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Original Research Article

Isolation and Characterization of *β*-Sitosterol, Palmitic acid and Rutin from the Root of Nigerian *Kalanchoe pinnata* (Lam.)

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ABSRTACT

In Nigeria, *Kalanchoe pinnata* (Lam) is a widely used medicinal plant in traditional medicine. The leaves are a popular therapeutic herb for a variety of ailments, but the root has gotten relatively little attention and limited scientific exploration, suggesting a potential untapped resource. Given the variety of biological activities linked to *Kalanchoe* species, it is conceivable that the root may contain special phytochemicals with beneficial medicinal qualities. The root extract of *K. pinnata* was obtained through cold maceration and triturated with n-hexane, ethyl acetate, and methanol according to polarity, where fractions TF1, TF2, and TF3 were obtained. TF3, based on its antimicrobial and phytochemical screening, was subjected to isolation and purification by column and thin layer chromatography and characterization by spectroscopic methods (^{1}H-NMR, ^{13}C-NMR, Infra-red spectroscopies, and Mass spectrometry). This led to the isolation of three compounds, which were identified as β-sitosterol, a known plant sterol; palmitic acid, a fatty acid; and rutin, a flavonol. This discovery may open up new avenues for the development of these compounds as medications for the treatment of ailments such as ulcers and inflammation.

Keywords: *Kalanchoe pinnata* root, Palmitic acid, Rutin, β-sitosterol, Spectroscopy

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Introduction

Kalanchoe pinnata, a plant with a rich history of ethnomedical use, has garnered increasing scientific interest due to its diverse array of phytochemicals and their associated medicinal properties. [1,2] Several isolated compounds, such as quercetin glycosides, have demonstrated promising therapeutic potential, exhibiting anti-nociceptive, anti-edematogenic, and anti-inflammatory effects. [3,4,5] Notably, *K. pinnata* also contains phytosterols, including β-sitosterol, a compound known for its anticancer activity against various cancer types. [6,7] Beyond its anticancer properties, β-sitosterol has been reported to possess a range of biological activities, including anti-cholesterolemic, anti-inflammatory, antioxidant, anti-diabetic, and wound-healing effects, as well as the induction of apoptosis. [8,9,10] This study aims to investigate the phytochemical composition of the root of *K. pinnata* sourced from Nigeria, seeking to identify bioactive compounds with potential therapeutic applications. By characterizing these constituents, it provides scientific validation for the plant's traditional ethno-medicinal uses and explores its potential as a source of novel drug leads.

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Materials and Methods

Kalanchoe pinnata plants were collected in the month of November, year 2022 from Yandev, Gboko Local Government Area (L.G.A.), Benue State, Nigeria (geographic coordinates: 9°02'34.84" E, 7°21'40.71" N) and identified at the Federal College of Forestry, Jos, Nigeria. A voucher specimen (FHJ 263) was deposited in the herbarium. Fresh roots were harvested, cleaned, and air-dried at room temperature on a bench, with regular turning to prevent moisture accumulation. The dried plant material was subsequently stored in sterile, desiccated containers.

Extraction

The dried *K. pinnata* root sample was crushed using a mortar and pestle; 1.50 kg of the root sample was macerated using 2.50 L of 70 % ethanol for 3 days with frequent agitation. This was then filtered and concentrated using rotary evaporator at 40 ºC to obtain solvent-free extract. The extraction was repeated twice and the extractives pooled together to obtain brownish crude extract. The percentage yield of the extract was calculated.

Isolation and characterization

The crude extract (40 g) was dissolved in 150 mL of hexane, warmed in a water bath, and filtered through Whatman filter paper. This process was repeated twice to obtain the n-hexane (n-Hex) fraction. The ethyl acetate (EtOAc) and methanol (MeOH) fractions were also obtained using the same hexane method. The MeOH fraction was then subjected to column chromatography using silica gel (60 – 120 mesh) as the stationary phase. Elution was performed using a gradient solvent system: 100 % n-Hex, mixtures of n-Hex/EtOAc, 100 % EtOAc, and mixtures of EtOAc/MeOH, with the proportion of the more polar solvent increasing in 5 % increments. Fractions of approximately 10 mL were collected, and the solvent volume was

reduced to approximately one-third by evaporation. These concentrated fractions were analyzed by thin layer chromatography (TLC). Fractions 51 – 55 (n-Hex/EtOAc, 60:40 v/v) exhibited similar R_f values and were combined and designated CF1. Similarly, fractions 255 – 286 (EtOAc/MeOH, 90:10 v/v) and 321 – 359 (EtOAc/MeOH, 80:20 v/v) were pooled and designated CF10 and CF20, respectively. These combined fractions (CF1, CF10, and CF20) were further analyzed using spectroscopic techniques (^{1}H, ^{13}C-NMR, IR and MS) for structural elucidation.

Instrument

The ^{1}H and ^{13}C-NMR spectra were obtained on Bruker AVIII (400 MHz) spectrophotometer using $CDCl_3$ and tetramethylsilane (TMS) as internal standard. The Infra-red spectra were recorded on FTIR Agilent Cary 630 (Agilent Technologies) and Mass spectra on JEOL Mstation JMS-700 spectrophotometer using high resolution Atmospheric Pressure Chemical Ionization method (APCIMS) with corona discharge electrode detector to ionize the sample at normal pressure. The NMR and APCIMS were carried out at the University of Glasgow, Scotland, United Kingdom and Universite Grenoble Alpes. FTIR analysis was carried out at the Department of Chemistry, Ahmedu Bello University (ABU) Zaria, Nigeria.

Results and Discussions

The percentage yield of the *K. pinnata* root methanolic fraction was found to be 47.98 %, and that of the isolated compounds, CF1, CF10 and CF20 were 0.01, 0.03 and 0.03 % respectively. The compound CF1 was obtained as a whitish crystalline substance with a melting point of 138 – 140 ºC, CF10 as a whitish powder with a melting point of 62 – 64 ºC, and CF20 as a yellowish powder with a melting point of 180 – 182 ºC. These values were comparable with the findings of literature.[11,12,13,14,15] The structures (Figures 1) of CF1, CF10 and CF20 were established based on NMR spectroscopic data as well as comparison with other existing data.

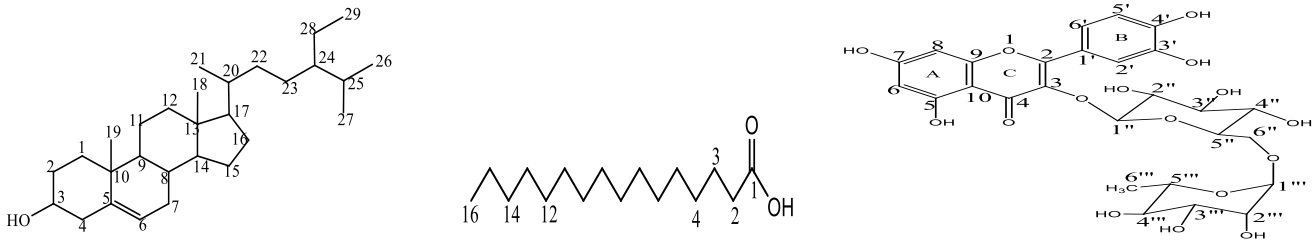

Figure 1: Structure of CF1 Structure of CF10 Structure of CF20

The NMR spectrum (Figure 2) for compound CF1 presented as ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.34 (dd, J = 4.48, 2.63 Hz, 1H), 3.51 (m, 1H), 2.29 (m, 2H), 1.83 (m, 2H), 0.67 (s, 3H), 0.91 (m, 3H), 0.82 (m, 3H) suggests the compound to be a phytosterol, which corresponds with literature values. [7,12,16] The ^{13}C NMR spectrum (Figure 3) also presented as ^{13}C NMR (125 MHz, $CDCl_3$): δ 140.92, 121.86, 72.49, 56.96, 55.52, 50.30, 46.79, 42.47, 42.47, 39.94, 37.42, 37.16, 37.12, 34.12, 32.08, 31.84, 29.85, 29.85, 28.40, 24.46, 24.46, 23.24, 21.85, 19.97, 19.20, 19.20, 18.97, 12.72 and 12.02 is indicative of a characteristic steroid. The infrared (IR) spectrum showed a broad peak at 3414 cm^{-1} characteristic of O-H stretching; this is assigned to the hydroxyl group at C-3 of the ^{1}H NMR spectrum. The spectral peaks at 1632 and 697 cm^{-1} are due to C=C vibrations, indicative of an olefinic proton (H-6). This distinguishes it from a stigmasterol with reported peaks of two olefinic protons. [17] Also, the peak at 2926 cm^{-1} indicates an aliphatic bond due to C-H vibration. Its molecular formula was determined to be $C_{29}H_{50}O$ from the APCIMS spectrum (Figure 3), which showed an ion peak of mass/charge (*m/z*) $[M+H]^+$ of 415.39.

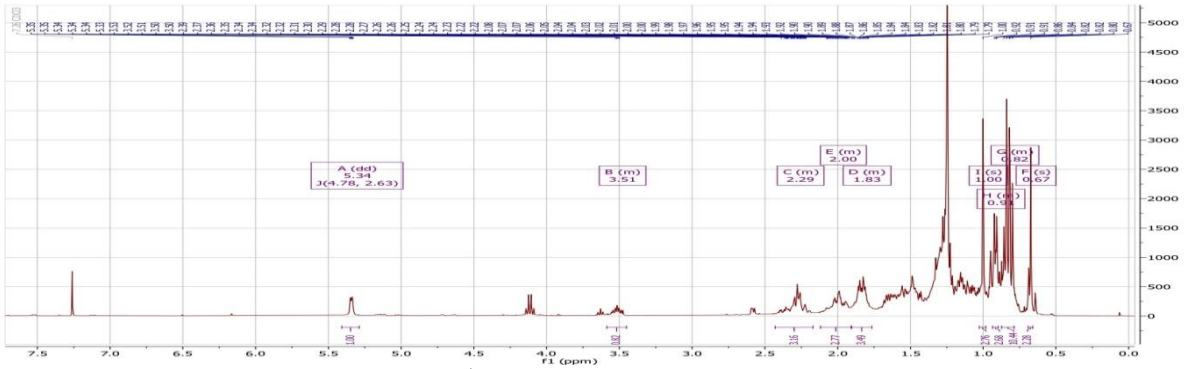

Figure 2: ^{1}H-NMR spectrum for compound CF1

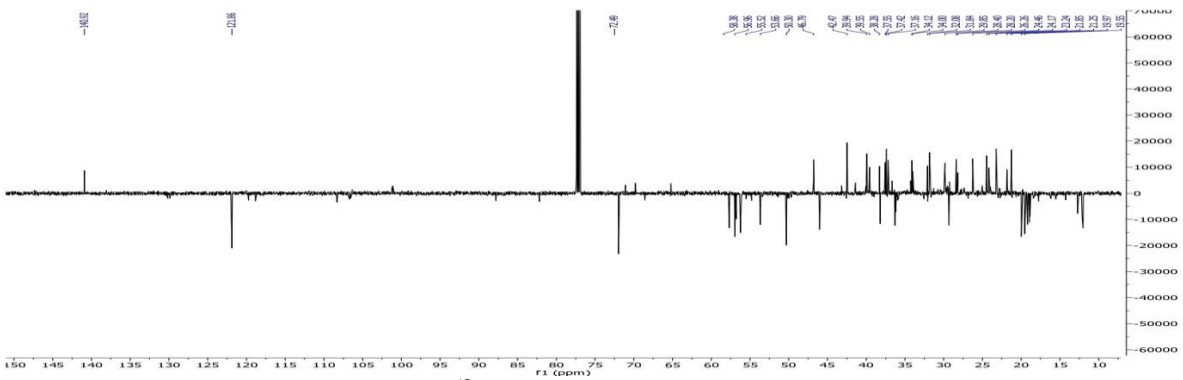

Figure 3: ^{13}C-NMR spectrum for compound CF1

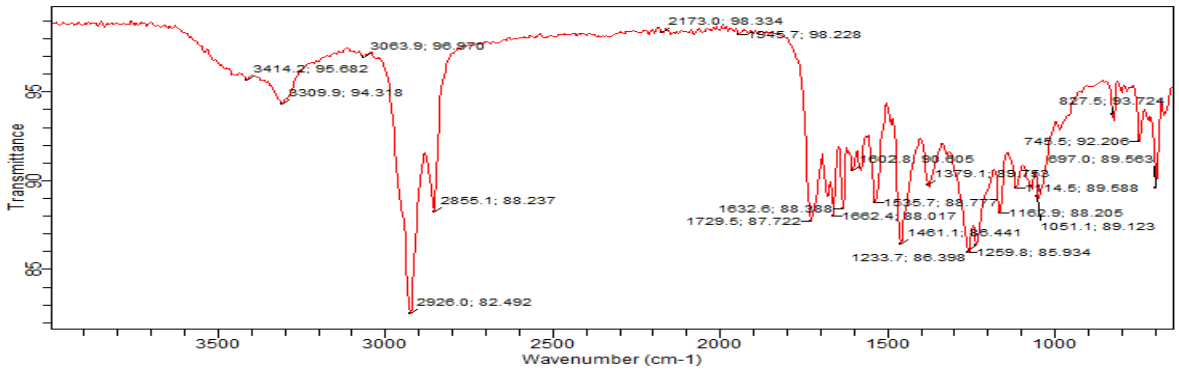

Figure 4: IR spectrum for compound CF1

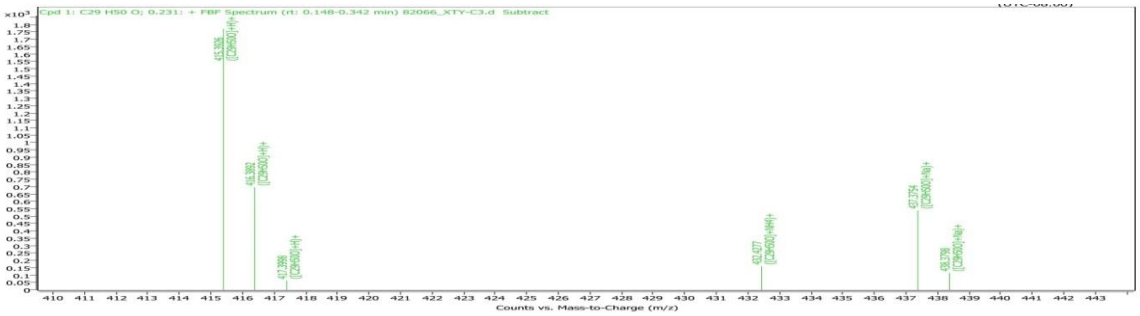

Figure 5: Mass Spectrum for Compound CF1

The ^{1}H NMR (Figure 6) spectral data of compound CF10 presented as ^{1}H NMR (400 MHz, MeOD): δ 2.29 (t, J = 7.49, 2H), 1.62 (q, J = 7.61, 2H), 1.32-1.34 (m, J = 5.82, 24H) 0.92 (t, J = 11.68,9.03, Hz, 3H). The ^{13}C NMR spectrum (Figure 7) showed the data as follows: ^{13}C NMR (100 MHz, MeOD) δ 176.35, 33.59, 24.71, 29.95, 13.06. The IR spectrum (Figure 8) showed a broad hydroxyl group peak at 3410 cm^{-1}. The peaks at 2922 cm^{-1} and 2855 cm^{-1} correspond to the C-H bond stretching for CH_3 and CH_2 respectively. The peak at 1725 cm^{-1} indicates the presence of C=O group, while 1461 cm^{-1} and 1379 cm^{-1} represent the presence of C-H bending for CH_3 and CH_2 bonds. The peaks at 1267, 1181, 1121, 1073 cm^{-1} is for C-O stretching of carboxylic acid group. The results correspond to that of literature.[18] From Figure 9, the *m/z* showed a $[M–H]^-$ to be 255.22 corresponding to the molecular formula $C_{16}H_{32}O_2$. Based on the spectral data and that of literature, compound CF10 was identified as palmitic acid.[18,14,19]

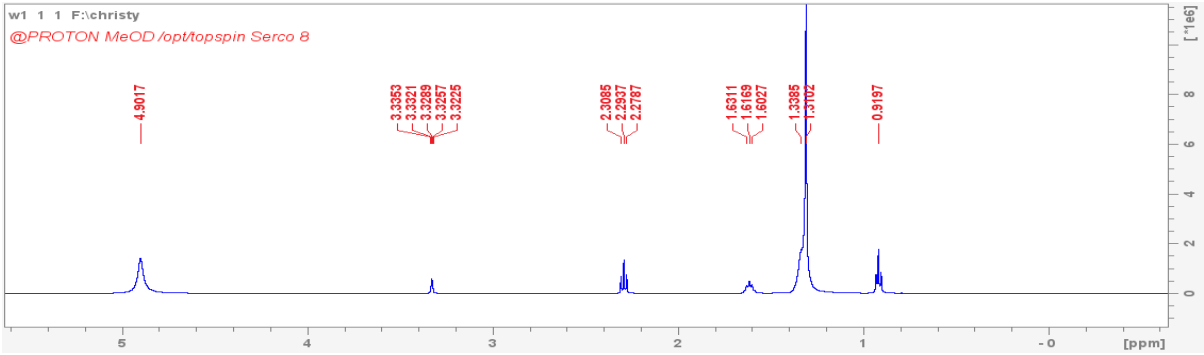

Figure 6: ^{1}H-NMR Spectrum for Compound CF10

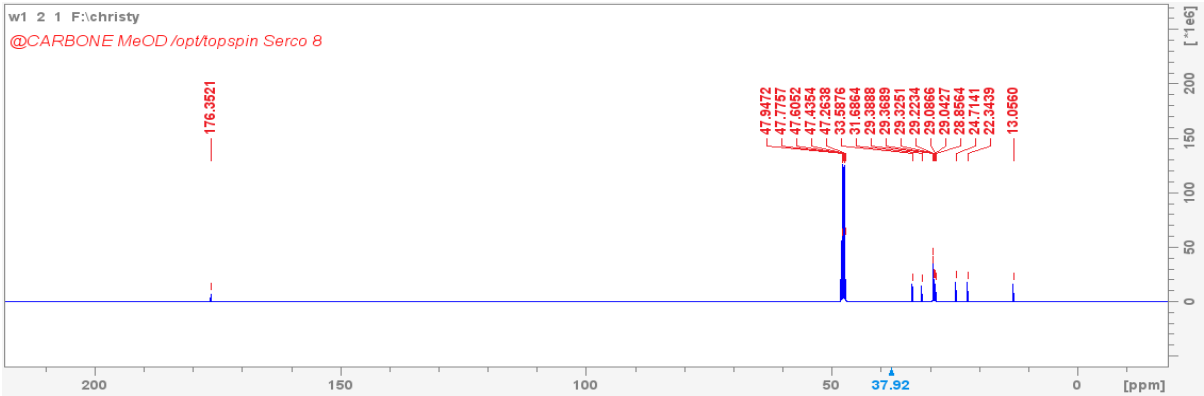

Figure 7: ^{13}C-NMR Spectrum for Compound CF10

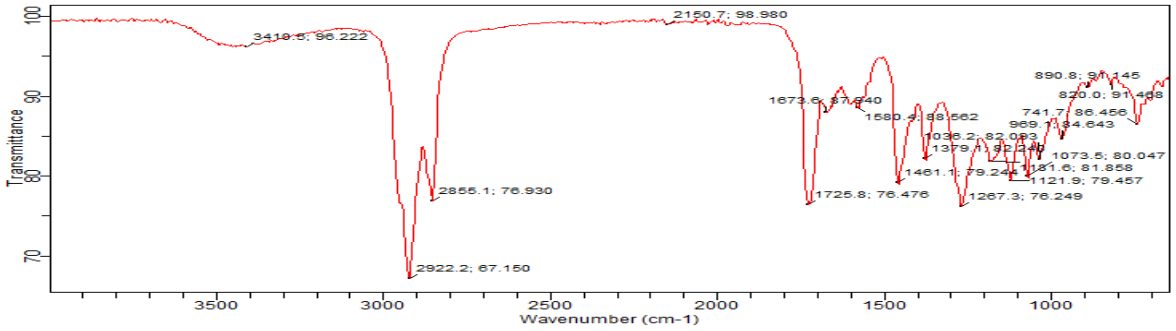

Figure 8: IR Spectrum for Compound CF10

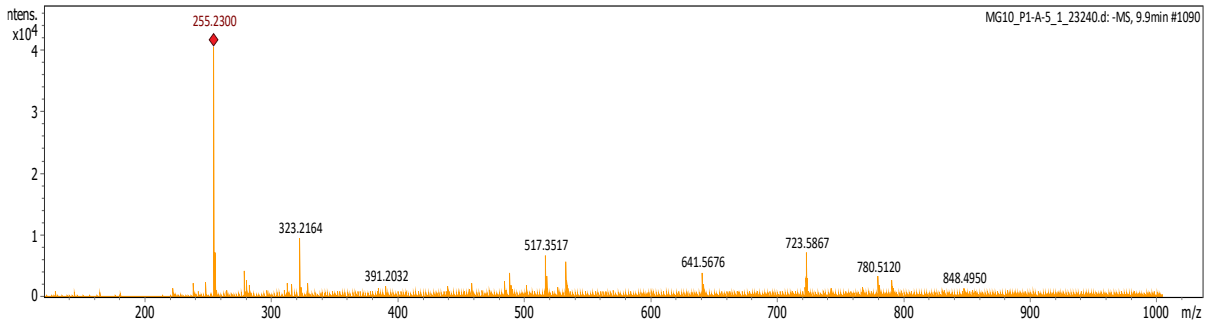

Figure 9: Mass Spectrum for Compound CF10

The compound CF20 ^{1}H NMR (400 MHz, MeOD) spectral data (Figure 10) showed characteristic signal peaks at δ 7.69 (dd, J = 8.5 Hz, 1H), 7.65 (dq, J = 2.2 Hz, 1H), 6.73 (d, J = 8.4 Hz, 1H), 6.41 (d, J = 2.1 Hz, 1H), 6.22 (d, J = 2.1 Hz, 1H), 5.13 (d, J = 7.8 Hz, 1H), 4.54 (d, J = 1.6 Hz, 1H), 1.14 (d, J = 6.2 Hz, 3H). These resonates with the aromatic protons which are visible between 6 to 8 ppm in the characteristic quercetin skeleton.[20] The anomeric proton chemical shift of rutinose which are visible between 3 to 4 ppm, can be seen in compound CF20; while, the peak at 1.14 (3H, d, J = 6.2 Hz) corresponds to the methyl group attached to the sugar moiety suggestive of rhamnose sugar.[21,22] The ^{13}C spectrum (Figure 11) is as follows: ^{13}C NMR (125 MHz, MeOD): δ 177.99, 164.59, 161.53, 157.94, 157.93, 148.40, 144.42, 134.23, 121.71, 122.17, 116.32, 114.67, 104.22, 103.33, 101.01, 98.56, 93.49, 76.76, 75.79, 74.32, 72.54, 70.84, 70.70, 69.98, 68.31, 67.16, 16.49. This data agrees with those obtained in literature. [22,23] The analysis of the ^{1}H and ^{13}C NMR spectra of CF20 and comparison with the values found in literatures led to the assignment of compound CF20 as rutin (quercetin-3-*O*-rutinoside). The IR spectrum (Figure 12) showed broad peak at 3348 cm^{-1}, representing the O–H bond stretching. The peak at 2920 cm^{-1} represents alkanes C–H bond stretching, and the peak at 1694 cm^{-1} indicates the presence of C=O bond vibration for aromatic rings. The strong peak at 1470 cm^{-1} represent C–H bond stretching which indicates the presence of aromatics and 1285 cm^{-1} represent C–O stretching (in-ring) attributed to benzene ring and sugars present. From Figure 13, the *m/z* showed a $[M+Na]^{+}$ to be 634.10 corresponding to the molecular formula $C_{27}H_{30}O_{16}$.

The three compounds: β-sitosterol ($C_{29}H_{50}O$), a phytosterol; palmitic acid ($C_{16}H_{32}O_2$), a fatty acid; and rutin ($C_{27}H_{30}O_{16}$), a flavonoid isolated from the roots of the Nigerian *K. pinnata* plant, clearly confirm the presence of active components in the crude extracts of the plant.[2,5] These phytochemicals (secondary metabolites), which occur naturally in plants, work in synergy and are also responsible for the curative properties of medicinal plants, as well as "lead compounds for the development of novel drugs". [2,24] β-sitosterol with a chemical structure similar to cholesterol competes with it for absorption in the gut, leading to reduced cholesterol levels. It exhibits anti-inflammatory, anti-oxidant and anti-cancer properties. [7,8] Palmitic acid is a major component of triglycerides, which are the primary energy storage molecules in the body. Rutin is a potent antioxidant, scavenging free radicals and protecting cells from oxidative stress, also possessing anti-inflammatory properties.[1,8]

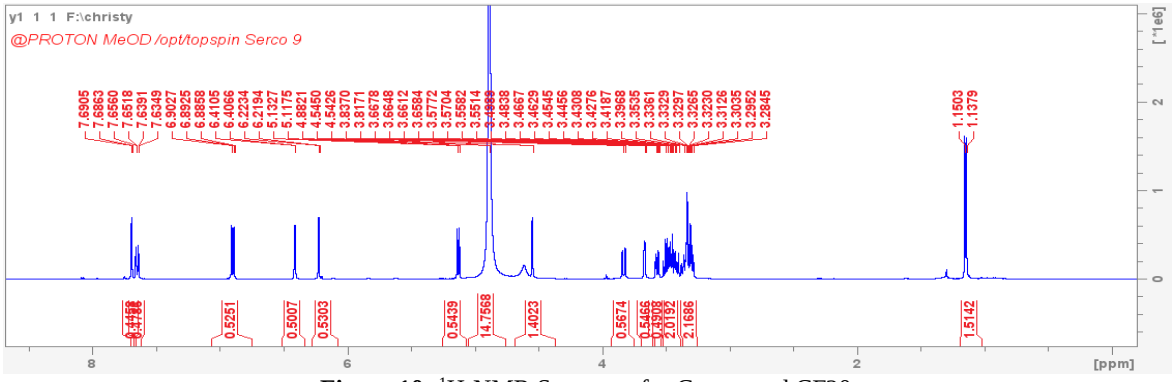

Figure 10: ^{1}H-NMR Spectrum for Compound CF20

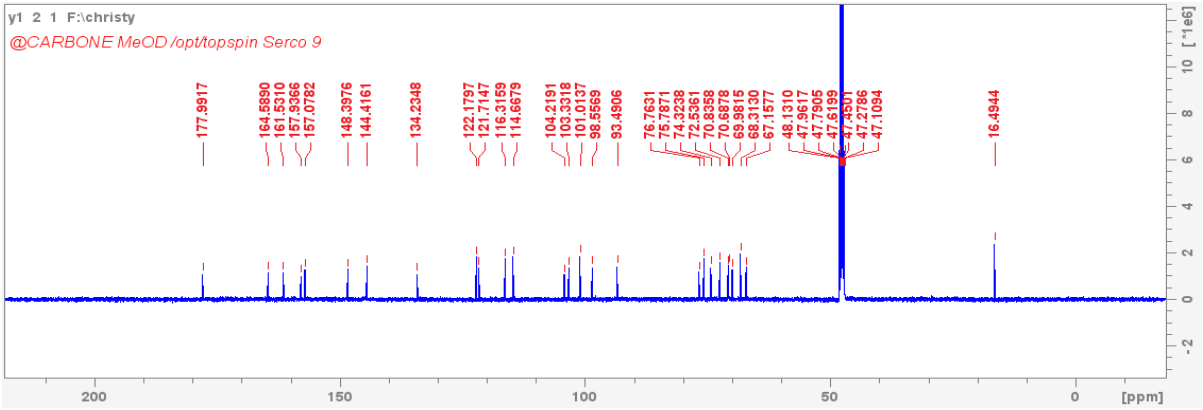

Figure 11: ^{13}C-NMR Spectrum for Compound CF20

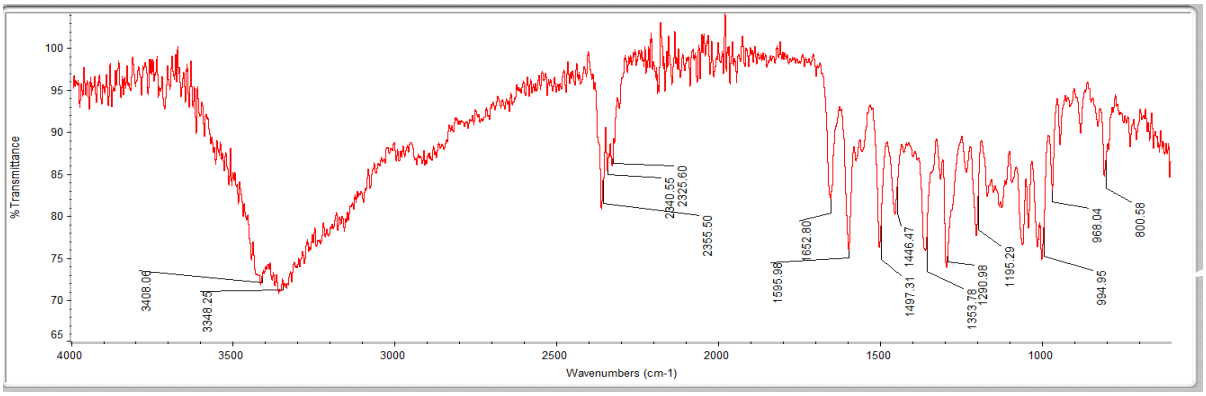

Figure 12: IR Spectrum for Compound CF20

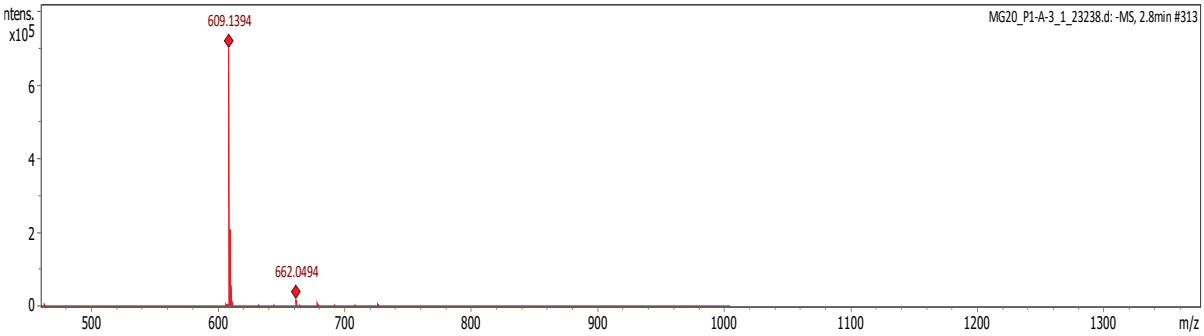

Figure 13: Mass Spectrum for Compound CF20

Conclusion

The compounds β-sitosterol, palmitic acid and rutin were isolated and characterized from the methanol fraction of Nigeria *K. pinnata* root. Although there has been reports from the leaves, this is the first time that β-sitosterol, palmitic acid and rutin has been reported to have been isolated from the Nigerian *K. pinnata* root. The presence of these compounds in the root of *K. pinnata* suggests potential medicinal uses for this plant; and may be responsible for its anti-oxidant, anti-microbial, anti-inflammatory, and anti-ulcer activities previously reported from the crude extracts. Based on the demonstrated potential in treating various ailments, this knowledge can inform the development of new therapeutic agents.

Conflict of Interest

The authors report no conflict of interest.

Authors' Declaration

The authors hereby attest to the originality of the work contained in this article and that they will be held responsible for any allegations pertaining to its content.

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References

1. Nascimento LBDS, Casanova LM, Costa SS. Bioactive compounds from Kalanchoe genus potentially useful for the development of new drugs. Life, 2023; 13(3): 646. https://doi.org/ 10.3390/life13030646
2. Kendeson AC, Kagoro LM, Adelakun AE. Phytochemical, antioxidant and antimicrobial evaluation of Nigerian *Kalanchoe pinnata* (Lam.) roots. Nig J. Pharm Appl Sci Res, 2024; 13(1): 19 – 25.

http://doi.org/10.60787/nijop hasr-v13-i1-531.
3. Coutinho MA, Muzitano MF, Cruz EA, Bergonzi MC, Kaiser CR, Tinoco LW, Bilia AR, Vincieri FF, Rossi-Bergmann B, Costa SS. Flowers from *Kalanchoe pinnata* are a rich source of T cell-suppressive flavonoids. Nat Prod Commun. 2012; 7(2): 175 – 178. https://doi.org/10. 1177/1934578X1200700211
4. Coutinho MA, Casanova ML, Nascimento SBL, Leal D, Palmero C, Toma KH, Dos Santos PE, Eurico L, Costa, S S. Wound healing cream formulated with *Kalanchoe pinnata* major flavonoid is as effective as the aqueous leaf extract cream in a rat model of excisional wound. Nat Prod Res. 2020; 35(24): 6034 – 6039. https://doi.org/10.1080/14786419.2020.1817012
5. Ferreira RT, Coutinho MAS, Malvar DC, Costa EA, Florentino IF, Costa SS, Vanderlinde FA. Mechanisms underlying the antinociceptive, antiedemato- genic and anti-Inflammatory activity of the main flavonoid from *Kalanchoe pinnata*. Evid Based Complement Alternat Med. (2014); 1 – 8. http://dx.doi.org/10.1155/2014/42 9256
6. Saeidnia S, Manayi A, Gohari R A, Abdollahi M. The story of beta-sitosterol- a review. Euro. J. Med. Plants, 2014; 4(5): 590 – 609.
7. Nyigo VA, Peter X, Mabiki F, Malebo HM, Mdegela RH, Fouche G. Isolation and identification of euphol and β-sitosterol from the dichloromethane extracts of *Synadenium glaucescens*. J Phytopharmacol, 2016; 5(3): 100 – 104. https://doi.org/10.31254/phyto.2016.5302
8. Alamgir ANM. Therapeutic use of medicinal plants and their extracts: Phytochemistry and bioactive compounds (Vol. 2), Gewerbestrasse: Springer 2018. 186 p. https://doi.org/10. 1007/978-3-319-92387-1
9. Farabi K, Harneti D, Nurlelasari, Mayanti T, Maharani R, Supratman U. Isolation, structure determination, and cytotoxic activity of steroid compound from the stem bark of *Aglaia cucullata* (Meliaceae). J. Sci. Appl Chem, 2023; 26(6): 217 – 223. https://doi.org/10.14710/jksa.26.6.217-223
10. Liu Y, Li Z, Li W, Chen X, Yang L, Lu S, Zhou S, Li M, Xiong W, Zhang X, Liu, Yu, Zhou J. Discovery of β-sitosterol's effects on molecular changes in rat diabetic wounds and its impact on angiogenesis and macrophages. Int. Immunopharmacol. 2024; 126, 111283. https://doi.org/10.1016/j.intimp.2023.111283
11. Aliba, M. O., Ndukwe, I. G. Ibrahim, H. Isolation and characterization of β-sitosterol from methanol extracts of the stem bark of large-leaved rock fig (*Ficus abutilifolia* Miq). J. Appl. Sci. Environ. Manage. 2018; 22(10): 1639 – 1642.
12. Farabi K, Harneti D, Nurlelasari, Maharani R, Hidayat TA, Supratman U, Awang K, Shiono Y. Cytotoxic steroids from the bark of *Aglaia argentea* (Meliaceae). Chiang Mai Univ J. Nat Sc, 2017; 16(4): 293 – 306. https://doi.org/10.12982/CMUJNS.2017.0024
13. Johannes E, Laga A, Litaay M, Husain RD, Haedar N, Tuwo M, Murua JH, Pasandin, J. The potentials of isolated Hexadecanoic acid of hydroid *Aglaophenia cupressina* Lamoureoux as an antifungal compound on the rotten Strawberries *Fragaria x ananassa* Dutch. and Mango *Mangifera indica* L. IJASEIT, 2022; 12(4): 1552 – 1557.
14. Mothana AA, Al-Shamahy HA, Mothana RA, Khaled MJ, Al-Rehaily JA, Al-Mahdi YA, Lindequist U. Streptomyces sp. 1S1 isolated from Southern coast of the Red Sea as a renewable natural resource of several bioactive compounds. Saudi Pharm J. 2022; 30(2): 162 – 171. https://doi.org/10. 1016/j.jsps.2021.12.01
15. Chang C, Song M, Ma M, Song J, Cao F, Qin Q. Preparation, characterization and molecular dynamics simulation of rutin–cyclodextrin inclusion complexes. Molecules, 2023; 28(3): 955. https://doi.org/ 10.3390/molecules28030955
16. Uttu AJ, Sallau MS, Ibrahim H, Iyun ORA. Isolation, characterization, and docking studies of campesterol and b-sitosterol from *Strychnos innocua* (Delile) root bark. J Taibah Univ Med Sc, 2023; 18(3): 566 – 578. https://doi.org/10.1016/j. jtumed.2022.12.003
17. Luhata LP, Munkombwe NM. Isolation and characterisation of stigmasterol and β-sitosterol from *Odontonema strictum* (Acanthaceae). JIPBS, 2015; 2(1): 88 – 96 *JIPBS*,
18. Bharath B, Perinbam K, Devanesan S, AlSalhi SM, Saravanan M. Evaluation of the anticancer potential of Hexadecanoic acid from brown algae *Turbinaria ornata* on HT–29 colon cancer cells. J. Molecul Struct, 2021; 1235: 130229. https://doi.org/10.1016/j.molstruc.2021.130229
19. Wakawa HY, Ahmad FB. Characterisation of chemical compounds from the root and leaf extract of *Abrus precatorius*: Borneo JRST, 2023; 13(2): 186 – 202. https:doi.org/10.33736/bjrst.5646.2023
20. Ganbaatar C, Gruner M, Mishig D, Duger R, Schmidt AW, Knölker H J. Flavonoid glycosides from the aerial parts of *Polygonatum odoratum* (Mill.) Druce growing in Mongolia. Open Nat. Prod. J., 2015; 8(1): 1 – 7.
21. Pivec T, Kargl R, Maver U, Bračič M, Elschner T, Žagar E, Gradišnik L, Kleinschek, KS. Chemical structure–Antioxidant activity relationship of water–based enzymatic polymerized rutin and its wound healing potential. Polymers, 2019; 11(10): 1566. https://doi.org/10.3390/polym11101566
22. Immaculate AA, Vimala JR, Takuwa DT. Isolation and characterisation of flavonoids (Rutin) from the roots of *Cadaba aphylla* (Thunb) and *Adenia glauca* of potential in anti-oxidant activity. Orient. J. Chem, 2022; 38(6): 1404 – 1413. http://dx.doi.org/10.13005/ojc/38 0610
23. Aisyah LS, Yun YF, Herlina T, Julaeha E, Zainuddin A, Nurfarida I, Hidayat AT, Supratman U, Shiono Y. Flavonoid compounds from the leaves of *Kalanchoe prolifera* and their cytotoxic activity against P-388 murine leukimia cells. Nat. Prod. Sci, 2017; 23(2): 139 – 145. https://doi.org/10.20307/nps.2017.23.2.139.
24. Miron A, Nahar L, Gille, E, Sarker SD. Antipsoriatic natural products. Ann Reports Med. Chem, 2020; 297–325, https://doi.org/10.1016/bs.armc.2020.02.005