Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 8, Issue 2, 2020

Isolation and Characterization of Phytosterol in the Leaf of *Tetracarpidium conophorum* (*Mull. Arg.*) Hutch and Dalziel

[*1]Bolarin-Akinwande, O.O., [1]Ayo, R.G., [1]Oyewale, A.O. and [1]Habila, J.

[1]Department of Chemistry, Ahmadu Bello University, Zaria, Nigeria.

[*]Corresponding Author: olubunmi35@gmail.com

ABSTRACT

The petroleum ether (60-80 $^{\circ}$C) extract of the leaf of *Tetracarpidium conophorum* (Euphorbiaceae) was subjected to column and thin layer chromatographic techniques, to obtain a compound, stigmasterol (labeled TC1) which is a phytosterol. TC1 was further subjected to spectroscopic analyses such as Fourier Transform–Infra Red (FT-IR), 1D and 2D- NMR experiments and Gas Chromatography–Mass Spectrometer (GC-MS). The IR spectrum of TC1 gave signals of O-H bond (3426.7 cm^{-1}), - CH_3 (2926. 1 cm^{-1}), -C=C- (1632.8 cm^{-1}) and -C-O- of an alcohol (1051. 2 cm^{-1}). In the ^{1}H NMR spectrum of TC1, H-3 proton appeared as a triplet of a double doublet (tdd) at 3.5 and H-6 olefinic proton showed a multiple at δ5.4. Two olefinic protons appeared downfield at δ5.3 (m) and δ5.4 (m), six methyl protons appeared at δ1.01, δ1.53. δ0.90, δ0.81, δ1. 56 and δ0.83 (3H each, s, CH_3). The ^{13}C NMR signals of -CH_3 at 12.05 ppm, 12.21 ppm; –CH_2 at 21.05 ppm, 24.37 ppm, 25.38 ppm, olefinic – CH proton signals at 129.7 ppm, 141.0 ppm, 138.7 ppm and 121.8 ppm. These assignments were confirmed with spectral analyses from literature. The Mass spectroscopy revealed a molecular ion peak at 412 which corresponds to the molecular formula $C_{29}H_{48}O$. The crude petroleum ether (60- 80 ^{o}C) extract of the leaf of *Tetracarpidium conophorum* as well as the pure compound TC1 were tested for their *in-vitro* inhibitory activities against some bacteria and fungi. The leaf of this plant appeared to be a potential source of antibiotic and antifungi.

Keywords: *Euphorbiaceae*, Leaf, Stigmasterol, *Tetracarpidium conophorum*.

INTRODUCTION

Chemical constituents represent cell contents which are either food storage products or by-products of metabolism or secondary metabolites (natural products). These include carbohydrates, proteins, fixed oils and fats, alkaloids, purines, glycosides, resins and tannins.

Secondary metabolites are often unique to a particular plant species or group of organisms and many act as anti- feedants, sex attractants or antibiotic agents [1].

In Southern Nigeria ethno-medicine, *Tetracarpidium conophorum* which is also known as African walnut, the leaves are used for the treatment of dysentery and to improve fertility in males [2]. Hence it is known as *Oke okpokirinya* meaning male string leaf (Igbo- Owerri), *Okumu* from *Umu* meaning 'babies call babies' (Igbo- Uburubu), *Asala* or *Awusa* (Yoruba) and *Gedan-yorubawa* (Hausa) [3].

The aim of this study is to report on the characterization of an isolated secondary metabolite found in the leaf of an extract of *Tetracarpidium conophorum.*

MATERIALS AND METHODS

Sample Collection and Preparation

The plant, *Tetracarpidium conophorum* was collected in September 2012 from a private cocoa farm in Itapagi **(**07° 58' N, 05° 10' E) located in Ikole Local Government Area, Ekiti State, Nigeria. It was authenticated by Malam Mohammed Musa of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. Its voucher specimen number is 2144. The leaves were air-dried and pulverized using a wooden mortar and pestle. Then the powder was stored in an airtight polythene bag in a cool dry place.

Extraction

The plant sample (300 g) was extracted exhaustively with 1.5 L of redistilled petroleum ether (60-80 °C) in a Soxhlet extractor until the colour of the extraction became colourless. The crude extract was concentrated in *vacuo* at 40 ^{o}C using a rotary evaporator. The crude extract was air dried until a constant weight was obtained of 18. 82 g (6.27 %).

Isolation

A portion of the petroleum ether extract (0.3 g) was dissolved in 2 cm^3 petroleum ether (60-80 ^{o}C) and the sample solution was spotted on Thin Layer Chromatography (TLC) silica gel pre-coated aluminum sheets. The spotted TLC plates were developed in various solvent systems and viewed by spraying with 10 % sulphuric acid followed by heating at 110 ^{o}C for 5-10 min. The most suitable solvent was n-hexane:ethylacetate (8:2). This solvent system was able to separate the crude extract initially into various discrete fractions containing compounds of various

polarities. Various solvent systems were used as trials. Some of them gave various fractions with close Retention factors (Rf) as well as incomplete separation of the fractions. Such solvent systems as ethylacetate:chloroform (2:8), petroleum ether:ethylacetate (7:3) etc. n-hexane is certainly the most well established industrial solvent for extraction of lipophilic natural products. The fractions obtained by n-hexane:ethylacetate (8:2) solvent system on TLC were further subjected to column chromatography. A total of 30 fractions were collected using a solvent system of n-hexane:ethylacetate (95:05). Each elute was monitored by TLC. Fractions numbered 1-8 were pooled as A1 because they were found to have similar Rf value of 0.6. They were conducted by preparativeTLC using pre-coated TLC plates of silica gel on aluminum sheets. And a solvent mixture of n- hexane: ethyl acetate (85: 15) as the mobile phase was used to develop [3,4]. TC1 was obtained as a single compound. The petroleum ether crude extract has proven to be effective against some selected human pathogenic micro-organisms and TC1 also exhibited similar inhibitory effects on the same micro-organisms [6].

Salkowski Reaction

A few crystals of TC1 were dissolved in chloroform and a few drops of concentrated sulphuric acid were added to the solution. There was the formation of a reddish- brown colour in the upper chloroform layer indicating presence of steroids [7].

Liebermann-Burchard Reaction

A few crystals of TC1 were dissolved in chloroform and a few drops of concentrated sulphuric acid were added to it followed by the addition of 2-3 drops of acetic anhydride. There was the formation of violet blue and finally green colour which indicates the presence of steroids [7, 8].
Ernst Leitz Welziar melting point apparatus was used to determine the melting point of TC1.

Spectroscopic Characterization

Different spectroscopic methods were used to elucidate the structure of TC1. The infra-red spectrum was recorded on FTIR- 8400s (Shimadzu, Japan) at National Research Institute for Chemical Technology, Bassawa, Zaria, Nigeria. ^{1}H- NMR and ^{13}C –NMR spectral were recorded on a Varian- 600 Hz NMR spectrometer (Varian, South Africa) at the School of Chemistry, University of Kwazulu-Natal, South Africa.^{1}H-NMR and ^{13}C-NMR spectral were recorded using $CDCl_3$ as solvent with tetramethylsilane (TMS) as an internal standard. Mass spectrum was recorded at a high resolution on a mass spectrometer on Agilent Technologies 1200 series Binary

SL (Agilent Technologies, Inc, Santa Clara, USA) at the School of Chemistry, University of Kwazulu-Natal, South Africa. The data are given in m/z values and compared with a Library search of the Mass Spectral of authenticated compounds.

RESULTS AND DISCUSSION

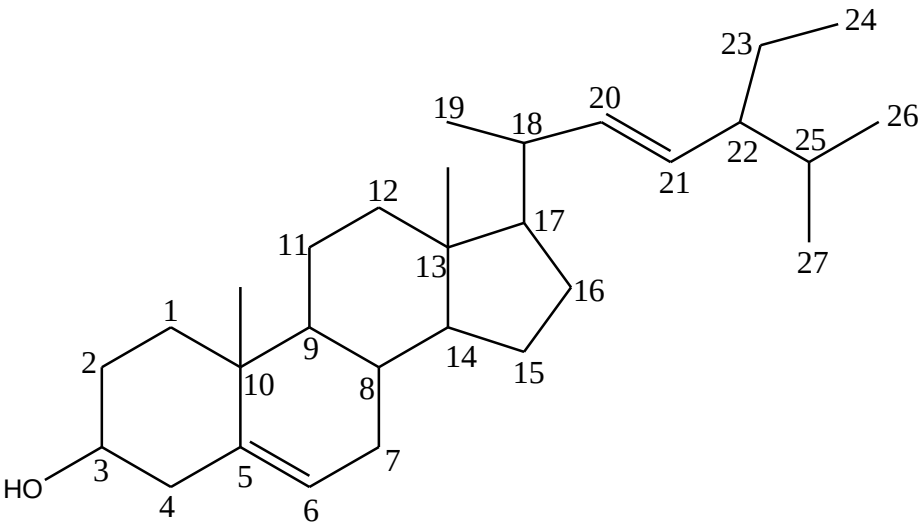
From the positive tests of steroids and methylated sterols given by TC1, it was assumed to be a sterol. The melting point of TC1 was 164-165 ^{o}C. Mass spectrum of TC1 revealed a parent molecular ion peak at m/z 412 which corresponds to the molecular formula $C_{29}H_{48}O$. IR: 3426.7 cm^{-1} (br, OH), 2926.1 cm^{-1} (C-C str.in CH_3), 1632.1 cm^{-1} (C=C str.) and 1458.2 cm^{-1} (bending vibration of -CH_2). These absorption frequencies resemble the absorption frequencies observed for stigmasterol [9].

^{1}H NMR ($CHCl_3$, 600 MHz) of TC1

^{1}H NMR gave signals of δ 3.5 (1H, m, H-3), δ 5.2 (1H, m, H-6), δ 5.0 (1H, m, H-23), δ4.6 (1H, m, H- 22). Two olefinic protons appeared downfield at δ 4.6 (m) and δ 4.7(m). The ^{1}H NMR gave signals of –CH_3 at 0.6 -1.0 ppm.

^{13}C NMR ($CHCl_3$, 600 MHz) of TC1

The ^{13}C NMR spectral data indicated signals for twenty-nine carbon atoms with the following functionalities; four olefinic carbons (δδ 121.7, 129.3, 138.3, 140.7); nine methylene carbons (δδ 21.0, 24.3, 25.4, 28.8, 31.7, 31.7, 37.2, 39.7, 42.2), six methyl carbons (δδ 12.10, 12.20, 19.4, 21.1, 25.4, 31.7) and three quaternary carbons (δδ140.7, 42.3, 36.5).These are characteristic resonances of a sterol with two olefins and an alcohol. The ^{1}H and ^{13}C assignments were verified by 2D Heteronuclear Single Quantum Coherence (HSQC) experiments while connectivities were verified by Heteronuclear MultiBond Coherence (HMBC) and Proton Correlation Spectroscopy (COSY). All spectral data were confirmed by comparison with spectral analysis data reported in literature. The physical and spectral data are consistent to the reported literature values [9, 10].



Stigmasterol ($C_{29}H_{48}O$; Mol wt. 412.69)
3β, 22E-stigmasta-5, 22-dien-3-ol

CONCLUSION

From the above findings, stigmasterol was isolated from petroleum ether extract of the leaves of *Tetracarpidium conophorum.* The chemical structure of TC1 was elucidated by means of various physical and spectral techniques and the compound elucidated exhibited some antimicrobial inhibitory effects.

REFERENCES

1. Cannel, R.J.P. (1998). *Methods in Biotechnology 4. Natural Products Isolation*. Humana Press. Totowa, New Jersey. p.473
2. Hardman, W. E. (2014). Walnut have potential for cancer prevention and treatment in mice. *The Journal of Nutrition*, 144(4), 555S-560S. Doi:10.3945/jn.113.188466
3. Odoemena, C.S.I., Udosen, J.R. & Sam, S.M. (2010). Anti-Diabetic Activity of *Tetracarpidium conophorum* Mull Arg. (Hutch and Dalziel). Ethanolic Seed Extract on Diabetic Rats. *Advances in Sciences and Technology*. 4(2), 120-124.
4. Grylling, H., Plat, J., Turley, S., Ginsberg, H.N., Ellegard, L., Jessup, W., Jones, J.P., Lutjohann, D., Maerz, W. Masana, L., Silbernagel, G., Staels, B., Boren, J., Catapano, A.L., De Backer, G., Deanfield,J., Descamps, O.S., Kovanen, P.T., Riccardi, G., Tokgozoglu, L. & Chapman, M.J. (2014). Plant Sterols and Plant Stanols in the Management of Dyslipidaemia and Prevention of Cardiovascular Disease. *Atherosclerosis*, 232(20), 346-360. DOI: 10.1016/j:Atherosclerosis.2013.11.043

5. Ajaiyeoba, E.O. & Fadare, D.A. (2006). Antimicrobial potential of extracts and fraction of the African Walnut .*African Journal of Biotechnology*. 5 (22), 2322-2325
6. Burkill, H.M. (1985). *The useful plants of West Topical Africa*. Royal Botanical Garden, Kew. 2, 127-128; 4, 444-445.
7. Srivastave, V.K. & Srivastave, K.K. (1987). An introduction to Chromatography Theory and Practice. S. Chand and company Ltd., Delhi, India. Vol.5, Pp.50-52.
8. Touchstone, J.C. (1992*). Practice of Thin Layer Chromatography*. John Wiley and Sons INC, USA, p.274.
9. Schlegel, H.G. (1993). *General Microbiology*. Press Syndicate of Cambridge University Press. The Pitt Building, Trumpington Street, Cambridge CB2 IRP. 40 West 20th Street, New York, NY. 10011-4211, USA. P.607.
10. Sharp, S.E. (6th edition*) Manual of Clinical Microbiology*. American Society for Microbiology, 1325 Massachussetts Avenue, N.W Washington, Pp.25-26.
11. Sofowora, A. (2009). Trease and Evans *Pharmacognosy*. Sauders Elsevier Limited, China, Pp.511-520.
12. Kamboj, A. & Saluja, K. (2011). Isolation of stigmasterol and ß -sitosterol from petroleum ether extract of aeriel parts of Ageratum conyzoides (Asteraceae), *International Journal of Pharmacy and Pharmaceutical Sciences.* 3 (1), 94-96
13. Chaturvedula, V.S.P. & Prakash, I. (2012). Isolation of stigmsterol and ß-sitosterol form the dichloromethane extract of Rubus suavissimus. *International Current Pharmaceutical Journal.* 1(9), 239-242.