
Spectrophotometric Identification of Chromene in *Phyllanthus muellerianus* Leaves Extract**Ogbuanu Cyril C*, Amujiogu Steve N**

Department of Industrial Chemistry, Enugu State University of Science and Technology (ESUT), P.M.B 01660, Enugu Nigeria

Original Research Article***Corresponding author**
Ogbuanu Cyril C**Article History**

Received: 18.09.2018

Accepted: 26.09.2018

Published: 30.10.2018



Abstract: *Phyllanthus muellerianus* is a large genus of the Euphobiaceae, popularly known as 'nvo nkwo' in Oghe traditional medicine. This species is used in the folk medicine for the treatment of sexually transmitted diseases. The aim of this study was to isolate, identify and characterize sample GH3 from leaves of *P. muellerianus*. Sample GH3 from leaves of *P. muellerianus* was analyzed for their absorption, functional groups (CH, H, OH and NH) and ¹H NMR resonance peaks (7.2 ppm and 6.7 ppm), resulting in the identification of benzopyran's groups, suggesting 2H-1-benzopyran (chromene). The identification of benzopyran's groups is here reported for the first time in *P. muellerianus*.

Keywords: *Phyllanthus muellerianus*; benzopyran (chromene); spectroscopic characterization; functional group; absorption.

INTRODUCTION

Phyllanthus muellerianus is a large genus of the Euphobiaceae family distributed essentially in Guinea-Bissau and Mali to West Cameroons and Fernando Po, and widespread in other areas of tropical Africa, containing from 750 [1] to 1200 [2]. This genus has been reported in the literature to possess various biological properties, such as treatment of gastroenteritis, urethritis, otitis media, and wound infections [3], antibacterial activities [4], Anti-inflammatory activity [5] and anti-diarrhoeal activity [6].

A brown dye obtained from the bark is used to dye mats and fishing line while a black dye is obtained from the whole plant and used to colour fibers [7].

Benzopyran (chromene) is a polycyclic organic compound that results from the fusion of a benzene ring to a heterocyclic pyran ring. There are many structural isomers owing to the multiple possible positions of the oxygen atom and the tetrahedral carbon atom [8]. Chromene derivatives used as food additives, cosmetic agents and potential biodegradable agrochemicals, are the important class of heterocycles being the chief components of many naturally occurring products.

For thousands of years Mother Nature has been a source of medicinal agents and modern drugs for thousands of years. Chromene have been known to exhibit a wide range of biological and pharmacological activities such as antioxidant [9, 10] anticancer [11], antimicrobial [12], hypotensive [13] and local anesthetic [14]. In addition, they can be used as cognitive enhancers [15, 16] for the treatment of neurodegenerative diseases, including Alzheimer's disease [17] and schizophrenia disorder [18]. This

research, therefore aims at spectroscopic characterization of sample GH3 isolated from *Phyllanthus muellerianus*.

MATERIALS AND METHODS**Plant material**

The leaves from *Phyllanthus muellerianus*, Euphobiaceae, were collected at Agbani near Faculty of Applied Natural Sciences of Enugu State University of Science and Technology on August 2015 and taken immediately to the laboratory where they were dried in a subdued sunlight and ground into powder form with the aid of Kika Grinding Mill (Model MP300-20 A11 basic). Plant identification was performed by Prof. J. C. Okafor, Department of Applied Biology and Biotechnology, Enugu State University of Science and Technology.

Extraction and isolation

Dried leaves from *P. muellerianus* (1.5 kg) were percolated in acetone and water (50/50 v/v) at the ratio of 1:3 for 48 hours and filtered. Acetone was distilled out leaving the aqueous filtrate. This was partitioned successively with n-hexane, chloroform,

ethyl acetate and n-butanol respectively. The fractions were concentrated using rotary evaporator. The concentrate was dried at 65°C in a Pickstone Thermostatic Oven series 30/300 (Model BD/AL) to a constant weight [19]. The partitioning of this extract yielded for ethyl acetate (1.15%) and n-butanol (0.61%). The ethyl acetate fraction was further purified by column chromatography using gradient of n-hexane increasing the polarity with ethyl acetate. Eight fraction were collected and TLC analysis conducted [20].

Subsequently, these combined-fractions were purified by sephadex 20 column eluted with methanol at the rate of 1mL/30 minutes. The fractions so collected were evaporated using rotary evaporator (RE-52CS).

Ultraviolet spectroscopic screening of sample GH3

Peak pick method was employed using Thermo Electron UV spectrometer equipped with vision pro-software's v4.10. The GH3 fraction of the dye in chloroform (mg/ml) were scanned through UV-visible spectrophotometer [21, 22].

Fourier transforms infrared spectroscopy analysis

FT-IR – 8400S Fourier transform infrared spectrophotometer was employed in functional group determination since different functional groups in molecule vibrate at distinctly different frequencies [23].

Nuclear magnetic resonance studies

In this study NMR (Model Agilent-NMR-vnmrs400) was used to determine the resonance of sample GH3.

Nuclear magnetic resonance (NMR) is an analytical technique used to probe the nature and chemical structure of the dyes employing proton (¹H) NMR [24].

RESULTS AND DISCUSSIONS

The result of UV/Visible analysis of sample GH3

The UV/visible spectrum of GH3 absorbs at 396, 500 and 533 (Fig-1). The absorption maximum of sample GH3 at 396 nm is within the range of UV-A (315-400nm) and in the visible region at 500 and 533 nm.

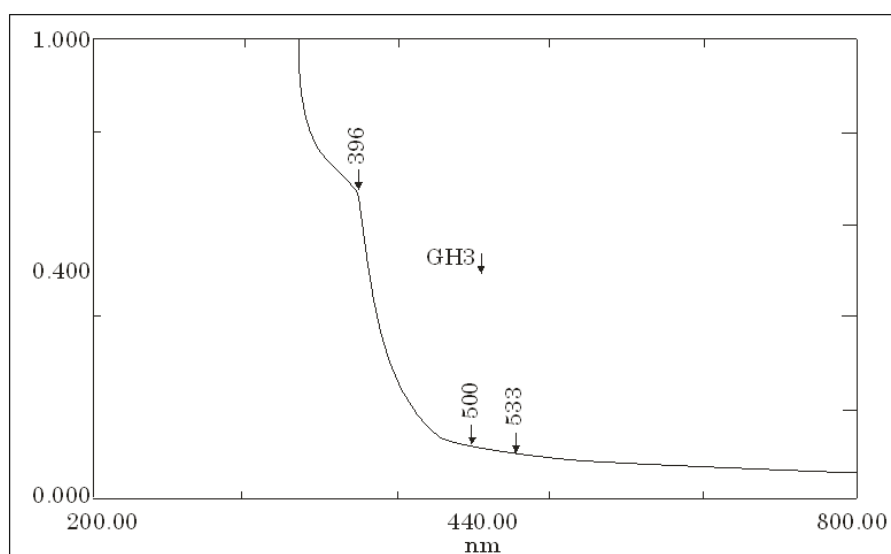


Fig-1: UV/visible spectra of sample GH3

Most plant natural constituent shows absorbance in the ultraviolet region but with exception of a few in the visible region that usually occurs in between 465 and 550 nm [25-27].

The result of FTIR analysis of sample GH3

The CH stretch vibrations for methyl and methylene are the most characteristic in terms of recognizing the compound as an organic compound containing at least one aliphatic fragment or center. The bending vibrations help to tell more about the basic structure.

The area between the bands at 1300 – 1000 cm^{-1} represents presence of stretching vibration of alcoholic and phenolic group's containing C-H, and C-O vibration of aromatic compounds [28]. Bands at 1600-1300 cm^{-1} represents C-O and C-C stretching vibrations of glucoside bonds. Ring stretching vibrations occur in the region between 1600-1300 cm^{-1} . The absorption involves stretching and contraction of all of the bonds in the ring and interaction between these stretching modes. The absorption at 1512 cm^{-1} GH3 sample therefore is due to stretching and contraction of all of the bonds in the ring of a hetero aromatic compound. This is region (1500-1520 cm^{-1}) of double bonds of which carbonyl group is the most

important that give the very strong intensity in their IR spectra. Weak IR absorption bands in this region reveal the existence of carbonyl group as impurities. The bands in GH3 sample, within this region reveal strong absorption bands and represents presence of carbonyl group (C=O) from aldehydes and ketones [28].

The position of the C = O stretching absorption varies predictably for different types of carbonyl compounds. It generally occurs at 1710–1715 cm^{-1} for simple ketones and at 1720–1725 cm^{-1} for simple aldehydes.

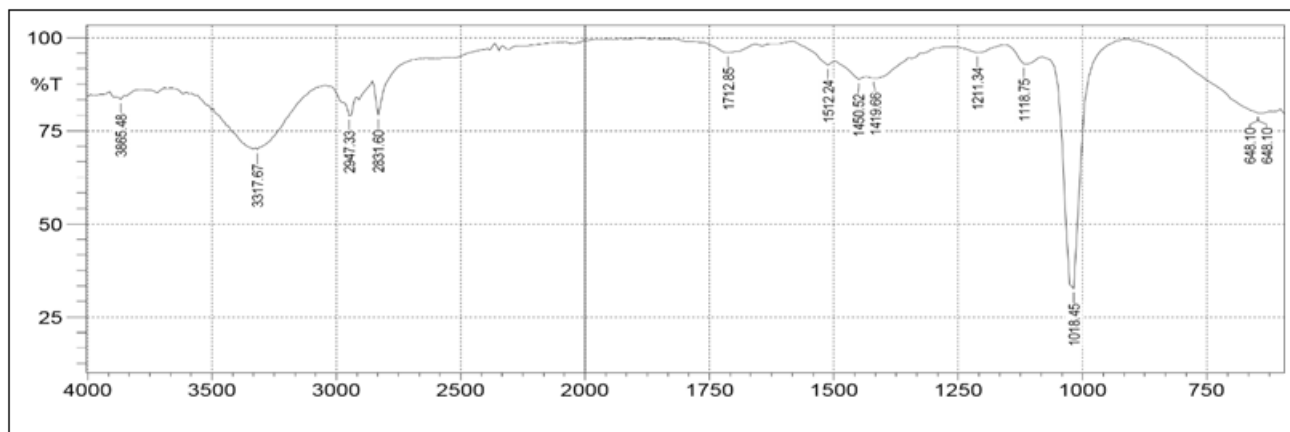


Fig-2: FTIR analysis result of sample GH3

The carbonyl absorption at 1720 cm^{-1} of the GH3 sample is clearly evident, for aliphatic and aromatic aldehydes producing carbonyl bands in the 1740–1720 cm^{-1} and 1720–1680 cm^{-1} ranges, respectively. The position of the C=O stretching wavenumber within these ranges is dependent on hydrogen bonding and conjugation within the molecule.

Conjugation with a C=C band results in delocalization of the C=O group, hence causing the absorption to shift to a lower wavenumber [29, 28].

Absorption bands between 2940–2920 cm^{-1} represents CH_2 while and 2870–2820 cm^{-1} represents CH_3 groups respectively. If the intensities of the peaks at 2940 and 2920 cm^{-1} are considerably stronger than those at 2960 and 2870 cm^{-1} it reveals that the sample (2924 cm^{-1}) contains many CH_2 groups with few

CH_3 groups [30]. In this study the intensities are nearly the same, CH_2 and CH_3 groups are evenly distributed.

Table-1: IR spectra data result of purified (GH3) sample

Peak	Intensity	Assignment
648.1	79.706	
648.1	79.706	
1018.45	32.615	C—H
1118.75	92.948	C—O Str
1211.34	95.901	C—O Str
1419.66	89.164	C—O Str.
1450.52	88.824	C—O Str.
1512.24	92.518	C=C Bending (Aromatic)
1712.85	95.934	C = O Str
2831.6	79.125	C—H Str
2947.33	78.934	C—H Str
3317.67	69.977	O-H and NH

The absorption bands of hydroxyl groups are situated in the 3650–3200 cm^{-1} region corresponds to stretching vibrations of OH groups. For the fact there is an absorption at 1300–1000 cm^{-1} that represents the presence of stretching vibration of alcoholic and phenolic group's containing C-O vibration of aromatic

compounds [30, 28] confirming that phenols is in the aromatic ring of the sample compound (GH3).

The result of ^1H NMR analysis GH3 sample

The goal of this research was to gain more information about the identification of this plant natural sample using nuclear magnetic resonance (NMR).

The spectrum of the GH3 sample is shown in Fig-3. The spectrum of the sample is suggestive of the presence aromatic signal in the 6-9 ppm region [31, 32].

The signal in sample GH3 (7.2 and 6.7) could be due to the presence of aromatic protons of a 2H-1-benzopyran.

The typical aromatic ^1H NMR resonance chemical shift of aromatic compounds are at 7.2-7.7

ppm while polyaromatic hydrocarbons are observed as far downfield as 8.7 ppm [33]. The resonance of sample GH3 at 7.2ppm is an aromatic peak. The typical pyrole ^1H NMR resonance are situated at 6.7ppm [34]. These resonances of aromatic peak at 7.2 ppm and that of pyrole at 6.7 ppm is suggestive that sample GH3 has 2H-1-Benzopyran (Chromene) as the major compound. The FTIR analysis coupled with the ^1H NMR suggests that the compound is most likely 4-amino 5, 7, 8 triol 2H chromene.

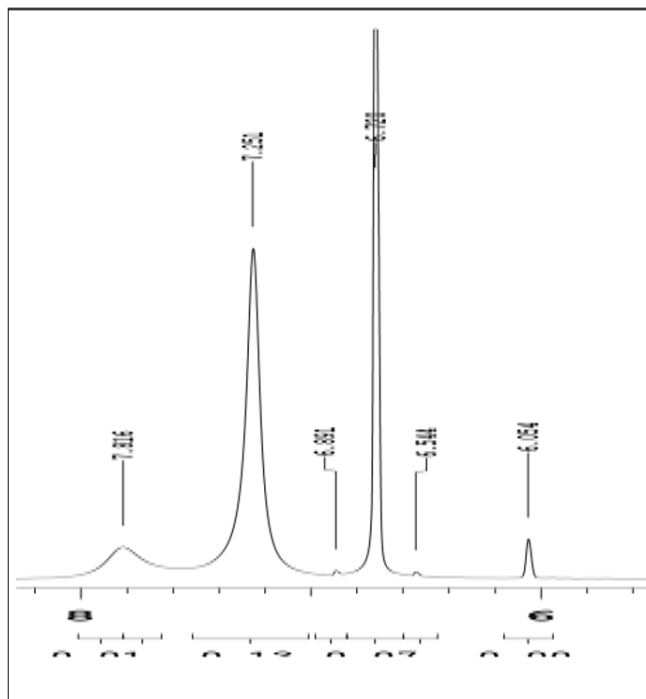


Fig-3: The result of ^1H NMR analysis of dye sample GH3

CONCLUSIONS

Three spectrophotometric method were used to try to identify, elucidate the structures, as well the functional groups and the absorbance of the isolated sample. Sample GH3 absorbs at 396, 500 and 533 nm. The FTIR analysis allowed for identification of functional groups that correspond to phenolic compounds associated to CH_3 , H, OH and NH in GH3 sample. They are also attributes substituent of benzopyran's group. A characteristic ^1H NMR spectra were obtained from GH3 sample of *Phyllanthus muellerianus* plant leaves. The analysis of GH3 sample revealed the presence of signal at 7.2 and 6.7 ppm. They are probably due to the presence of 2H-1-benzopyran present in GH3 sample.

The functional groups (CH, H, OH and NH) coupled with the ^1H NMR spectrum of GH3 (7.2 ppm and 6.7 ppm) are attributes benzopyran's groups, suggesting 2H-1-benzopyran (chromene).

REFERENCES

1. Mabberley, D. J. (2008). Mabberley's Plant-Book third edition, Cambridge University Press.
2. Kathriarachchi, H., Hoffmann, P., Samuel, R., Wurdack, K. J., & Chase, M. W. (2005). Molecular phylogenetics of Phyllanthaceae inferred from five genes (plastid atpB, matK, 3' ndhF, rbcL, and nuclear PHYC). *Molecular Phylogenetics and Evolution*, 36(1), 112-134.
3. Doughari, J. H., & Sunday, D. (2008). Antibacterial Activity of Phyllanthus muellerianus. *Pharmaceutical biology*, 46(6), 400-405.
4. Ofokansi, K. C., Attama, A. A., Uzor, P. F., & Ovri, M. O. (2013). Antibacterial activities of the combined leaf extract of Phyllanthus muellerianus and ciprofloxacin against urogenital isolates of Staphylococcus aureus. *Clinical Pharmacology and Biopharmaceutics*, 1(4), 1-5.
5. Boakye, Y. D., Agyare, C., Abotsi, W. K. M., Ayande, P. G., & Ossei, P. P. S. (2016). Anti-inflammatory activity of aqueous leaf extract of

- Phyllanthus muellerianus (Kuntze) Exell. and its major constituent, geraniin. *Journal of ethnopharmacology*, 187, 17-27.
6. John-Africa, L., Adzu, B., Dzarma, S., & Gamaniel, K. (2009). Anti-diarrhoeal activity of the methanolic leaf extract of Phyllanthus muellerianus. *International Journal of Biological and Chemical Sciences*, 3(5).
 7. Tropical Plants Database, Ken Fern. tropical. theferns.info. 2018-09-10. <tropical.theferns.info/viewtropical.php?id=Phyllanthus+muellerianus>
 8. Favre, H. A., & Powell, W. H. (2013). *Nomenclature of organic chemistry: IUPAC recommendations and preferred names 2013*. Royal Society of Chemistry.
 9. Alvey, L., Prado, S., Huteau, V., Saint-Joanis, B., Michel, S., Koch, M., ... & Janin, Y. L. (2008). A new synthetic access to furo [3, 2-f] chromene analogues of an antimycobacterial. *Bioorganic & medicinal chemistry*, 16(17), 8264-8272.
 10. Symeonidis, T., Chamilos, M., Hadjipavlou-Litina, D. J., Kallitsakis, M., & Litinas, K. E. (2009). Synthesis of hydroxycoumarins and hydroxybenzo [f]-or [h] coumarins as lipid peroxidation inhibitors. *Bioorganic & medicinal chemistry letters*, 19(4), 1139-1142.
 11. Foye, W. O. (1991). *Principi di Chemico Farmaceutica Piccin. Padova, Italy*, 416.
 12. Morgan, L. R., Jursic, B. S., Hooper, C. L., Neumann, D. M., Thangaraj, K., & LeBlanc, B. (2002). Anticancer activity for 4, 4'-dihydroxybenzophenone-2, 4-dinitrophenylhydrazone (A-007) analogues and their abilities to interact with lymphoendothelial cell surface markers. *Bioorganic & medicinal chemistry letters*, 12(23), 3407-3411.
 13. Tandon, V. K., Vaish, M., Jain, S., Bhakuni, D. S., & Srimal, R. C. (1991). Synthesis, carbon-13 NMR and hypotensive action of 2, 3-dihydro-2, 2-dimethyl-4H-naphtho [1, 2-b] pyran-4-one. *Indian J. Pharm. Sci*, 53, 22-23.
 14. Longobardi, M., Bargagna, A., Mariani, E., Schenone, P., Vitagliano, S., Stella, L., ... & Marmo, E. (1990). 2H-[1] benzothiepine [5, 4-b] pyran derivatives with local anesthetic and antiarrhythmic activities. *Farmaco (Societa chimica italiana: 1989)*, 45(4), 399-404.
 15. Bedair, A. H., El-Hady, N. A., El-Latif, M. A., Fakery, A. H., & El-Agrody, A. M. (2000). 4-Hydroxycoumarin in heterocyclic synthesis: Part III. Synthesis of some new pyrano [2, 3-d] pyrimidine, 2-substituted [1, 2, 4] triazolo [1, 5-c] pyrimidine and pyrimido [1, 6-b][1, 2, 4] triazine derivatives. *Il Farmaco*, 55(11-12), 708-714.
 16. Heravi, M. M., Bakhtiari, K., Zadsirjan, V., Bamoharram, F. F., & Heravi, O. M. (2007). Aqua mediated synthesis of substituted 2-amino-4H-chromenes catalyzed by green and reusable Preyssler heteropolyacid. *Bioorganic & medicinal chemistry letters*, 17(15), 4262-4265.
 17. Brühlmann, C., Ooms, F., Carrupt, P. A., Testa, B., Catto, M., Leonetti, F., ... & Carotti, A. (2001). Coumarins derivatives as dual inhibitors of acetylcholinesterase and monoamine oxidase. *Journal of Medicinal Chemistry*, 44(19), 3195-3198.
 18. Kesten, S. R., Heffner, T. G., Johnson, S. J., Pugsley, T. A., Wright, J. L., & Wise, L. D. (1999). Design, synthesis, and evaluation of chromen-2-ones as potent and selective human dopamine D4 antagonists. *Journal of medicinal chemistry*, 42(18), 3718-3725.
 19. Singh, R. (2005). Isolation and synthesis of anthraquinones and related compounds of Rubia cordifolia. *Journal of the Serbian Chemical Society*, 70(7), 937-942.
 20. Adebayo, G. B., Adekola, F. A., Olatunji, G. A., & Oguntoye, S. O. (2005). Chromatographic separation and spectroscopic characterization of a white African mineral hair dye/ Proceedings of the 28th International Conference of the Chemical Society of Nigeria 2(1): 121-126.
 21. Perkampus, H. H. (1995). *Encyclopedia of spectroscopy*. Weinheim: VCH.
 22. Espinosa-Morales, Y., Reyes, J., Hermosín, B., & Azamar-Barrios, J. A. (2012). Characterization of a Natural dye by spectroscopic and chromatographic techniques. *MRS Online Proceedings Library Archive*, 1374, 49-59.
 23. Adebayo, G. B., Olatunji, G. A., & Adekola, F. A. (2009). Isolation and characterization of the organic components of a black indigenous mineral hair dye. *International Journal of Physical Science*, 4(12): 770-776.
 24. Claridge, T. (1999). *High resolution NMR techniques in organic chemistry (1st edition)*, Elsevier, UK.
 25. Kafle, B. P., Pokhrel, B. R., Gyawali, R., Kafle, A., Shrestha, T. M., Shrestha, R., & Adhikari, R. M. (2014). Absorbance of natural and synthetic dyes: Prospect of application as sensitizers in dye sensitized solar cell. *Absorbance Nat. Synth. Dye. Prospect Appl. as sensitizers Dye sensitized Sol. cell*, 5(1), 8-12.
 26. Huria, K. A., Deepti, D., Lajya, D., & Sunder, S. S. (2005). Domestic violence in pregnancy in North Indian women. *Indian journal of medical sciences*, 59(5), 195-199.
 27. Eder, R. (2000). *Pigments*. In Food Analysis by HPLC, 2 ed.; NOLLET, L. M. L., Ed. Marcel Dekker Inc. Monticello, NY, 845-880.
 28. Baciu, A., Ranga, F., Fetea, F., Zavoi, S., & Socaciu, C. (2013). Fingerprinting food supplements and their botanical ingredients by coupled UV/Vis/FTIR spectrometry. *Bulletin of*

- University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca: Food Science and Technology*, 70(1), 8-15.
29. Stuart, B. H. (2004). *Infrared spectroscopy: Fundamentals and Applications*. John Wiley and Sons, Ltd, England, 25-31.
30. Young-Cheng, N. (2011). *Interpretation of Organic Spectra*. John Wiley & Sons Singapore, (1); 87-127.
31. Tulio Jr, A. Z., Reese, R. N., Wyzgoski, F. J., Rinaldi, P. L., Fu, R., Scheerens, J. C., & Miller, A. R. (2008). Cyanidin 3-rutinoside and cyanidin 3-xylosylrutinoside as primary phenolic antioxidants in black raspberry. *Journal of Agricultural and Food Chemistry*, 56(6), 1880-1888.
32. Ozgen, M., Wyzgoski, F. J., Tulio, A. Z., Gazula, A., Miller, A. R., Scheerens, J. C., ... & Wright, S. R. (2008). Antioxidant capacity and phenolic antioxidants of midwestern black raspberries grown for direct markets are influenced by production site. *HortScience*, 43(7), 2039-2047.
33. Simpson, J. H. (2008). *Organic structure determination using 2-D NMR spectroscopy: A problem based approach*. Elsevier Inc. London UK.
34. Charles, J. P., & Jacquelyn, B. (1993). *The Aldrich Library of 13C and 1H FT NMR Spectra*. Aldrich Chemistry Company Inc., New York, 2.