

ORIGINAL RESEARCH ARTICLE

Isolation and Structural Elucidation of Stigmasterol, β -Sitosterol, and Tripalmitolein from the Leaf of *Vitex Chrysocarpa* (Lamiaceae)

Safwan Yakubu Ruba^{1*}, Mohammed Ibrahim Sule², Yahaya Muhammad Sani²,Adetunji Adebayo Sadam² and Muhammad Sada Uzairu³¹Department of Chemistry, Sule Lamido University, Kafin Hausa, Jigawa, Nigeria²Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria, Nigeria³Department of Chemistry, National Open University of Nigeria

ARTICLE HISTORY

Received July 15, 2024

Accepted October 10, 2024

Published October 16, 2024

ABSTRACT

Vitex chrysocarpa (Lamiaceae) is used in traditional medicine to treat fever, dysmenorrhea, venereal diseases, and dysentery. However, more research needs to be done on the compounds responsible for these activities. This study aims to isolate and elucidate the main bioactive compounds from the plant's methanol leaf extract. The maceration method was utilized for the extraction, while column and thin-layer chromatographic techniques were employed for purification and isolation. As a result, Tripalmitolein (4.7 mg) and a combination of Stigmasterol and β -sitosterol (7 mg) were isolated. Careful examination of the 1D and 2D NMR data allowed for the structural elucidation of the compounds, and additional confirmation of the compounds were obtained by comparing the results with those of the existing literature. This study presents the first isolation and structural elucidation of Stigmasterol, β -sitosterol, and tripalmitolein from *Vitex chrysocarpa*.

KEYWORDS

Vitex chrysocarpa, Lamiaceae, Extraction, NMR, Stigmasterol, β -sitosterol



© The authors. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License

(<https://creativecommons.org/licenses/by-nc/4.0/>)

INTRODUCTION

Nature is blessed with abundant biological resources that can be employed as fillers in pharmaceutical formulations or as treatments for various illnesses and infections (Akwada et al., 2020). The use of plants for medicinal purposes has been there throughout antiquity and could be regarded as the forerunner of contemporary medicine. Around the world, the usage of medicinal plants to cure illnesses is still multiplying, with many people resorting to this kind of product for the prevention and treatment of various pathogens (Romano et al., 2021).

Vitex comes from “Vieo” (Latin), meaning binding or linking (Devika, 2017). *Vitex chrysocarpa* belongs to the genus *Vitex* of the family Lamiaceae, which consists of about 250 species known globally.

Traditionally, different plant parts are used to treat various ailments (Atiku et al., 2021). The plant *Vitex chrysocarpa* is a medicinal plant that taxonomically belongs to the kingdom Plantae, phylum-Magnoliophyta, class-Magnoliopsida, order-Lamiales, family-Lamiaceae, genus-*Vitex* and specie-*chrysocarpa* (GBIF, 2021). It is a small

spreading tree in a fringing forest and frequently on riverbanks in Mali and northern and southern Nigeria (Meerts, 2020). It is traditionally used to treat diseases related to insects, fungi, bacteria, venereal diseases (Ilodibia et al., 2016), and dysentery (Nna et al., 2022).

Stigmasterol and β -sitosterol are known for their anti-inflammatory, antioxidant, anticancer, and cholesterol-lowering properties (Ashraf and Bhatti, 2021; Saeidnia et al., 2014), while tripalmitolein has potential applications in cosmetics and food preservation and drugs (Wu et al., 2017). However, these compounds have not been previously isolated from *Vitex chrysocarpa*. Several studies have isolated Stigmasterol and Sitosterol from various plants (Table 1), but no study reported the isolation of these compounds from the *Vitex chrysocarpa* plant; thus, this study aims to fill this gap by attempting to isolate and characterize a few of the phytoconstituents present in the leaf of this plant.

Correspondence: Safwan Yakubu Ruba. Department of Chemistry, Sule Lamido University, Kafin Hausa, Jigawa, Nigeria.

✉ safwanyakubu.ruba@slu.edu.ng, Phone Number: +234 703 527 9041

How to cite: Ruba, S. Y., Sule, M. I., Sani, Y. M., Sadam, A. A., & Uzairu, M. S. (2024). Isolation and Structural Elucidation of Stigmasterol, β -Sitosterol, and Tripalmitolein from the Leaf of *Vitex Chrysocarpa* (Lamiaceae). *UMYU Scientifica*, 3(4), 84 – 93. <https://doi.org/10.56919/usci.2434.008>

Table 1: Summary of related literature

Plant name	Part	Solvent/Extraction method	Isolated compounds	Author
Macadamia	Standard nuts oil	-	Tripalmitolein	(Retief et al., 2009)
Stylochiton lancifolius	Rhizome	Pet-ether, methanol Successive extraction	Stigmasterol, Sitosterol	(Pateh et al., 2009)
Rubus suavissimus	Leaf	Dichloromethane(DCM) / successive extraction	Stigmasterol, Sitosterol	(Chaturvedula & Prakash, 2012)
Odontema strictum	Leaf	Methanol, DCM	Stigmasterol, Sitosterol	(Pierre & Moses, 2015)
Ocimum tenuiflorum	Aerial part	Methanol/ maceration	Stigmasterol, Sitosterol	(Nhan et al., 2018)
Adenodolichoe peniculatus	Leaf	Ethylacetate exhaustive extraction	Stigmasterol, Sitosterol	(Ibrahim et al., 2018)
Magnifera indica	Root	Hexane, Ethylacetate/ Soxhlet extraction	Stigmasterol, Sitosterol	(Nna et al., 2019)
Emilia coccinea	Leaf	Hexane, Ethylacetate/ Soxhlet extraction	Stigmasterol, Sitosterol	(Akwada et al., 2020)

MATERIALS AND METHOD

Collection and Identification of the Plant Material

A plant sample was collected from the riverbank area of Dani village in the Itas-Gadau local government area of Bauchi state. Mallam Namadi Sanusi identified and authenticated the plant sample at the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria. The voucher specimen number 018669 was obtained for documentation purposes.

Extraction and Partitioning

The maceration process extracted 1.5 Kg of powdered leaves with 80% methanol over three days (Nn, 2015). After draining and filtering the extract through No. 1 Whatman filter paper, the filtrate was concentrated using a rotary evaporator and left to dry. A dark green residue of 102.4 g remained. The n-hexane, chloroform, and ethyl acetate fractions were obtained by chromatographic partitioning with n-hexane, chloroform, and ethyl acetate, in that order, as described in the subsequent section.

Chromatographic Separation

The n-hexane fraction (8g) was run through a 250ml column chromatography process using silica gel (mesh size 60-120). The wet slurry method was used to pack the

column, and it was given time to settle. After dissolving the sample in a small amount of hexane, a small amount of silica gel was added to create a paste, which was then adsorbed on silica gel. The paste was loaded onto the previously packed column after it had dried and been triturated. Gradient elution was used to elute the column, starting with 100% hexane, then 95:5 % hexane: ethyl acetate, then gradually increasing the polarity by 10% to 100% ethyl acetate. The column was then washed with methanol. Eluents in 100 milliliters were collected, yielding seventy-six (76) collections. The various column collections were combined based on similarity in their TLC (thin layer chromatography) retention factor using hexane and ethyl acetate (8 : 2, 7 : 3 and 1 : 1) as solvent system to give 20 significant column fractions coded A-T for further purifications.

After being pooled collectively, the pooled fractions E-F, which displayed three spots on TLC, were subjected to silica gel column chromatography and gradient elution, which involved commencing with 100% hexane and ending with increasing order of polarity to reduce the complexity of the compounds. The column collections (95 collections) were pooled in order of similarities of their retention factor on TLC, of which collections 74-89 then formed crystals and were washed using methanol to obtain a clear white crystal which, employing the same solvent

system, produced a single spot on TLC and was designated SR1, and subjected to infrared and nuclear magnetic resonance (NMR) spectroscopy to verify its purity.

Similarly, fraction G consists of 3 significant spots partitioned utilizing gradient elution silica gel column chromatography using hexane and Ethyl acetate as solvents of choice. Collections between the ranges of 84-92 gave a single homogenous spot. They were coded as SR2 and subjected to infrared and nuclear magnetic resonance (NMR) spectroscopy and chemical tests to verify their purity.

RESULTS

Identification and Characterization of Compound SR1

Compound SR1 (7mg) was isolated as a white crystalline powder with an R_f (retardation factor) value of 0.49 using hexane: Ethyl acetate (3:1) as the solvent system (Plate 1). Its melting point was found to be between 145-147°C and gave appositive Liebermann-Burchard's test for steroids.



Plate 1: TLC chromatogram of SR1

The IR absorption spectrum of SR1 (Figure 1) showed absorption peaks at 3634 cm^{-1} (O-H str), 3421 cm^{-1} (C=C cyclic olefinic), 2866 cm^{-1} (C-H str), 1660 cm^{-1} (C=C olefinic str), other absorption frequencies include 1461 cm^{-1} (C-H ben), 1058 cm^{-1} (cyclo alkane).

The ^1H NMR (400 MHz, CDCl_3) spectrum of SR1 (Figure 2) showed signals at δ H 3.55 (m), 5.36 (s), 5.15 (m), 1.08 (s), 0.94 (d) and 0.87 (d).

The ^{13}C -NMR (δ ppm, 125 MHz, CDCl_3) (Figure 3) & DEPT experiments shows the presence of 29 carbon

atoms; 140.99(q), 138.33(CH), 129.28(CH), 121.74(CH), 71.83(HC-OH), 56.87(CH), 55.96(CH), 51.24(CH), 50.14(CH), 42.54(CH_2), 42.30(q), 40.51(CH), 39.69(CH_2), 37.26(CH_2), 36.15(q), 31.93(CH_2), 31.93(CH), 31.66(CH_2), 29.16(CH), 28.93(CH_2), 25.42(CH_2), 24.31(CH_2), 23.07(CH_3), 21.23(CH_2), 21.09(CH_3), 19.41(CH_3), 18.99(CH_3), 12.06 (CH_3), 11.87(CH_3).

Identification and Characterization of Compounds in SR2

Compound SR2 (4.7mg) was isolated as a yellowish oil with an R_f value of 0.37 using hexane: dichloromethane (3:1) as solvent system (Plate 2).



Plate 2: TLC chromatogram of SR2

The IR absorption spectrum of SR2 (Figure 4) revealed major bands at 2922 cm^{-1} (C-H str), 2855 cm^{-1} (C-H str), 1740 cm^{-1} (C=O str), and 1162 cm^{-1} (C-O). Other significant absorptions include that observed at 723 cm^{-1}

The ^1H NMR spectrum (400 MHz, CDCl_3) of SR2 (Figure 5) revealed the following chemical shift values 5.34 (t, $J=5.68$ Hz), 5.26 (t, $J=4.6$ Hz, 4.8 Hz), 4.29 (dd $J=3.96$ Hz, 4.36 Hz), 4.14 (dd $J=5.64$ Hz, 6.16 Hz), 2.31 (t $J=7.64$ Hz, 7.32Hz), 2.0 ($J=5.56$ Hz), 1.60, 1.29, 0.88 ($J=7.12$ Hz, 6.16 Hz).

The ^{13}C NMR (Figure 6) and DEPT (400 MHz, CDCl_3) spectrum of SR2 showed absorption signals at 173.28 (q), 170.61 (q), 130.04 (CH), 129.7 (CH), 68.88 (CH_2), 62.11 (CH_2), 34.21 (CH_2), 34.04 (CH_2), 31.93 (CH_2), 31.80 (CH_2), 29.77 (CH_2), 29.71 (CH_2), 29.67 (CH_2), 29.49 (CH_2), 29.37 (CH_2), 29.33 (CH_2), 29.28 (CH_2), 29.13 (CH_2), 29.06 (CH_2), 27.23 (CH_2), 27.18 (CH_2), 24.87 (CH_2), 22.70 (CH_2), 22.48 (CH_2), 14.12 (CH_3)

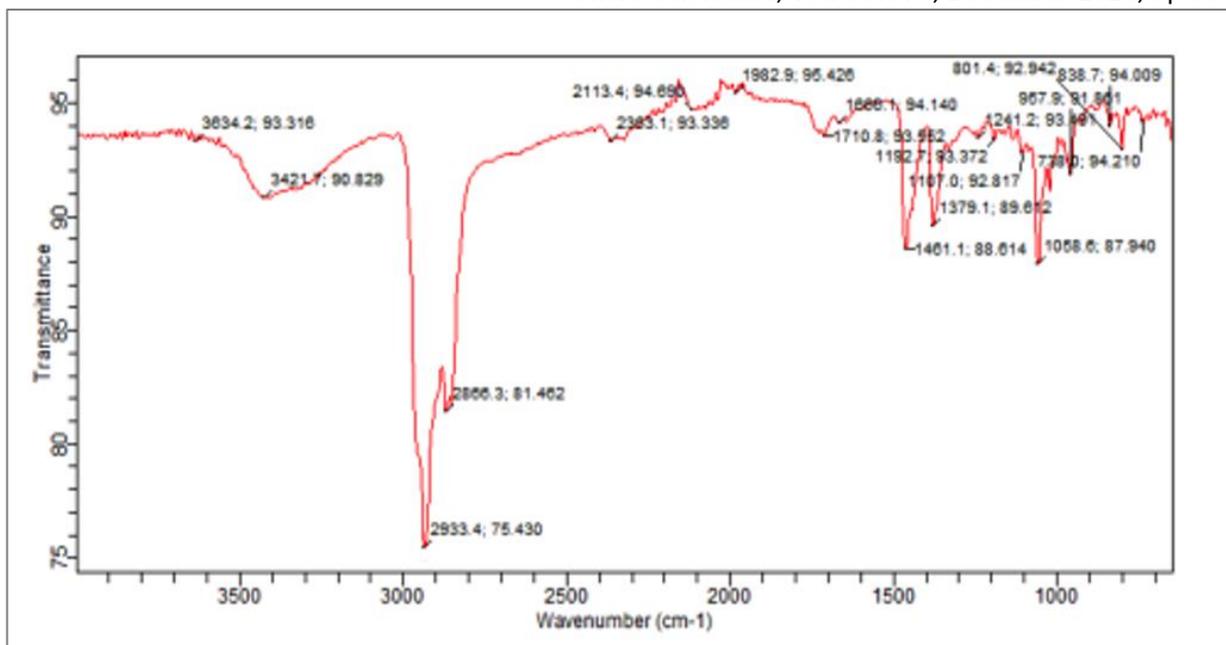


Figure 1: IR spectrum of SR1

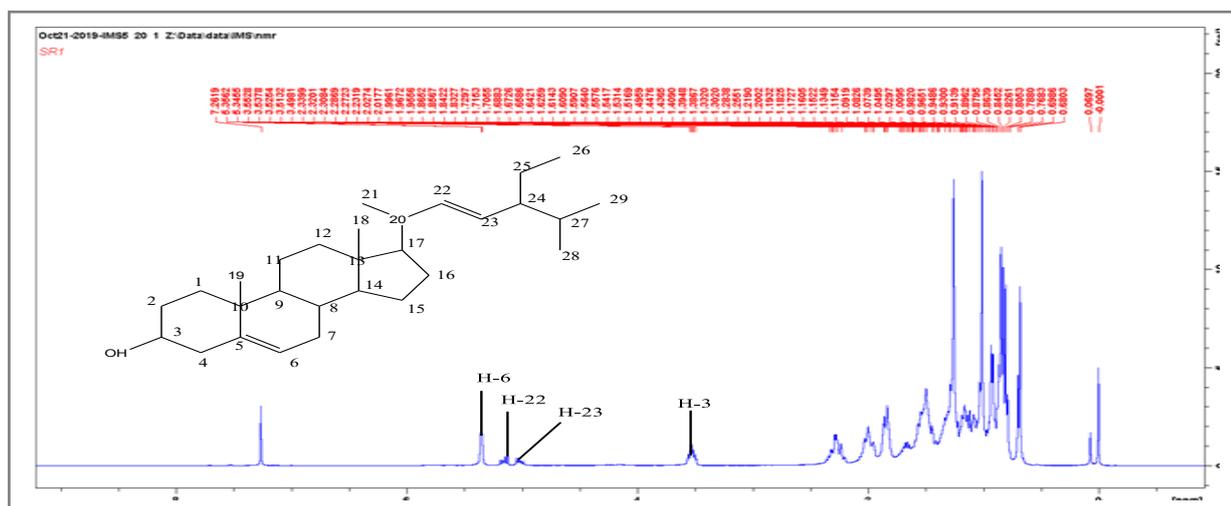


Figure 2: ¹H-NMR spectrum of SR2 in CDCl₃

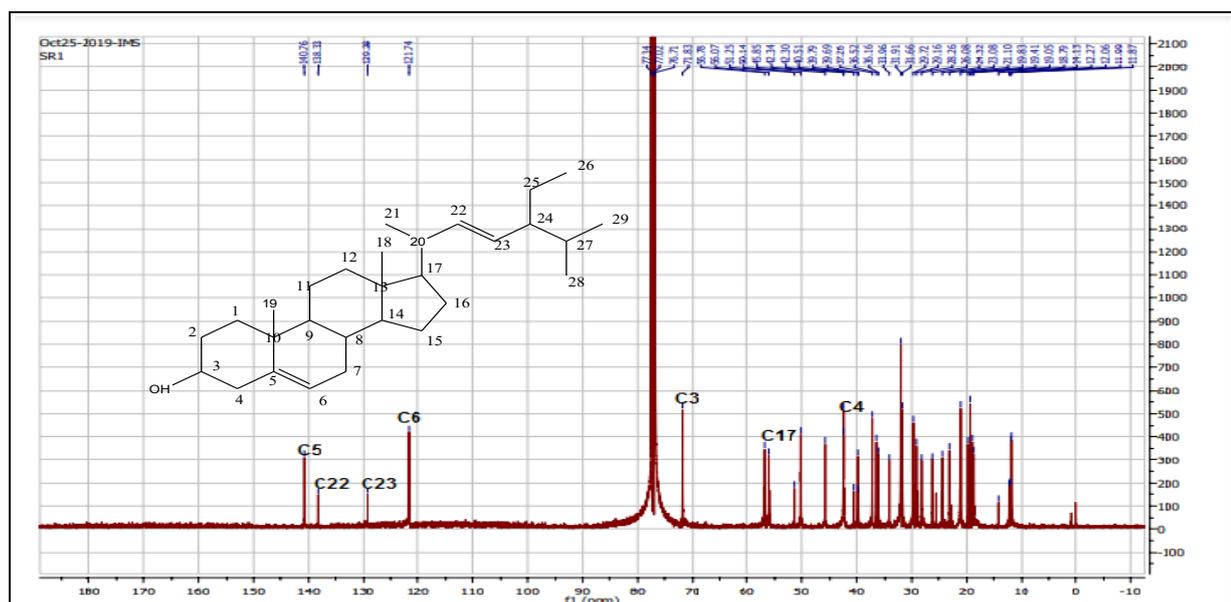


Figure 3: ¹³C-NMR spectrum of SR1

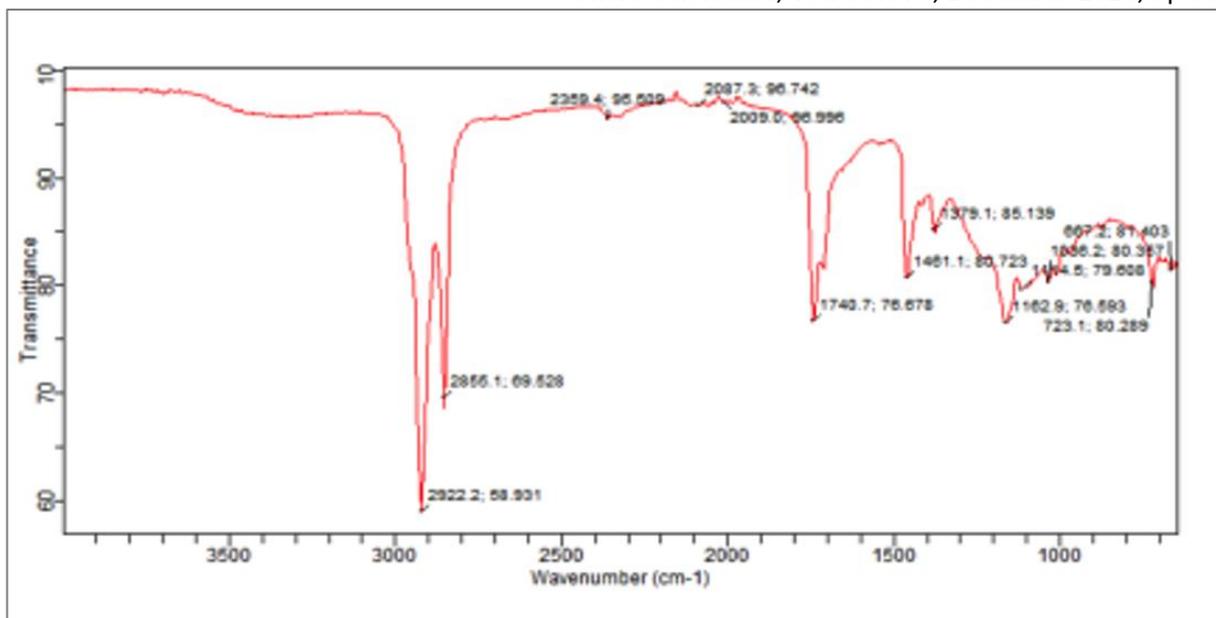


Figure 4: IR spectrum of SR2

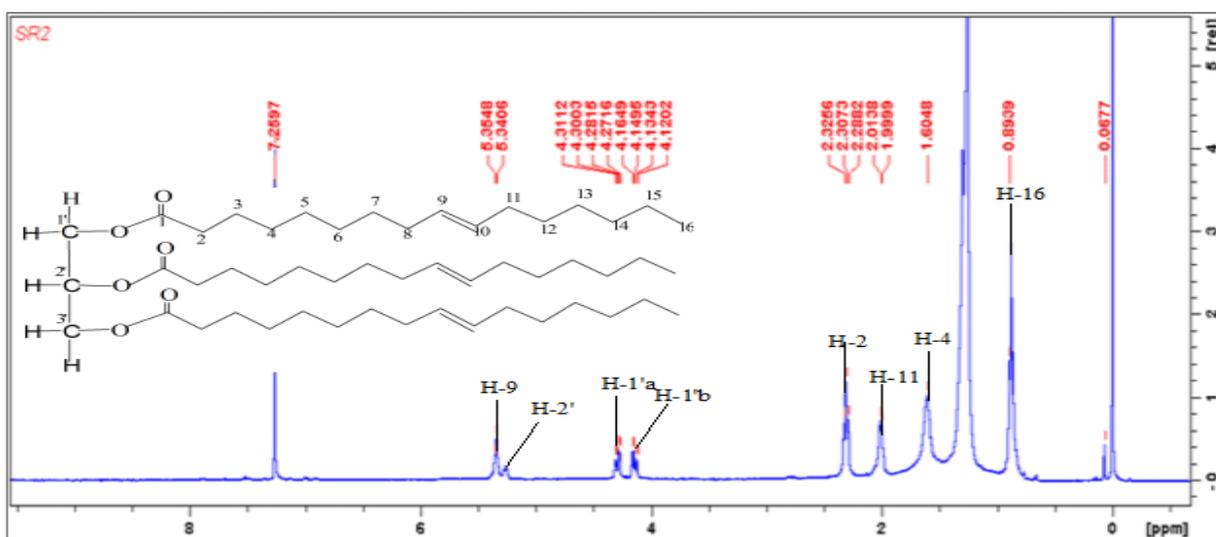


Figure 5: ¹H-NMR spectrum of SR2

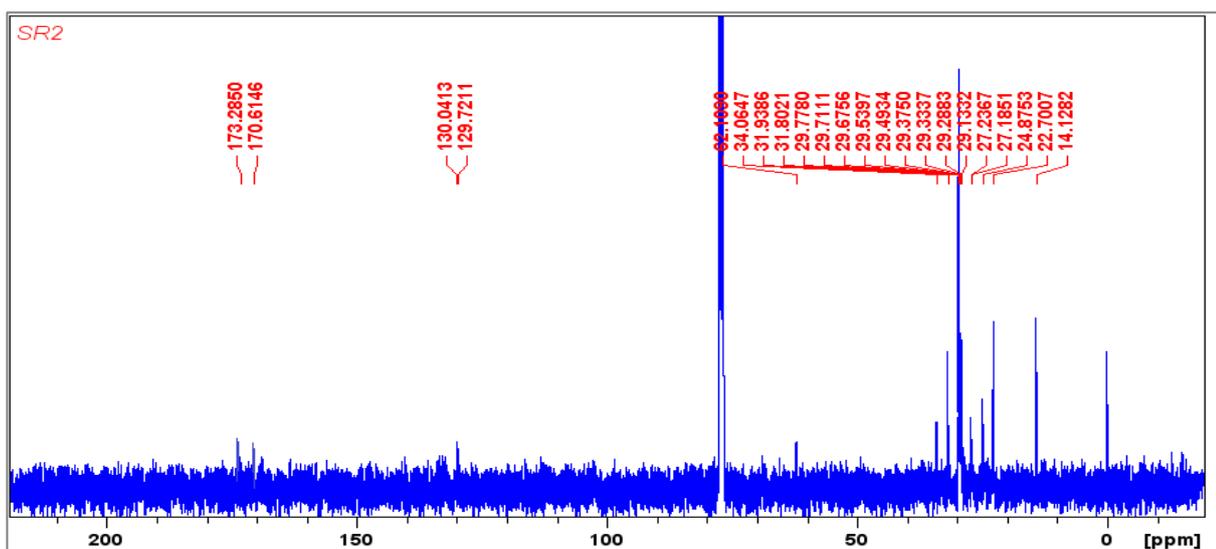


Figure 6: ¹³C-NMR spectrum of SR1

Table 2: NMR data of SR1 compared with the literature

Position	¹³ C-NMR SR1	¹ H-NMR SR1	¹³ C-NMR	¹ H-NMR (Nhan et al., 2018)
	Experimental			
1	37.3	-	37.2	
2	31.7	-	31.6	
3	71.8	3.53 (m)	71.8	3.53 (m)
4	42.3	-	42.3	
5	140.7	-	140.7	
6	121.7	-	121.7	
7	31.9	-	31.9	
8	31.9	-	31.9	
9	51.3	-	50.1	
10	36.2	-	36.5	
11	21.1	-	21.07	
12	39.7	-	39.7	
13	42.3	-	42.3	
14	56.9	-	56.8	
15	24.3	-	24.3	
16	28.9	-	28.9	
17	56.1	-	55.9	
18	11.9	0.69 (s)	12.0	0.68 (s)
19	21.2	1.01 (s)	19.4	1.01 (s)
20	40.5	-	40.4	
21	21.2	1.03 (d)	21.09	1.02 (d)
22	138.3	-	138.3	
23	129.3	-	129.3	
24	51.3	-	51.2	
25	31.9	-	31.8	
26	21.2	0.788 (d)	21.2	0.84 (d)
27	19.0	0.845 (d)	18.9	0.83 (d)
28	25.4	-	25.4	
29	12.1	0.805 (t)	12.2	0.86 (t)

Table 3: NMR spectral data of SR2

Carbon	Position on glycerol backbone	$\delta^{13}\text{C}$	$\delta^1\text{H}$	Dept	Cosy	HMBC
C1	1', 3'	173.28	-	C	-	-
	2'	170.61	-	C	-	-
C2	1', 3'	34.06	2.31	CH ₂	H3	C1, C3, C5
	2'	34.21	2.31	CH ₂	-	-
C3	1', 3'	24.87	1.60	CH ₂	H2, H4	C2, C4
	2'	24.87	1.60	CH ₂	-	-
C4	1', 3'	29.06	1.29	CH ₂	H3	-
	2'	29.06	1.29	CH ₂	-	-
C5	1', 3'	29.13	-	CH ₂	-	-
	2'	29.13	-	CH ₂	-	-
C6	1', 3'	29.28	-	CH ₂	-	-
	2'	29.33	-	CH ₂	-	-
C7	1', 3'	29.49	-	CH ₂	-	-
	2'	29.67	-	CH ₂	-	-
C8	1', 3'	27.18	2.0	CH ₂	-	-
	2'	27.18	2.0	CH ₂	-	-
C9	1', 3'	130.06	5.34	CH	-	-
	2'	129.73	5.34	CH	-	-
C10	1', 3'	130.06	5.34	CH	H11	C11
	2'	129.73	5.34	CH	-	-
C11	1', 3'	27.23	2.0	CH ₂	H10, H12	C10, C12
	2'	27.23	2.0	CH ₂	-	-

To be continued next page

Table 3 continued

Carbon	Position on glycerol backbone	$\delta^{13}\text{C}$	$\delta^1\text{H}$	Dept	Cosy	HMBC
C12	1', 3'	29.71	-	CH ₂	H11	-
	2'	29.71	-	CH ₂	-	-
C13	1', 3'; 2'	29.77	-	CH ₂	-	-
C14	1', 3'	31.80	1.29	CH ₂	-	-
	2'	31.80	1.29	CH ₂	-	-
C15	1', 3'	22.70	1.26	CH ₂	H16	C14
	2'	22.48	1.26	CH ₂	-	-
C16	1', 3'	14.12	0.88	CH ₃	H15	C14, C15
	2'	14.12	0.88	CH ₃	-	-
CHO		68.88	5.26	CH	H 1',3'a; H 1',3'b	-
CH ₂ O		62.11	4.14	CH ₂	H 1',3'b,H2'	C1, C2'
			4.29		H 1',3'a, H2'	

Table 4: NMR data of SR2 in comparison with literature

Carbon	Position on glycerol backbone	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$
		Experimental	(Retief et al., 2009)
C1	1', 3'	173.28	173.20
	2'	170.61	172.79
C2	1', 3'	34.06	34.02
	2'	34.21	34.18
C3	1', 3'	24.87	24.87
	2'	24.87	24.87
C4	1', 3'	29.06	29.08
	2'	29.06	29.04
C5	1', 3'	29.13	29.16
	2'	29.13	29.18
C6	1', 3'	29.28	29.10
	2'	29.33	29.11
C7	1', 3'	29.49	29.69
	2'	29.67	29.70
C8	1', 3'	27.18	27.16
	2'	27.18	27.16
C9	1', 3'	130.06	129.69
	2'	129.73	129.66
C10	1', 3'	130.06	129.98
	2'	129.73	129.99
C11	1', 3'	27.23	27.22
	2'	27.23	27.22
C12	1', 3'	29.71	29.72
	2'	29.71	29.72
C13	1', 3'; 2'	29.77	29.98
C14	1', 3';	31.80	31.78
	2'	31.80	31.78
C15	1', 3'	22.70	22.65
	2'	22.48	22.65
C16	1', 3'	14.12	14.09
	2'	14.12	14.09
CHO		68.88	68.87
CH ₂ O		62.11	62.08

DISCUSSION

Spectral Analysis of SR1

In the IR spectra of SR1 (Figure 1), absorption bands were visible at 3634 cm⁻¹ due to (O-H stretching), at 3421 cm⁻¹

due to (cyclic olefinic C=C), at 2933-2866 cm⁻¹ due to (C-H stretching). Additional absorption frequencies are 1660 cm⁻¹ due to (C=C olefinic stretching), at 1461 cm⁻¹ due to (C-H bending), and the absorption at 1058 cm⁻¹ signifies cycloalkane. Stigmasterol's absorptions are like these.

The ^1H NMR spectra of SR1 (Figure 2) show three (3) olefinic protons δ 5.36, δ 5.15, and δ 5.03, proposing the existence of three (3) protons, which would be consistent with the trisubstituted and disubstituted olefinic bond configurations; an oxygenated carbon with a proton on it at δ 3.55 and a group of resonance up field between δ 2.53 and δ 0.69, suggesting that it is a steroidal nucleus.

The ^{13}C NMR (Figure 3) and DEPT of the compound's structure were revealed by experiments to consist of an unsaturated molecule with 29 carbons, which are three quaternary carbons (C), six methyl's (CH_3) groups, nine methylene (CH_2) groups, and eleven methines (CH). This further indicates the compound. The resonance observed downfield at 140.76 (C-5), 121.74 (C-6), 138.33 (C-22), and 129.28 (C-23) indicates unsaturation, and the hydroxylated carbon (HC-OH) at 71.83 (C-3) further suggest the compound to be Stigmasterol.

This compound's 1D NMR spectral features, summarized in Table 2, validated the structure and demonstrated an excellent fit in contrast to a reference NMR result.

Literature has shown that Stigmasterol and β -sitosterol usually come as a mixture and are challenging to obtain in pure form. The existence of the C22-C23 single bond in β -sitosterol and the C22=C23 double bond in Stigmasterol is the only distinction between the two substances. Despite using different solvent systems, Stigmasterol and β -sitosterol did not give any difference in their R_f values. Therefore, compound SR1 is a mixture of Stigmasterol (66.7%) and β -sitosterol (33.3%); these substances are phytosterols found to exist naturally in various plants. However, this is the first time that it has been documented from *V. chrysocarpa* leaves.

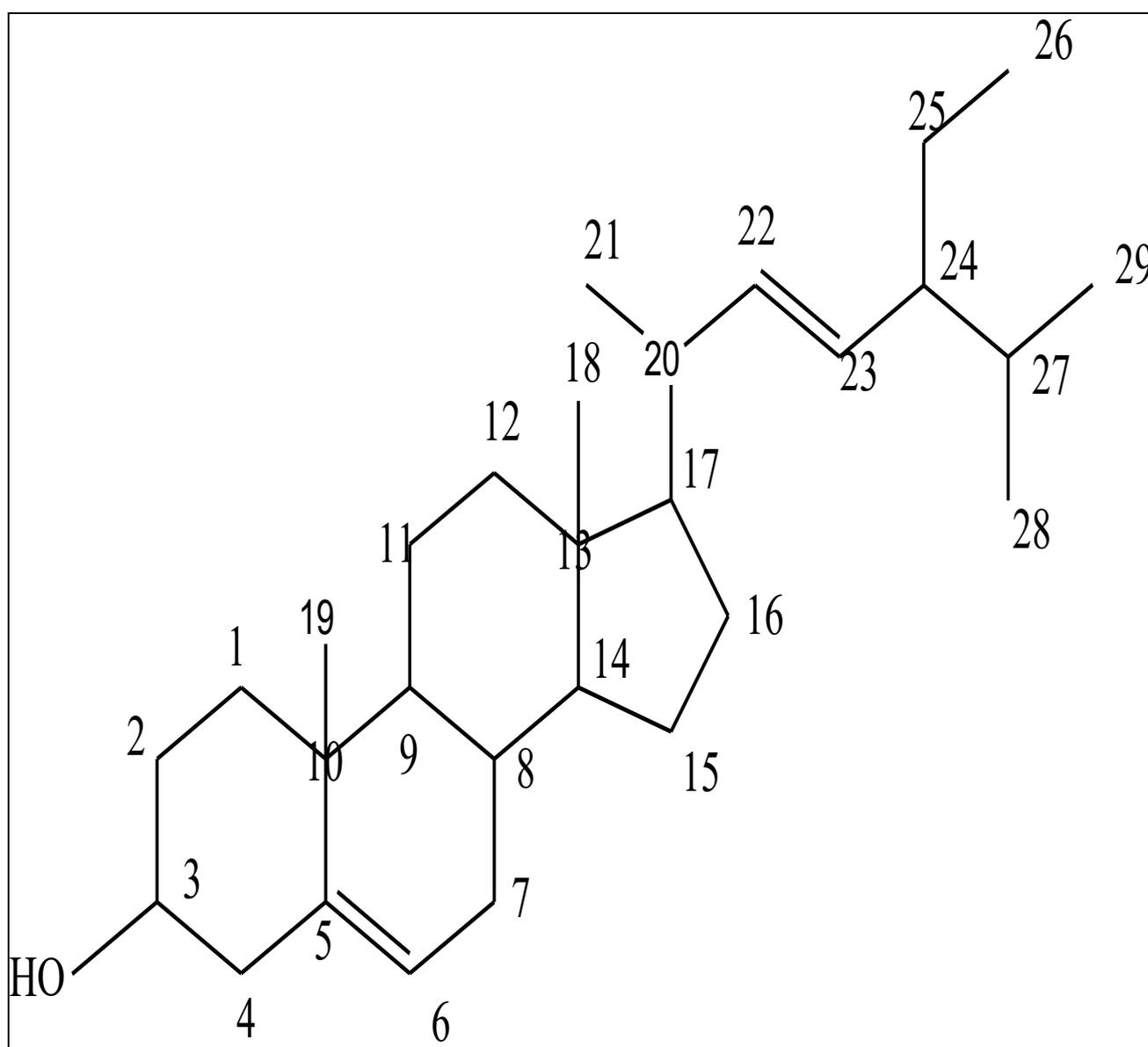


Figure 7: Stigmasterol (Stigmast-5, 22-dien-3 β -ol)

Spectral Analysis of SR2

Compound SR2 gave a single homogenous spot on TLC using different solvent systems, which shows that it was obtained pure.

In the IR spectra of SR2 (Figure 4), absorption bands were visible at $2922\text{--}2855\text{ cm}^{-1}$, which is due to (C-H stretching), 1740 cm^{-1} due to (C=O stretching), 1461 cm^{-1} due to (C-H bending), at $1162\text{--}1114\text{ cm}^{-1}$ which is due to (C-O stretching). Other vibrations observed include those at 1086 cm^{-1} and 723 cm^{-1} .

The ^1H NMR spectra of SR2 (Figure 5) revealed a prominent peak at $\delta 1.25\text{--}1.31\text{ ppm}$, indicating that SR2 is likely a fatty acid ester (Wu et al., 2017) representing the protons of acyl groups (Lu et al., 2013).

The ^{13}C NMR (Figure 6) and DEPT experiments showed two carbonyl carbons at 173.23 and 170.61 ppm, two olefinic carbons at 130.04 and 129.73 ppm, one methine carbon at 68.88 ppm, one methylene carbon at 62.11 ppm, a prominent peak at 29.06–29.77 ppm representing most of the methylene carbon (Wu et al., 2017). Other methylene carbons were observed at 34.21 ppm, 31.93 ppm, 27.1 ppm, 224.87 ppm, and 22.7 ppm (Retief et al., 2009); one methyl carbon at 14.12 deduces one type of fatty acid terminal for a straight chain acyl terminal (Wu et al., 2017).

The result of the 2D homonuclear $^1\text{H}\text{--}^1\text{H}$ COSY and heteronuclear correlation HSQC spectroscopy verifies the connection between the molecule's protons and carbons. From the HSQC spectrum of SR2, two signals at $\delta 4.14$ and $\delta 4.29\text{ ppm}$ with double protons (doublet of a doublet) observed ^1H NMR were attached on the methylene carbon

at 62.11 ppm. This shows that the carbon signal 62.11 ppm was assigned to two methylene carbons, C1' and C3'.

The proton signal at $\delta 1.25\text{--}1.31\text{ ppm}$ observed in the ^1H NMR was assigned to most of the methylene in the fatty acid chain. The proton signal at $\delta 5.26$ in the ^1H NMR was assigned to the methine carbon at 68.88 (the H2' proton on the glycerol) (Di Pietro et al., 2020). This shows that the carbon at 68.88 was assigned to C2'. The proton at $\delta 5.34\text{ ppm}$ was assigned to the alkene carbon at 129.73 ppm, the proton at $\delta 2.31$ was assigned to the methylene carbon alpha to the ester group, the proton at $\delta 1.60$ was assigned to the methylene carbon beta to the ester group and the proton signal at $\delta 2.0$ was assigned to the methylene near the double bonds. The $^1\text{H}\text{--}^1\text{H}$ COSY experiment established the correlations between the protons at H16 (0.88) # H15 (1.26), H3 (1.60) # H4 (1.29), H3 (1.60) # H2 (2.31), H12 (1.29) # H11 (2.00), H11 (2.00) # H10 (5.34), H1',3'a (4.14) # H1'3'b (4.29), H1',3'a (4.14) # H2' (5.26), H1',3'a (4.29) # H2' 5.26).

The cross peaks detected on the heteronuclear multiple bond correlation (HMBC) confirmed the correct assignment of the protons, carbons, and their linkages. Some of the significant correlations that were observed between protons and carbons include.

Proton H16 correlated with C14 and C15, proton H15 showed a correlation with C14, proton H3 correlated with C2 and C4, proton H11 correlated with C10 and C12, proton H2 correlated with C1, C3 and C5, proton H1', H3'a showed correlation with C1 and C2' while the proton H10 showed correlation with C11.

Compound SR2 was verified as a triglyceride (Propane-1, 2, 3-triyl tri [(9Z)-hexadec-9-enoate]) by the 1D and 2D NMR experiment results, which are described in Table 3. In contrast to a reference, the NMR results (Retief et al., 2009) provided more support for the suggested structure.

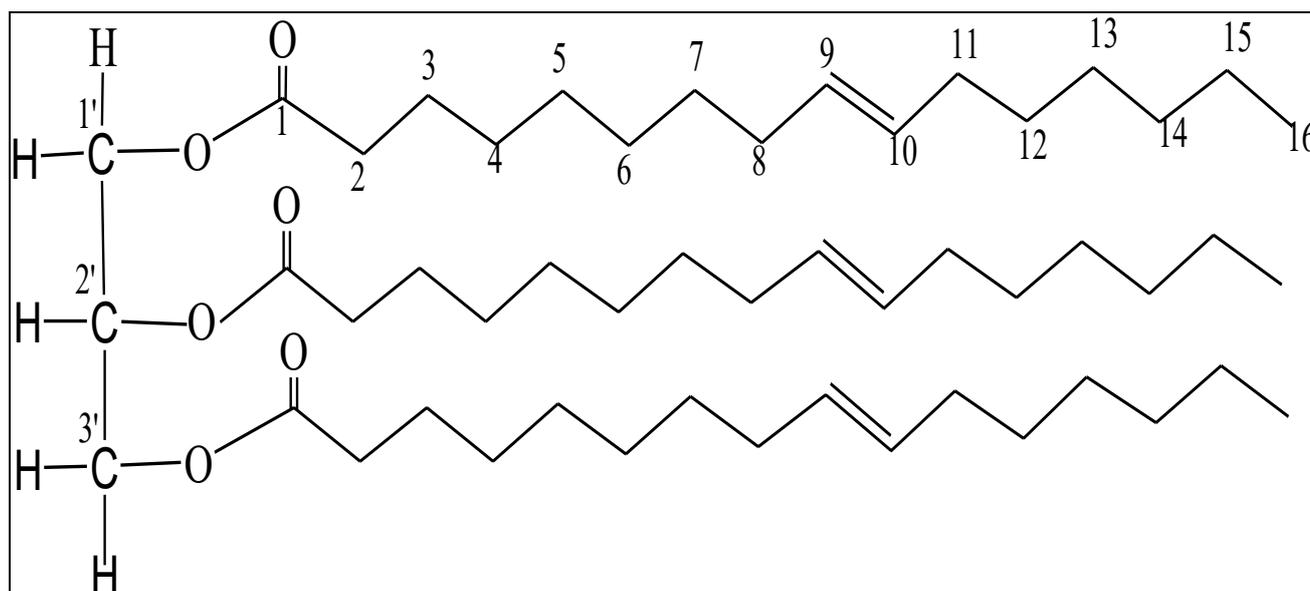


Figure 9: Tripalmitolein (Propane-1, 2, 3-triyl tri [(9Z)-hexadec-9-enoate])

CONCLUSION

Two compounds coded (SR1 and SR2) were isolated from *Vitex chrysocarpa* methanol leaf extract. The isolates were characterized using spectroscopic techniques and compared with the reported literature. The isolation of tripalmitolein, β -sitosterol, and Stigmasterol from *Vitex chrysocarpa* creates new opportunities to investigate this plant's pharmacological potential. Future research ought to concentrate on these chemicals' bioactivity and extensive extraction. This is the first time *Vitex chrysocarpa* has been used to isolate Stigmasterol, β -sitosterol, and Tripalmitolein.

REFERENCE

- Akwada, U. C., Igwe, O. U., Chisom, F., & Diala, C. I. (2020). Isolation and Characterization of Stigmasterol and β -Sitosterol from the leaves of *Emilia coccinea* (Sims) G. Don. *Communication in Physical Science*, 6(2), 863–868.
- Ashraf, R., Bhatti, H. N. (2021) Chapter 10-Stigmasterol. *A century of Valuable Plant Bioactives* (213-232). Elsevier. [\[Crossref\]](#)
- Atiku, I., Pateh, U. U., Sule, M. I., & Isyaka, M. S. (2021). Isolation of squalene and its derivative from the leaf of *Ficus sycomorus* L. (Moraceae). *International Journal of Chemical and Biochemical Sciences*, 19(1466), 65–69.
- Chaturvedula, V. S. P., & Prakash, I. (2012). Isolation of Stigmasterol and β -Sitosterol from the dichloromethane extract of *Rubus suavissimus*. *International Current Pharmaceutical Journal*, 1(9), 239–242. [\[Crossref\]](#)
- Devika, R. (2017). *A Review on Genus Vitex -A Novel Medicinal Plant*. 5(6), 1904–1908. [\[Crossref\]](#)
- Di Pietro, M. E., Mannu, A., & Mele, A. (2020). NMR determination of free fatty acids in vegetable oils. *Processes*, 8(4). [\[Crossref\]](#)
- GBIF Secretariat (2021). GBIF Backbone Taxonomy. Checklist dataset <https://doi.org/10.15468/39omei> accessed via GBIF.org on 2022-02-02.
- I. Ibrahim, I. A. Bello, I. G. Ndukwe, I. G. K. (2018). ISOLATION AND CHARACTERIZATION OF STIGMASTEROL AND β -SITOSTEROL FROM ETHYL ACETATE EXTRACT OF *Adenodolichos paniculatus* (FABACEAE). *FUW Trends in Science & Technology Journal*, 3(2), 770–772.
- Ilodibia, C. V., Okafor, J. C., Akachukwu, E. E., Igboabuchi, N. A., Chukwuma, U. M., & Adimonyemma, N. R. (2016). Phytochemical and Antimicrobial Studies on *Vitex Chrysocarpa* (Planch ex Benth.). *American Journal of Life Science Researches*, 4(4), 127–131. [\[Crossref\]](#)
- Lu, Y., Wang, J., Deng, Z., Wu, H., Deng, Q., Tan, H., & Cao, L. (2013). Isolation and characterization of fatty acid methyl ester (FAME)-producing *Streptomyces* sp. S161 from sheep (*Ovis aries*) faeces. *Letters in Applied Microbiology*, 57(3), 200–205. [\[Crossref\]](#)
- Meerts, P. (2020). The genus *Vitex* (Lamiaceae) in the flora of Cameroon. *Phytotaxa*, 434(2), 131–150. [\[Crossref\]](#)
- Nhan, Dang Thi Thanh Trang, H. T., & Y, N. D. (2018). ISOLATION OF STIGMASTEROL AND β -SITOSTEROL FROM *OCIMUM TENUIFLORUM* L. (LAMIACEAE). *Journal of Science and Education, College of Education, Hue University*, 01(45), 39–44.
- Nieva-Echevarría, B., Goicoechea, E., Manzanos, M. J., & Guillén, M. D. (2014). A method based on 1H NMR spectral data useful to evaluate the hydrolysis level in complex lipid mixtures. *Food Research International*, 66, 379–387. [\[Crossref\]](#)
- Nieva-Echevarría, B., Goicoechea, E., Manzanos, M. J., & Guillén, M. D. (2016). A study by 1H NMR on the influence of some factors affecting lipid in vitro digestion. *Food Chemistry*, 211, 17–26. [\[Crossref\]](#)
- Nna, A., Halliru, A., & Wada, D. (2022). Phytomedicine Plus Ethnobotanical survey: A comprehensive review of medicinal plants used in treatment of gastro intestinal diseases in Kano state, Nigeria. *Phytomedicine Plus*, 2(1), 1–5. [\[Crossref\]](#)
- Nna, P. J., Tor-Anyin, T. A., & Mulu, E. (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. [\[Crossref\]](#)
- Pierre, L. L., & Moses, M. N. (2015). Isolation and Characterisation of Stigmasterol and β -Sitosterol from *Odontonema strictum* (Acanthaceae). *Journal of Innovations in Pharmaceuticals and Biological Sciences*, 2(2349–2759), 88–95.
- Retief, L., McKenzie, J. M., & Koch, K. R. (2009). A novel approach to the rapid assignment of 13C NMR spectra of major components of vegetable oils such as avocado, mango kernel and macadamia nut oils. *Magnetic Resonance in Chemistry*, 47(9), 771–781. [\[Crossref\]](#)
- Romano, B., Lucariello, G., & Capasso, R. (2021). Topical collection “pharmacology of medicinal plants.” *Biomolecules*, 11(1), 1–6. [\[Crossref\]](#)
- Saeidnia, S., Manayi, A., Gohari A. R., and Abdollahi M. (2014). The story of Beta-sitosterol- A Review. *European Journal of Medicinal Plants*, 4(5), 590-609. [\[Crossref\]](#)
- Salmerón-Manzano, E., Garrido-Cardenas, J. A., & Manzano-Agugliaro, F. (2020). Worldwide research trends on medicinal plants. *International Journal of Environmental Research and Public Health*, 17(10), 1–20. [\[Crossref\]](#)
- U. U. Pateh, A. K. Haruna, M. Garba, I. Iliya, I. M. Sule, M. S. A. and A. A. A. (2009). Isolation of Stigmasterol, β -Sitosterol And 2-Hydroxyhexadecanoic acid methyl ester from the rhizomes of *Stylochiton lancifolius*, Pyer & Kotchy (Araceae). *Nigerian Journal of Pharmaceutical Sciences*, 8(1), 19–25.
- Wu, X., Xu, L., Yuan, G., Wang, Y., & Xu, X. (2017). Triglycerides Isolated from *Streptomyces* sp. ZZ035 and Their Nuclear Magnetic Resonance Spectroscopic Characters. *Spectral Analysis Review*, 05(01), 1–10. [\[Crossref\]](#)