hz, H-5) and 7.57 (1H, d, J= 2.3 Hz, H-6). It also showed a methoxy group signal at δ_H 3.91 (3H, s, 3-OCH₃). This is typical of a cinnamic acid derivative and with one methoxy group substituent; it must be ferulic acid.



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Table 2. Experimental and Literature data for β -Sitosterol¹³C NMR

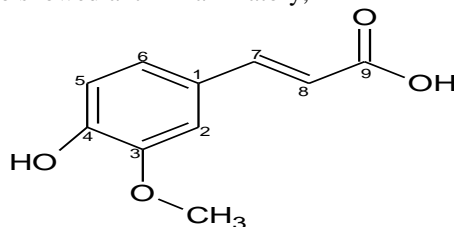
Position	Experimental data ¹³ C(δ ppm)	(Firouz et al., 2006) ¹³ C(δ ppm)	(Arjun et al., 2010) ¹³ C(δ ppm)	(Soumia et al., 2012) ¹³ C(δ ppm)	(WU et al., 2014) ¹³ C(δ ppm)	(Ahmed et al., 2013) ¹³ C(δ ppm)
1	37.20	37.70	37.28	37.20	37.26	37.29
2	31.60	32.30	31.69	31.60	31.65	31.95
3	71.79	72.20	71.82	71.80	71.78	71.84
4	42.25	42.80	42.33	42.300	42.29	42.36
5	140.72	141.20	140.70	140.70	140.76	140.8
6	121.73	122.10	121.72	121.70	121.69	121.73
7	31.85	32.10	31.69	31.90	31.91	31.71
8	31.89	32.30	31.93	31.80	31.91	31.95
9	50.07	50.60	50.17	50.10	50.15	50.19
10	36.47	36.90	36.52	36.50	36.51	36.18
11	21.05	21.50	21.10	21.10	21.09	21.12
12	39.72	40.20	39.8	39.70	39.79	39.82
13	42.29	42.80	42.33	42.300	42.32	42.36
14	56.72	57.20	56.79	56.70	56.77	56.81
15	24.28	24.70	24.37	24.30	24.30	24.33
16	28.24	28.70	28.25	28.20	28.24	28.26
17	55.98	56.50	56.09	56.00	56.08	56.11
18	11.84	12.40	11.86	11.80	11.85	11.88
19	19.82	19.80	19.40	19.40	19.39	19.41
20	36.13	36.60	36.52	36.10	36.14	36.54
21	19.00	19.20	18.79	18.80	18.78	19.07
22	33.88	34.40	33.98	33.90	33.96	34.00
23	25.95	26.50	26.14	26.00	26.12	26.16
24	45.77	46.20	45.88	45.80	45.85	45.89
25	29.70	29.60	28.91	29.10	29.18	29.23
26	18.75	20.20	19.80	19.80	19.81	19.83
27	19.39	19.50	18.79	19.00	19.05	18.81
28	23.01	23.50	23.10	23.00	23.08	23.12
29	11.97	12.30	11.99	12.00	11.98	12.01

Table 3. Experimental and Literature data for β -Sitosterol¹H NMR

Position	Experimental data ¹ H (δ ppm)	(Firouz et al., 2006) ¹ H(δ ppm)	(Arjun et al et al., 2010) ¹ H(δ ppm)	(Soumia et al., 2012) ¹ H(δ ppm)	(Ahmed et al., 2013) ¹ H(δ ppm)	(WU et al., 2014) ¹ H(δ ppm)
1	1.29		1.47	1.13		1.08
2	1.67		1.56	1.56		1.85
3	3.54	3.56	3.52	3.58	3.51	3.50
4	2.27		2.28	2.34		2.28
5						
6	5.34	5.39	5.36	5.40	5.34	5.35
7	1.62		2.03	1.50		1.54
8	1.97		1.67	2.03		2.00
9	0.99		1.48	0.98		0.96
10						
11	1.52		1.52	1.55		1.54
12	1.54		1.49	1.21		1.16
13						
14	1.31		1.50	1.04		1.04
15	1.61		1.60	1.63		1.61
16	1.85		1.84	1.89		1.86
17	1.15		1.49	1.16		1.13
18	0.66	0.72	0.68	0.74	0.67	0.68
19	1.03	1.05	1.02	1.06	1.00	1.01
20	1.49		1.64	1.40		1.35
21	0.93	0.96	0.94	0.93	0.92	0.93
22	1.06		0.88	1.07		1.04
23	1.18		1.04	1.21		1.19
24	0.96		1.50	0.97		0.95
25	1.65		1.65	1.71		1.71
26	0.82	0.87	0.83	0.88	0.83	0.82
27	0.87	0.85	0.85	0.87	0.79	0.83
28	1.26		1.04	1.31		1.27
29	0.89	0.89	0.88	0.89	0.85	0.85

The proton chemical shifts (Table 4) were in good agreement with literature reports for the compound. Seyed et al., 2012 Reported antioxidant and anti-inflammatory properties of ferulic acid. β -Sitosterol was reported to reduce carcinogen-induced cancer of the colon. It also showed anti-inflammatory,

anti-pyretic, antiarthritic, anti-ulcer, insulin-releasing and oestrogenic effects and inhibition of spermatogenesis (Arjun et al., 2010). Beta-sitosterol is mainly known and used for its cholesterol lowering property (Arjun et al., 2010).



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Table 4. Experimental and Literature data for Ferulic acid ¹H NMR

Position	Experimental data ¹ H (δ ppm)	(Takeo et al., 2015) ¹ H (δ ppm)	(Nguyen et al., 2004) ¹ H (δ ppm)	(Seyed et al., 2012) ¹ H (δ ppm)	(Josh and Hixson 2012) ¹ H (δ ppm)	(Mohamed et al., 2015) ¹ H (δ ppm)	(Subramani et al., 2016) ¹ H (δ ppm)
2	7.05	6.93	7.05	7.09	7.33	7.3	7.08
5	6.76	6.67	6.35	6.97	6.87	6.18	6.79
6	7.19	6.83	7.17	7.14	7.14	7.1	7.08
7	7.57	7.44	7.57	7.75	7.6	7.52	7.5
8	6.25	6.15	6.3	6.34	6.38	6.69	6.34
3-OCH ₃	3.91	3.64	3.89	3.98	3.92	3.81	3.81

Conclusion

β-sitosterol and ferulic acid were isolated from the hexane extract *Bryophyllum pinnatum* stem. They were isolated by microwave-assisted extraction and identified by spectral techniques.

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Conflict of interest

The author hereby declares no conflict of interest.

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References

- Adamo, C., & Barone, V. (1999). Toward reliable density functional methods without adjustable parameters: The PBE0 model. *Journal of Chemical Physics*, 110(13), 6158–6170. <https://doi.org/10.1063/1.478522>
- Abd El Azim, M. H. (2015). Phenolic Compounds and Cytotoxic Activities of Methanol Extract of Basil (*Ocimum basilicum* L.). *Journal of Microbial & Biochemical Technology*, 07(04). <https://doi.org/10.4172/1948-5948.1000202>
- Ahmed, Y., Yaakob, Z., Akhtar, P., Rahman, M., & Islam, F. (2015). In vitro Antibacterial and in vivo Brine Shrimp Lethal Active Compounds Isolated from the Leaves of *Saurauia roxburghii*. *International Journal of Pharmacology*, 11(7), 821–827. <https://doi.org/10.3923/ijp.2015.821.827>
- Begum, H. A. (2023). Exploring the pharmacological potential of *Trametes hirsuta* (White Rot Fungi): Analgesic, anti-inflammatory, antispasmodic and antimicrobial activities. *Pure and Applied Biology*, 12(2). <https://doi.org/10.19045/bspab.2023.120121>
- Cyprian, T. A., Sewuese, S., & Akacha, L. U. (2019). Preliminary Phytochemical Screening and Thin Layer Chromatography Analysis of Stem Bark Extracts of African Mistletoe Parasitic on

- Vitellaria paradoxa*, *Piliostigma thonningii* and *Combretum fragrans*. *Asian Journal of Applied Chemistry Research*, 1–6. <https://doi.org/10.9734/ajacr/2019/v3i230087>
- Dikko, Y., Khan, M., Tor-Anyiin, T., Anyam, J., & Linus, U. (2016). In vitro Antimicrobial Activity of Fruit Pulp Extracts of *Azanza garckeana* (F. Hoffm.) Exell & Hillc. and Isolation of One of its Active Principles, Betulinic Acid. *British Journal of Pharmaceutical Research*, 14(1), 1–10. <https://doi.org/10.9734/bjpr/2016/30152>
- Ebrahim Sajjadi, S., Shokoohinia, Y., & Moayedi, N. S. (2012). Isolation and Identification of Ferulic Acid From Aerial Parts of *Kelussia odoratissima* Mozaff. *Jundishapur Journal of Natural Pharmaceutical Products*, 7(4), 159–162. <https://doi.org/10.5812/jnpp.4861>
- Eluwa, M., Ngwu, E., Asuquo, O., Akpantah, A., Isamoh, T., & Ekanem, T. (2014). Effect of Ethanolic Extracts of Lemon Grass and Mango Bark on the Cerebral Astrocytes of Adult Wistar Rats. *British Journal of Pharmaceutical Research*, 4(16), 1956–1962. <https://doi.org/10.9734/bjpr/2014/11772>
- Golea, L., Haba, H., Lavaud, C., Long, C., & Benkhaled, M. (2012). Chemical constituents from *Lotus pusillus* Medik. *Biochemical Systematics and Ecology*, 45, 12–15. <https://doi.org/10.1016/j.bse.2012.07.001>
- Gupta, M. M., Lal, R. N., & Shukla, Y. N. (1981). 5α-Stigmast-9(11)-en-3β-ol, a sterol from *Costus speciosus* roots. *Phytochemistry*, 20(11), 2557–2559. [https://doi.org/10.1016/0031-9422\(81\)83093-2](https://doi.org/10.1016/0031-9422(81)83093-2)
- Hixson, J. L., Sleep, N. R., Capone, D. L., Elsey, G. M., Curtin, C. D., Sefton, M. A., & Taylor, D. K. (2012). Hydroxycinnamic Acid Ethyl Esters as Precursors to Ethylphenols in Wine. *Journal of Agricultural and Food Chemistry*, 60(9), 2293–2298. <https://doi.org/10.1021/jf204908s>
- Ibibia, E. T., Peter Obaloluwa, A., Olasunkanmi, A. M., Ijeoma, M. A., & Olugbemiga, O. S. (2023). Total phenolic, flavonoid and mineral contents of the methanolic leaf extract of *parinari curatellifolia* planch. ex benth. *International Journal of Chemistry Research*, 13–18. <https://doi.org/10.22159/ijcr.2023v7i2.218>
- Moghaddam, F. M., Farimani, M. M., Salahvarzi, S., & Amin, G. (2007). Chemical Constituents of Dichloromethane Extract of Cultivated *Satureja khuzistanica*. *Evidence-Based Complementary and Alternative Medicine*, 4(1), 95–98. <https://doi.org/10.1093/ecam/nel065>

- Nguyen Minh, T., Nguyen Thi, M., Tran Thi, H., Nguyen Vo Tra, M., Tran Thi Quynh, T., Le Thi, L., Tran Binh, T. (2021). Prevalence of depression and the associated factors among the elderly in some communities of thua thien hue province. *Journal of Medicine and Pharmacy*, 60–69. <https://doi.org/10.34071/jmp.2021.2.9>
- Okwu, O. J., Obinne, C. P. O., & Agbulu, O. N. (2006). A Paradigm for Evaluation of Use and Effect of Communication Channels in Agricultural Extension Services. *Journal of Social Sciences*, 13(1), 31–36. <https://doi.org/10.1080/09718923.2006.11892527>
- Phenolic Profile and In Vitro Antioxidant Potential of Four Medicinal Plants. (2021). *Tropical Journal of Natural Product Research*, 5(11), 2037–2042. <https://doi.org/10.26538/tjnpr/v5i11.24>
- Yoshioka, T., Inokuchi, T., Fujioka, S., & Kimura, Y. (2004). Phenolic Compounds and Flavonoids as Plant Growth Regulators from Fruit and Leaf of *Vitex rotundifolia*. *Zeitschrift Für Naturforschung C*, 59(7–8), 509–514. <https://doi.org/10.1515/znc-2004-7-810>
- Zhang Chun-Shu, Kai Gui-Yun, Wang Zhi, Wang Chao, Sun Ting-Ting, Zhang Wei-Gang, Dong Xiao-Yi. (2005). Temperature and strain sensing property of grapefruit microstructure fiber Bragg grating. *Acta Physica Sinica*, 54(6), 2758. <https://doi.org/10.7498/aps.54.2758>

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