Extraction and Characterisation of Steroidal Compounds from *Gmelina arborea* Stem Bark for Pharmaceutical Applications

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ABSTRACT

Medicinal plants have remained a source of therapeutic agents in nature till date. There has been increasing concern in the use of some existing drugs due to their side effects, resistance and other shortcomings, hence the need for new drugs with better therapeutic properties. In order to contribute to the quest for novel medicines, this research sought to isolate, characterise, and identify putative therapeutic mediators from the stem bark of Gmelina arborea. Using a Soxhlet device, the powdered stem bark was extracted with hexane and ethyl acetate. On a silica gel column, the extracts were separated, and increasing concentrations of hexane in ethyl acetate were used to elute them. 40 fractions were produced after TLC analysis, and five combined fractions were created based on their characteristics (8 fractions to one) and coded PHE 001,002,003,004 and 005 respectively. The five fractions were analyzed by NMR (1D and 2D) to confirm the structures of their constituents. The result showed that PHE-004 was confirmed as mixtures steroidal compounds (stigmasterol and sitosterol)). The spectroscopic characterization of fraction PHE-004 reveals a chemical shift of the ^{1}H-NMR ranging from 0-80-5.36 ppm and ^{13}C-NMR ranging from 12.02-146.93 ppm. Melting point of 131-133^{0}C and Rf value of 0.53. The results shows that the plant has some pharmaceutical potentials and the stem bark extract could serve as an entrepreneurial tool for trado-medical practitioners to justify its traditional medical claims.

INTRODUCTION

The unique interactions of medications with biomolecules in the body, such as proteins and nucleic acids, increase the functional effects of pharmaceuticals in the body since medicinal plants are the source of many important drugs. As a result, different medicines are helpful for various reasons, making plants ideal as drug development precursors (Ajibesin, 2011). In essence, plants have vital roles in aromatherapy, homoeopathy, and traditional or herbal medicine. For instance, foods derived from medicinal herbs and spices are utilised in human meals to boost immune function (Akinpela and Onukoya, 2006). Due to some biological and pharmacological activities of these plants, such as their potency as anti-inflammatory, diuretics, laxatives, anti-plasmodics, antihypertensive, and antimicrobial agents, a wide variety of

information has been gathered regarding the utility of plants as food and medicine for humans (Adesokan *et al.*, 2007).

Huge amounts of modern drugs have their source from natural medicinal agents making traditional medicine an important means that are actually useful ingredients for the development of chemotherapeutics (Jiri, 2003). Hence, the prospect of modern medicine and therapeutics perhaps will be associated with rudimentary medicinal practices as majority of novel drugs have remained heartened by natural products as well as compounds isolated from plants (Lahlou, 2007). Gmeliana arborea has a number of traditional applications that make it useful as a raw material in the pharmaceutical and cosmetic industries (Bafi-Yeboa et al., 2005). It is regarded as diaphoretic, analgesic, and antiseptic. It is common practise to treat malaria, fever, sickle cell anaemia, TB, paralysis, edoema, and generalised bodily weakness using the root or stem bark macerations, decoctions, or infusion (Negi et al., 2011). It is often used to treat intestinal issues including dysentery, intestinal worms, gonorrhoea, and urethritis, but it is also used to treat migraines and neuralgia as well as pain associated with childbirth. The roots are administered topically to wounds, leprosy and syphilitic sores, swellings, haemorrhoids, abscesses, snake bites, yaws, rheumatic and arthritic discomfort, and hernias (Lourenco et al., 2011). When eaten, the roots and stem bark have a warm, aromatic, and numbing impact on the palate. They are also often used to treat dental cavities, sore gums, and toothaches. The roots' decoction is applied to the throat and used as mouthwash. The aim of this study was to isolate, characterize and identify any bioactive compound(s) present in the in the stem bark of the plant investigated using spectroscopic methods such as NMR, IR, etc.

Sample collection and Preparation

The stem bark of G. arborea was collected in August 2021 from Primary School 2 Bunu Tai in Tai Local Government Area, Rivers State, Nigeria. A botanist at the Department of Plant Science and Biotechnology at Rivers State University in Port Harcourt identified and authenticated the stem bark, which was then given the voucher specimen number RSU/2021/GA-32 and kept in the herbarium. The sample was dried in the air for two weeks before being ground into powder using a mortar and pestle. After that, it was placed in a glass container and sent to Glasgow, United Kingdom's Strathclyde Institute of Pharmacy and Biomedical Sciences laboratory for extraction and further examination**.**

Extraction of Sample

G. arborea's pulverised stem bark, weighing about 400g, was put in a soxhlet apparatus and continuously extracted over the course of two days using hexane and ethylacetate. In a rotary evaporator set at 400C, the extracts were dried out. All dry extracts were combined and stored in various sample vials with the labels HX/EA-01 for hexane extracts and EA/HX-01 for ethylacetate extracts, respectively. For column chromatography, 40g of each crude extract were dissolved in silica gel and left to dry**.**

Isolation and Characterisation

Dichloromethane was used to dissolve 30 g of the dried extract of G. arborea, which was then put into a small beaker. The extract was mixed with 10 g of silica gel, agitated for about 10 minutes, and then left to dry in a fume cupboard. The use of silica gel allowed the extract to be absorbed. A slurry was created by continuously stirring 100 g of silica gel with 500 mL of

hexane until it was produced. To prevent contamination, the column was washed three times with ethyl acetate and hung on a retort stand. A little amount of hexane was added to the column, and two minutes later the slurry was added. The column tap was then gently opened, and the mixture was allowed to settle while the solvent was drilled out. Adsorbed extract slurry was placed into the column with the tap closed until there was approximately 10 cm of solvent above the silica packing. The tap was then opened to enable any remaining solvent to drain out of the column and land on the silica. Hexane and 5% ethylacetate were added to the column, and the tap was let to run at a rate of around 10-15 drops per minute. Using vials with serial numbers, evenly 25–30 mL portions were collected. The eluates were maintained in a dust-free fume hood and allowed to dry (Nande and Igoli, 2017). After TLC analysis, comparable column fractions were pooled (Hostettmann et al.,1998). When burned with concentrated sulphuric acid, ethyl acetate produced a TLC profile with a single creamy spot that was comparable to the PHE-001, PHE-002, PHE-003, and PHE-004 fractions and had an Rf value of 0.53. A substance known as PHE-004 was produced by recrystallizing the mixed portions 001-004 in ethyl acetate. The substance was analysed using spectroscopy (NMR-Spectroscopy), and its melting point was found to be between 131 and 1330 C. (Table 1).

RESULTS AND DISCUSSION

Table 1: ^{1}H-NMR and ^{13}C-NMR Chemical Shift for PHE-004

Spectroscopic Technique	Data
Mp	131-133^{0}C
Rf	0.53
1R (Cm^{-1})	3547, 3230, 3025, 2850, 1640,
^{1}HNMR	δ 0.80, 0.82, 0.83, 0.84, 1.00, 1.01, 1.40, 1.81, 2.02, 2.21, 1.42,1.37,1.10,1.09, 1.67,2.03, 3.51, 3.52, 5.35, 5.36, 5.33 ppm
^{13}CNMR	δ 36.67, 29.86, 71.97, 42.47, 146.93, 121.86, 31.83, 29.32, 46.01, 37.42, 18.94, 39.96, 41.42, 57.09, 25.03, 28.40, 56.93, 12.72, 19.97, 42.49, 23.09, 142.91, 129.87, 46.61, 34.12, 21.25, 23.24, 26.26, 12.02 ppm

The spectroscopic characterization of fraction PHE-49 reveals a chemical shift of the 1HNMR ranging from 0-80-5.36ppm and 13CNMr ranging from 12.02-146.93ppm. Melting point of 131-133^{0}C and Rf value of 0.53 as shown in table 1.

Characterization of PHE-004 as a Mixture of Steroidal Compound

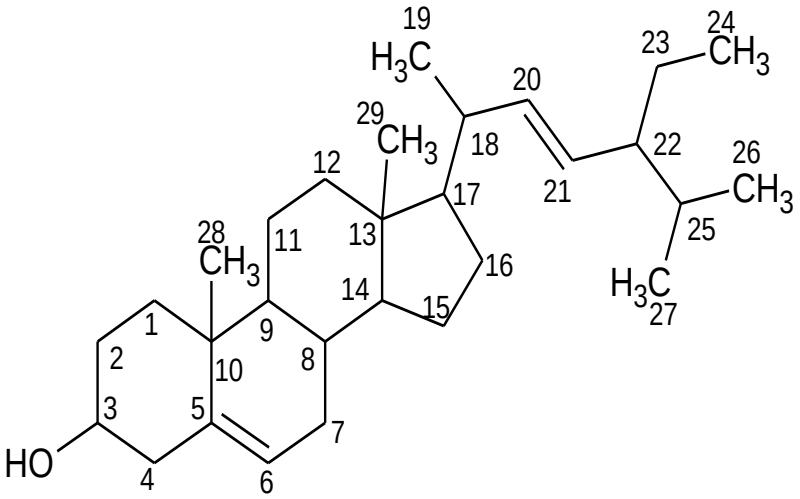
Fraction PHE-004 was obtained as white crystalline solid from the hexane extract of *Gmelina arborea*. It was subjected to IR analysis and absorption bonds of 3547cm^{-1} was observed that is a characteristic of OH stretching. Absorptions at 3230cm^{-1} was done to cyclic olefinic carbon, 3025cm^{-1} for C=CH structure and 2850 cm^{-1}, C-H. Other absorption frequencies included 1640cm^{-1} as a result of C=C absorption. It suncorrected melting point was 132-133^{0}C (Lit 132-134^{0}C, Anjoor and Ajar, 2011). The fraction's 1H-NMR analysis revealed a combination of two chemicals. The spectrum revealed signals that were indicative of sterols, including olefinic hydrogen H 5.35 (H-6) and carbonolic hydrogen 3.52 (M-H-3), as well as an accumulation of signals between 0.80 and 2.03 ppm that were indicative of sitosterol and were caused by many

different groups of hydrogens, including methyl, methylene, and methane (Okoro et al., 2017). Six methyl protons with signals of 0.82, 1.00, 1.01, 0.84, 0.83, and 1.67 correspondingly were present to show this. The olefinic hydrogens of the double bond with the stereochemistry moiety are represented by the doublet at 5.35 and 5.36. (Table 1). Three quaternary carbons, eleven methane, nine methylene, and six methyl carbons were seen in the 13C-NMR spectrum (Rajput and Rajput, 2012). The 13C-NMR spectra showed the sitosterol and stigmasterol signature signals at c 121.86, 146.93, 129.87, and 142.92. (Goulart et al., 1993). The resonance at c 71.97 in the 13C-NMR spectra was attributed to C-13 (Jamal et al., 2009). Signals seen at c 142.92 and c 129.87 are for C-20 and C-21, respectively. Inferred from the 1HNMR spectrum signals for the olefinic protons H-22, H-23, and H-6 that both stigmasterol and sitosterol were present in the combination in an equal amount was verified by their integration as follows: When stigmasterol and sitosterol were combined, a doublet of doublet at 3.52 that was attributable to H-3 was integrated for 2. The sitosterol and stigmasterol proton at H 5.35, corresponding to H-6, integrated for 2. While stigmasterol's H-ss and H-23, which had signals at H 4.17 and 5.35, were each integrated for one proton. As a result, the mixture has a stigmasterol:sitosterol ratio of 1:1. A thorough set of cosy and HMBC experiments confirmed that the relative configuration of fraction PHE-49 was consistent with that expected for a steroidal derivative. H-20 (H 1.56) and the methyl at C-10 demonstrated a single bond correlation (37.42). H-12 (H 1.40) demonstrated correlations with C-11 (c 8.94), but H-21 (H 1.01) had cross-peaks with C-14 (c 57.09), C-17 (c 56.93), and C-24 (c 46.0) in the HMBC. Other correlations included those between H-2 (H 1.82) and C-1 (c 36.67) and C-3 (c 71.97), H-4 (H 2.25) and C-7 (c 31.83), H-6 (H 5.35) and C-4 (c 52.47 and C-7 (c 31.83), H-14 (H 1.35) and C-15 (c 25.03), H-17 (H 0.90) and C-12 (39.94) and C-14 (c 57.09), PHE-49 was determined to be a combination of stigmasterol and -sitosterol using the ID NMR, 2D NMR, IR, melting point, and their comparison (Ajoor & Ajar, 2011). Phytosterols like stigmasterol and sitosterol are often found in the plant world. Sitosterol contains a single bond at C-22 whereas stigmasterol has two bonds at C-23. Thus, it is challenging to find sitosterol in its purest form (Habib et al., 2007). stigmasterol-containing mixtures are recognised in the 1HMNR spectrum by singles referring to the vinyl hydrogens (H-22 and H-23) that appear as two doublets between H 4.17 and H 5.35. (Nna et al., 2019). In every instance, H-6 appears as a wide doublet at 5.35 in the 1H-NMR spectrum, whereas H-3 hydrogen displays a multiple at about H 3.52. These might be free, esterified, or glycosylated phytosterols (Pierre and Moses, 2015).

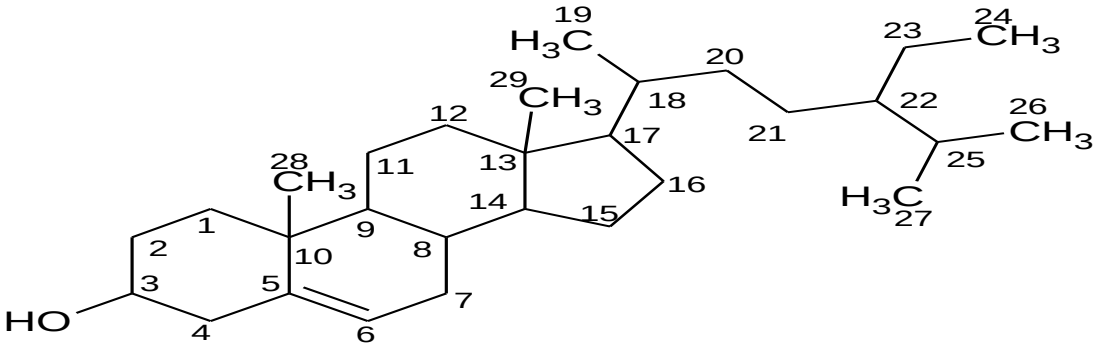
Table2: NMR data for PHE-004

Position	**Experimental**		**Literature Nna et al. (2019)**	
	^{1}H(δ)	**^{13}C(δ)**	**^{1}H(δ)**	**^{13}C(δ)**
1	1.40	36.67	1.42	36.72
2	1.81	29.86	1.82	29.71
3	3.52	71.97	3.53	71.97
4	2.23	42.47	2.25	42.35
5		146.93		140.94
6	5.36	121.86	5.38	121.32
7	1.90	31.83	1.98	31.71
8	1.22	29.32	1.20	29.24

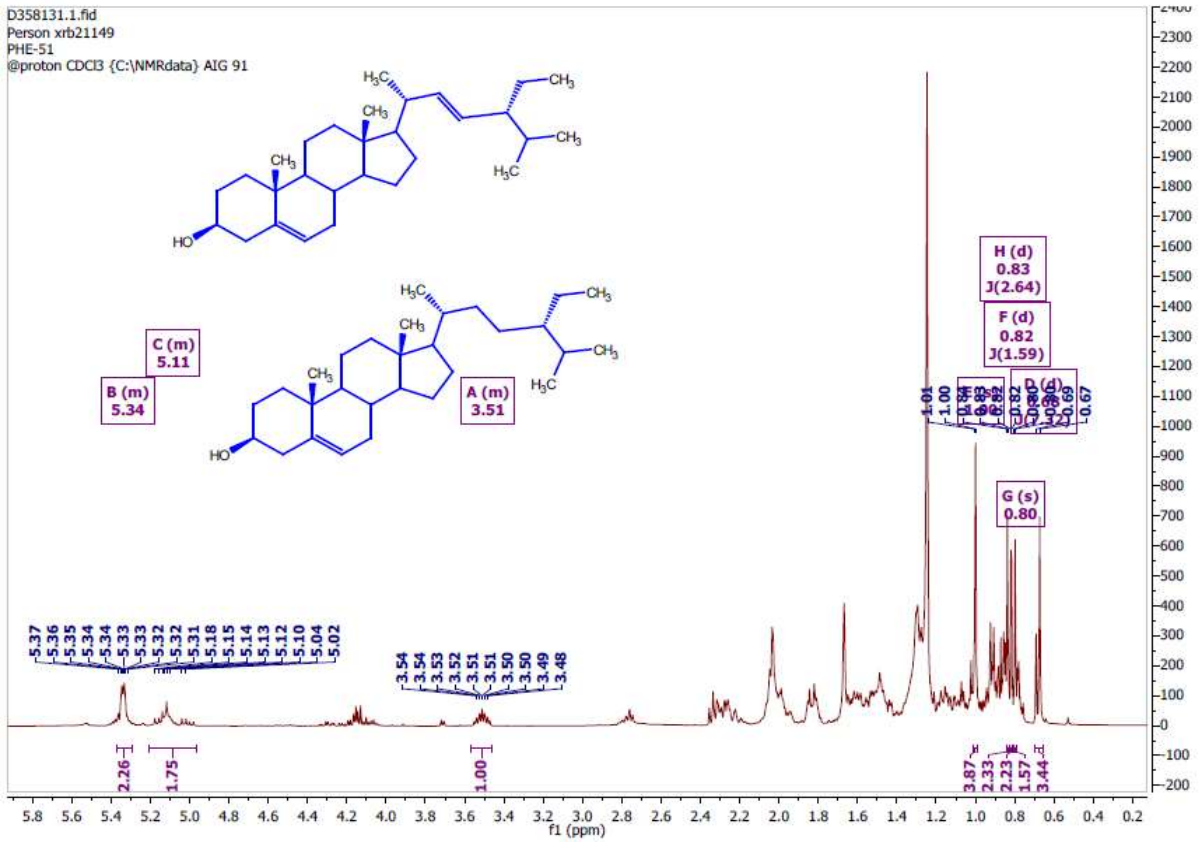
9	2.02	46.01	2.00	50.03
10	2.21	37.42		36.16
11	1.81	18.94	1.80	24.32
12	1.42	39.94	1.40	39.82
13		41.42		40.45
14	1.37	57.09	1.35	56.90
15	1.10	25.03	1.12	24.32
16	1.09	28.40	1.07	28.90
17	0.90	56.93	0.90	56.03
18	1.67	12.72	1.29	12.06
19	0.80	19.97	0.74	19.06
20		142.92	1.55	138.40
21	2.03	129.87	1.20	129.34
22	5.35	42.49	5.07	36.83
23	5.33	23.09	5.20	23.12
24	0.84	46.01	2.05	51.26
25		34.12	1.04	34.01
26	0.82	21.25	0.85	21.12
27	0.83	23.24	0.96	22.84
28	1.00	26.26	1.04	25.30
29	1.01	12.02	1.01	12.06



Stigmasterol



Sitosterol



Conclusion

The compounds isolated, characterized and identified are phytosteroids that has been reported for display antioxidant, anticancer, antifeedant, antibacterial, antifungal, antiparasitic and anti-inflammatory activities (Nna et al., 2019). They have been found to show hepatoprotective activity. The means of actions of phytosteroids are gambled to encompass the illness of the cell membrane activity. The compound has also been designated for its antimicrobial activities against some clinical pathogens (Nna et al., 2019). The prior account of the medicinal properties

of the isolated compounds in this study is an evident that it is an important medicinal compound. Its isolation from the root of *G. arborea* shows it to be a principal medicinal source behind ethnomedical claims of the stem bark in antifungal, antibacterial, anti-inflammatory, anti-cancerous, antiparasitic and other infectious diseases. This study has shown that the stem bark of *G. arborea* to be a complete source for these compounds with a hopeful antimicrobial activity which justifies the trado-medical potencies and claims of the plant part investigated. In essence, the paper justifies that extracts from the stem bark of this plant could serve as pharmaceutical and entrepreneurial tool for traditional practical practitioners owing to the usefulness of the compounds isolated and reported in this work.

REFERENCES

Adesokan, A.A., Akanji, M.A. and Yakubu, M.I. (2007). Antibacterial potentials of aqueous extracts of *Enantia chlorantha* stem bark. *Africa Journal of Biotechnology,* 6(22): 2502-2505.

Ajibesin, K.K. (2011). *Dacryodes edulis*: A review of its medicinal, phytochemical and economic properties, *Journal of Traditional Medicine 51*: 32-41.

Akinpelu, D.A. and Onukoya, Z.T.M (2006). Antimicrobial activities of medicinal plants used in folklore remedies in South-Western Nigeria. *African Journal of Biotechnology*, 7(5): 1078-1081.

Anjoo, K. and Ajar, K.S (2011). Isolation of stigmasterol and β-sitosterol from petroleum ether extract of aerial parts of *Ageratum conyzoides* (Asteraceae). *International Journal of Pharmach and Pharmaceutical Sciences*, 3(1): 94-96.

Bafi-Yeboa, N.F.A, Arnason, J.T., Bakar, J. and Smith, M.L (2005). Anti-fungal constituents of Northern prickly ash. *Zanthoxylum americanum*. *Phytomedicine*, 12(5): 370-377. Complementary and Alternative Medical Research, 7(4):1-10.

Goulart, M.O.F., Saut'Ana, A.E.G. Lima, R.A., Cavalcante, S.H., Carvalho, M.G.and Braz-Filho, R. (1993). Fitoconstituents quimos isolados de *Jatropha elliptica*. Attibucao dos deslocamentos quimicos dos atomos de carbone hidrogenio dos diterpenes jatrofolonas AeB. *Quimica Nova*, 16: 95-100.

Hostettmann, K., Marston, A. and Hostettman, M. (1998). Preparative chromatography techniques: Applications in natural product isolation. 2nd edition. Springer-Verlag publication. Berlin. 39-43.

Jamal, A.K., Yaacob, W.A. and Din, L.B. (2009). A chemical study on *Phyllanthus columnaris*. European Journal of Scientific Research, 28: 76-81.

Jiri, P. (2003). Biologically active Pentacyclic triterpenes and their current medicine significance. *Journal of Applied Biomedicine,* 1: 7-12.

Lahlou, M. (2007). Screening of Natural Products for Drug Discovery. *Expert Opinion on Drug Discovery*, 2(5): 697-705.

Lourenco, L.B., Santana, C.V., Lafaiete, C.A, Tadeu, A.C and Coasta, E.S (2011). Extraction with supercritical fluid and comparism of chemical composition from adults and young leaves of *Zanthoxylum tin goassuiba*. *Brazilian Journal of Pharmacognosy*, 21(2): 564-567

Nande, W.U and Igoli, J.O (2017). Isolation and Characterization of Bioactive compounds from plant materials: The Nigerian situation. *Nigerian Journal of Pure and Applied Sciences*, 9(1): 132-143.

Negi, J.S., Bisht, V.K., Singh, P. and Sundniyal, R.C (2011). Chemical Constituents and Biological activities of the genus *Zanthoxylum*. A review. *African Journal of Pure and Applied Chemistry*, 5(12): 412-416.

Nna, P.J., Tor-Anyiin, T.A. and Khan, E.M. (2019): Stigmasterol and β-Sitosterol from the Root of *Mangifera indica* and their Biological Activities against some Pathogens. *Journal of Complementary and Alternative Medical Research*, 7(4): 1-10.

Okoro, I.S., Tor-Anyiin, T.A. and Igoli, J.O. (2017): Isolation and Characterisation of Stigmasterol and β- Sitosterol from *Anthocleista djalonensis A. Cev*. *Asian Journal of Chemical Sciences*. 3(4): 1-5.

Pierre, L.L. and Moses, M.N.(2015): Isolation and Characterisation of Stigmasterol and β-sitosterol from *Odontoneman strictum* (Acanthaceae). *Journal of Innovative Pharmaceutical and Biological Sciences*, 2(1): 88-95.

Rajput, A. and Rajput, T. (2012). Isolation of stigmasterol and β-sitosterol from Chloroform extract of leaves of *Corchorus fascicularis* Lam. *International Journal of Biology and Chemistry*, 6: 130-135.