ChemSearch Journal 10(1): 20 – 24, June, 2019
Publication of Chemical Society of Nigeria, Kano Chapter

Received: 27/02/2019 Accepted: 12/04/2019

Isolation of Chemical Constituents from *n*-Hexane Leaf Extract of *Cassia singueana* del. (Fabaceae)

[1,2]Saidu Jibril, [2]Hasnah Mohd Sirat, [1]Abdu Zakari, [1]Isyaka Mohammed Sani, [1]Christiana A. Kendeson, [1]Zaharadeen Abdullahi and [3]Aminu Muhammed

[1]Department of Chemical Sciences, Faculty of Science, Federal University Kashere, P.M.B. 0182, Gombe, Nigeria

[2]Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia

[3]Department of Pure and Industrial Chemistry, Faculty of Physical Sciences, Bayero University Kano, P.M.B. 3011, BUK, Kano, Nigeria

Email: saidujb2015@gmail.com; amuhammad.chm@buk.edu.ng

ABSTRACT

Cassia singueana Del. (Fabaceae) is a tropical plant species widely distributed across northern Nigeria. It is used by traditional herbalists in the treatment of ulcer, diabetes and other diseases. In this work, the *n*-hexane leaf extract of *C. singueana* was fractionated and purified by column chromatography using *n*-hexane, $CHCl_3$, and EtOAc. This process led to the isolation of five (5) compounds namely, stigmasterol (**1**), stigmast-4-en-3-one (**2a**), stigmast-4, 22-dien-3-one (**2b**), 1-heneicosanol (**3**) and hexyl heneicosanoate (**4**). Compounds **2a** and **2b** were isolated as mixture. The structures of the isolated compounds were elucidated using 1D and 2D NMR spectral analysis and comparison of the spectral data with literature values was undertaken.

Keywords: *Cassia singueana*, column chromatography, isolation, phytochemicals, stigmasterol

INTRODUCTION

Cassia species are used worldwide as traditional medicines and in commercial pharmaceuticals (Lim, 2012, Ayo, 2010). They spread across tropical and sub-tropical regions (Bhalerao and Kelkar, 2012). *Cassia singueana* Del. (Fabaceae), commonly known as winter *Cassia* is a shrub or tree with numerous medicinal values across Africa (Hiben, *et. al.*, 2016) including Nigeria were it is widely found in the northern part of the country. The leaf juice is traditionally used to treat syphilis, ulcer, malaria, pneumonia, snake bite and eye infection (Schmelzer *et. al.*, 2008). Furthermore, in northern Nigeria, the leaf is used to treat ulcer (Ode and Asuzu, 2011), diabetes mellitus (Etuk *et. al.*, 2010) and to enhance blood circulation in nursing mothers (Ifeanyi and Ode, 2012). Studies have demonstrated that leaf extract of *C. singueana* possess anti-ulcer (Ode, 2011; Ode *et. al.*; Ode and Asuzu, 2011; Ode and Onakpa, 2010), antimalarial (Hiben *et. al.*, 2016; Saidu *et. al.*, 2011), antioxidant (Ibrahim and Islam, 2013; Ifeanyi and Ode, 2012; Mebrahtom, 2007) activities. In terms of the compounds isolated from this plant, only luteolin has been reported from the leaf extract of *C. singueana* (Ode and Asuzu, 2011). In this study, we describe the isolation and structural elucidation of stigmasterol (**1**), stigmast-4-en-3-one (**2a**), stigmasta-4, 22-dien-3-one (**2b**), 1-heneicosanol (**3**) and hexyl heneicosanoate (**4**) (Fig. 1) from the *n*-hexane leaf extract of *C. singueana.*

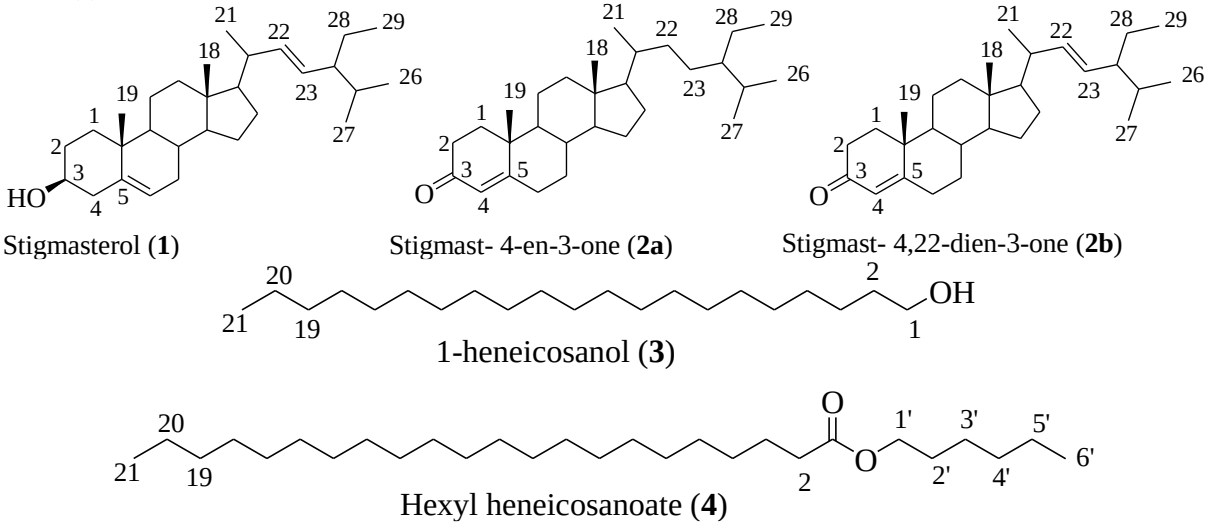

Fig. 1: Compounds Isolated from *n*-Hexane Leaf Extract of *C. Singueana*

EXPERIMENTAL

General Experimental Procedure

The NMR spectra were recorded on a Bruker Avance instrument, (400 MHz for ^{1}H and 100 MHz for ^{13}C) using $CDCl_3$ as solvent. Silica gel 60 (70-230 mesh, for Column Chromatography (CC) from ASTM, Merck, New Jersey, USA) was used.

Plant Material

The leaf part of *C. singueana* was collected in August, 2014, from Kumuyel, Alkaleri Local Government Area of Bauchi State, Nigeria. The plant was identified by Mr. Baha'uddeen Said Adam of the Department of Plant Biology, Bayero University Kano. Voucher specimen, BUKHAN 0316 was then deposited at the Herbarium of Department of Plant Biology, Bayero University Kano, Nigeria.

Extraction and Isolation

The air-dried and powdered leaf of *C. singueana* (200 g) was macerated with *n*-hexane for three (3) days (3 × 4 L) at room temperature. The extract was filtered and concentrated under reduced pressure to yield the *n*- hexane crude extract (CSLH, 3.2 g, 1.6%). The *n*-hexane crude extract (CSLH, 3 g, 1.50%) was subjected to Column Chromatography (silica gel, 120 g) using *n*-hexane, *n*-hexane/ $CHCl_3$, $CHCl_3$, *n*-hexane /EtOAc, EtOAc in a polarity gradient manner. Fractions were pooled together base on their TLC profile to obtain thirty fractions (CSLH 1- CSLH 30). Fraction CSLH 8 - 17 (1.1 g) was loaded on a silica gel column chromatography (70 g) and eluted using *n*-hexane/$CHCl_3$/EtOAc in an increasing polarity to give fifteen sub-fractions. Sub-fractions 5-10 were combined and further purified on column chromatography to yield compound (**1**) and compound (**3**) as white amorphous substances. Sub-fractions 11 – 15 were pooled together and re-chromatographed by column chromatography using *n*- hexane, *n*-hexane/$CHCl_3$, $CHCl_3$, $CHCl_3$/EtOAc in gradient manner, affording compound (**2**) as white amorphous substance. Fraction CSLH 18 - 30 (0.8 g) was absorbed on silica gel (70 - 230 mesh, 55 g) and eluted in *n*-hexane/$CHCl_3$/EtOAc in a step wise gradient manner, to give compound (**4**) as white amorphous substance. Structure of compounds **1-4 (**Fig. 1**)** were identified using spectroscopic methods and comparison of the data obtained with published literature was undertaken.

Compound **1**: yield, 25 mg; ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.65, 0.77, 0.80, 0.84, 0.98, 1.03, (each 3H, s, CH_3), 2.02 (1H, m, H-20), 3.57 (1H, m, H-3), 5.20 (1H, dd, J = 15.2, 8.8 Hz, H-22), 5.06 (1H, dd, J = 15.2, 8.8 Hz, H-23), 5.38 (1H, d, J = 5.2 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.2 (C-29), 12.0 (C-18), 19.0 (C-27), 19.4 (C-19), 21.2 (C-11), 21.2 (C-26), 21.2 (C-21), 24.4 (C-15), 25.4 (C-28), 29.0 (C-16), 31.6 (C-2), 31.9 (C-7), 32.0 (C-8/C-25), 36.4 (C-10), 37.2 (C-1), 40.5 (C-12), 39.7 (C-20), 42.2 (C-13), 42.2 (C-4), 50.1 (C-9), 51.3 (C-24), 56.0 (C-17), 56.8 (C-14), 71.8 (C-3), 121.5 (C-6), 129.2 (C-23), 138.4 (C-22), 140.8 (C-5).

Compound **2a**: yield, 18 mg; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.71 (3H, s, H-18), 0.82 (3H, d, J = 6.8 Hz, H-27), 0.84 (3H, d, J = 6.8 Hz, H-26), 0.85 (3H, m, H-29), 0.92 (3H, d, J = 6.5 Hz, H-21), 1.18 (3H, s, H-19), 5.72 (1H, s, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.9 (C-29), 12.2 (C-18), 17.3 (C-19), 18.7 (C-21), 19.0 (C27), 19.8 (C-26), 21.1 (C-11), 23.0 (C-28), 24.1 (C-15), 26.0 (C-23), 28.2 (C-16), 29.1 (C-25), 32.0 (C-22), 32.9 (C-7), 33.8 (C-6), 33.9 (C-2), 35.6 (C-1), 35.6 (C-8), 36.1 (C-20), 38.6 (C- 10), 42.3 (C-13), 45.8 (C-24), 53.8 (C-9), 55.8 (C-14), 55.9 (C-17), 123.7 (C-4), 171.8 (C-5), 199.7 (C-3).

Compound **2b**: yield, 18 mg; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.73 (3H, s, H-18), 0.80 (3H, d, J = 6.0 Hz, H-26), 0.81 (3H, m, H-29), 0.85 (3H, d, J = 6.0 Hz, H-27), 1.02 (3H, d, J = 7.5 Hz, H-

21), 1.18 (3H, s, H-19), 5.03 (1H, dd, *J* = 9.0, 15.5 Hz, H-23), 5.15 (1H, dd, *J* = 9.0, 15.5 Hz, H-22), 5.72 (1H, s, H-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ 11.9 (C-29), 12.1 (C-18), 17.3 (C-19), 18.9 (C-26), 21.0 (C-11), 21.1 (C-27), 21.1 (C-21), 24.2 (C-15), 25.4 (C-28), 29.7 (C-25), 31.8 (C-7), 32.0 (C-6), 33.9 (C-2), 35.6 (C-1), 35.6 (C-8), 38.6 (C-10), 39.5 (C-12), 40.4 (C-20), 42.2 (C-13), 51.2 (24), 53.8 (C14), 55.8 (C-9), 55.9 (C-17), 123.7 (C-4), 129.4 (C-23), 138.1 (C-22), 171.8 (C-5), 199.7 (C-3).

Compound **3**: yield, 21 mg; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.88 (3H, t, *J* = 6.4 Hz, H-21), 1.27 (2H, overlapping, H-20), 1.60 (2H, overlapping, H-2), 3.68 (2H, t, *J* = 6.4 Hz, H-1). ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-21), 23.7 (C-20), 31.9 (C-19), 63.1 (C-1).

Compound **4**: yield, 25 mg; ^{1}HNMR (400 MHz, $CDCl_3$): δ 0.88 (6H, t, *J* = 6.8 Hz, H-21/6'), 1.65 (2H, overlapping, H-3/2'), 2.33 (2H, t, *J* = 6.8 Hz, H-2), 4.09 (2H, t, *J* = 6.8 Hz, H1'), ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1 (C-21/6'), 22.7 (C-20/5'), 25.0 (C-3), 25.9 (C-2'), 28.6 (C-3'), 31.9 (C-19/4'), 34.4 (C-2), 64.4 (C-1'), 174.0 (C=O).

RESULT AND DISCUSSION

The ^{1}H NMR spectrum data of compound **1** showed a well resolved multiplet at the low-field region and a complicated resonance signals in the high-field region, which suggested a steroid skeleton. A pair of doublet of doublets signals at δ_H 5.20 and 5.06; a doublet signal at δ_H 5.38 and a multiplet signal at δ_H 3.57 strongly suggested compound **1** to be a phytosterol (Forgo and Kover, 2004). The signals at δ_H 5.20, and 5.06 (1H, dd, *J* = 8.8, 15.2 Hz) were assigned to the olefinic protons at H-22 and H-23 respectively, while signal at δ_H 5.38 (1H, d, *J* = 5.2 Hz), was attributed to olefinic proton (H-6). The multiplet signal at δ_H 3.57 represented the proton located at position C-3. The highly overlapped signals in the upfield region, contain the six methyl signals. The COSY spectrum analysis showed the ^{1}H-^{1}H interaction between H-22 (δ_H 5.20) and H-23 (δ_H 5.06). The ^{13}C NMR spectrum revealed the total of 29 carbons. The DEPT spectra analysis confirmed the presence of six methyl groups, three methylene groups, an olefinic quaternary carbon and one oxygenated carbon. Compound **1** was eventually identified as stigmas-5, 22-dien-3β-ol also known as stigmasterol, based on the comparison of its NMR data with those of stigmasterol previously reported (Forgo and Kover, 2004).

Compound **3** was obtained as white solid. The ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) displayed triplet signals at δ_H 3.68 assignable to the methyl group carrying the hydroxyl group. Triplet signal at δ_H 0.88 was attributed to the terminal methyl protons. The ^{1}H-^{1}H COSY spectrum indicated correlation between H-1 and H-2; between H-21 and H-22. Thus, H-2 proton signal was observed on the ^{1}H NMR spectrum to overlap at δ_H 1.58, while H-20 proton signal overlapped at δ_H 1.27. The HMQC spectrum showed correlation between H-21 and C-21; between H-1 and C-1. Thus signal at δ_C 14.1 and 63.1 on the ^{13}C NMR spectrum were assigned to C-21 and C-1 respectively. The long-range ^{1}H-^{13}C HMBC spectrum indicated cross peaks linking H-21 to C-19 and C-20. Hence, signals at δ_C 31.9 and 22.7 on the ^{13}C NMR spectrum were assigned to C-19 and C-20 respectively. The DEPT spectrum further confirmed C-21 as sp^3 carbon atom. Compound **3** was elucidated as 1-heneicosanol (**3**), based on the 1D and 2D NMR spectra analysis and the data obtained were in agreement with those from literature (Luz *et. al.*, 2016).

Compound **4** was isolated as white solid substance. The ^{1}H NMR spectrum data of compound **4** showed triplet signal at δ_H 4.06 assigned to the oxo-methylene protons. Similarly, triplet signal at δ_H 2.29 was attributed to the methylene protons alpha to the carbonyl carbon. The triplet signal upfield at δ_H 0.88 was assigned to the terminal methyl protons in compound **3**. From the 2D NMR analysis of compound **3**, the COSY spectrum data showed correlation between H-1' and H-2'; between H-2 and H-3; between H-20 and H-21; between H-6' and H-5'. From these correlations, it is obvious that H-2' and H-3 protons overlapped as observed in the ^{1}H NMR spectra of compound **4** at δ_H 1.65. The HMQC spectrum revealed the ^{1}H - ^{13}C correlation between H-21/H6' and C-21/C-6'; between H-2 and C-2. Thus, from the ^{13}C NMR spectrum, C-2, C-21 and C-6' were assigned at δ_C 34.4, 14.1 and 14.1 respectively. Furthermore, analysis of the HMBC spectrum of compound **4** showed cross peaks linking H-1' to C-2'; H-2 to C-3; H-21 to C-20 and C-19; H-6' to C-5' and C-4'; H-1' to C=O and H-2 to C=O. Therefore, the ^{13}C NMR spectrum signal at δ 25.9, 25.0, 22.7,, 22.7, 31.9, 31.9 and 174.0 were assigned to C-2', C-3, C-20, C-5', C-19, C-4', and C=O respectively. The DEPT spectrum indicated the presence of one carbonyl carbon while DEPT spectrum confirmed the presence of sp^3 carbon (C-21 and C-6'). Based on the 1D and 2D NMR spectra analysis and comparison with those previously reported in literature (Niko and Dragan, 2014), compound **4** was established as hexyl heneicosanoate (**4**).

Compound **2a** (stigmast-4-en-3-one) and Compound **2b** (stigmast-4, 22-dien-3-one) were obtained as white solid mixture. The ^{1}H and ^{13}C NMR spectra data (Table **1**) depicted mixture of stigmast-4-en-3-one (**2a**) and stigmast-4, 22-dien-3-one (**2b**). The ^{1}H NMR revealed signals representing six methyl groups at δ_H 1.18, 0.92, 0.85, 0.84, 0.82, 0.71 and one olefinic proton at δ_H 5.72.The ^{13}C NMR spectrum data displayed 29 carbon atom signals consisting of two olefinic carbons at δ_C 123.8 and 171.7 and carbonyl carbon at δ 199.7. The DEPT spectrum further confirmed the presence of carbonyl carbon. These NMR

spectra data suggests stigmast-4-ene-3-one (**2a**) structure. Furthermore, signals from the ^{1}H NMR spectrum revealed three olefinic protons at δ 5.72, 5.15, 5.03 and six methyl protons. The ^{13}C NMR spectrum still accounted for another 29 carbon atoms with four olefinic carbons. The HMBC spectrum revealed ^{1}H-^{13}C correlation between H-22/C-22 and H-23/C-23. The HMQC spectrum further showed correlations between H-4 with C-6 and C-10, while H-22 and H-23 correlated with C-20/21/24 and C-20 respectively. Hence, this data was attributed to stigmast-4, 22-dien-3-one (**2b**). From the NMR spectra analysis and comparison of the data with literatures (N.T. Hoa, 2014), it was obvious that stigmast-4-en-3-one (**2a**) and stigmasta-4,22-dien-3-one (**2b**) have been isolated as a mixture.

Table 1: ^{1}H and ^{13}C NMR spectroscopic data for compound **2a** and **2b** in $CDCl_3$

Position of C	Compound **2a**		Compound **2b**	
	δ_C	δ_H (m, *J* in Hz)	δ_C	δ_H (m, *J* in Hz)
1	35.616		35.616	
2	33.987		33.987	
3	199.768		199.768	
4	123.736	5.72 (1H, s)	123.736	5.72 (1H, s)
5	171.839		171.800	
6	33.875		32.035	
7	32.963		31.879	
8	35.684		35.684	
9	53.831		55.889	
10	38.622		38.622	
11	21.108		21.017	
12	39.620		39.519	
13	42.391		42.275	
14	55.878		53.813	
15	24.194		24.254	
16	28.204		28.881	
17	55.986		55.986	
18	12.262	0.71 (3H, s)	12.147	0.73 (3H, s)
19	17.388	1.18 (3H, s)	17.388	1.18 (3H, s)
20	36.122		40.490	
21	18.709	0.92 (3H, d, *J* = 6.5 Hz)	21.169	1.02 (3H, d, *J* = 7.5 Hz)
22	32.047		138.139	5.15 (1H, dd, *J* = 8.8, 15.5 Hz)
23	26.045		129.445	5.03 (1H, dd, *J* = 8.8, 15.5 Hz)
24	45.818		51.237	
25	29.132		29.714	
26	19.833	0.84 (3H, d, *J* = 6.8 Hz)	18.989	0.80 (3H, d, *J* = 6.0)
27	19.025	0.82 (3H, d, *J* = 6.8 Hz)	21.108	0.85 (3H, d, *J* = 6.0 Hz)
28	23.060		25.413	
29	11.957	0.85 (3H,m)	11.983	0.81 (3H,m)

CONCLUSION

The extraction of leaf of *C. singueana* in *n*-hexane and the subsequent fractionation and purification of the crude *n*-hexane extract has afforded three steroids (stigmasterol (**1**), stigmast-4-en-3one (**2a**) and stigmast-4,22-dien-3-one (**2b**)) and two fatty acid derivative compounds (1-heneicosanol (**3**) and hexyl heneicosanoate (**4**)). These compounds as an entity or in synergy could be responsible for the ethno-medicinal/bioactivities reported in literature from the leaf of *C. singueana*.

ACKNOWLEDGMENT

The authors are grateful to Tertiary Education Trust Fund-Nigeria (TETFund) for the fellowship sponsor of Saidu Jibril. The authors would like to thank the Ministry of Higher Education (MOHE) Malaysia, for financial support under the Vote 4F635 and the Faculty of Science, Universiti Teknologi Malaysia for the research facilities.

REFERENCES

Ayo, R.G. (2010). Phytochemical constituents and bioactivities of the extracts of *Cassia nigricans* Vahl: A review. *J. Med. Plant Res.*, 4, 1339-1348.

Bhalerao, S.A., and Kelkar, T.S. (2012). Traditional medicinal uses, phytochemical profile and pharmacological activities of *Cassia fistula* Linn. *Int. Res. J. Biolo. Sci.*, 1, 79-84

Etuk, E.U., Bello, S.O., Isezuo, S.A., and Mohammed B.J. (2010). Ethnobotanical survey of medicinal plants used for the treatment of Diabetes mellitus in the north western region of Nigeria. *Asian J. Exp. Biol. Sci., 1*(1), 55-59.

Forgo, P., and Kover, K.E. (2004). Gradient enhanced selective experiments in the ^{1}H NMR chemical shift assignment of the skeleton and side-chain resonances of stigmasterol, a phytosterol derivative. *Steroids*, 69, 43-50.

Hiben, M.G., Sibhat, G.G., Fanta, B.S., Gebrezgi, H.D., and Tesema, S.B. (2016). Evaluation of *Senna singueana* leaf extract as an alternative or adjuvant therapy for malaria. *J. Tradit. Complement Med.*, 6, 112-117.

Hoa, N.T., Dien, P.H., Quang, D.N. (2014). Cytotoxic Steroids from the Stem Barks of *Pandanus tectorius*. *Res. J. Phytochem.*, 8, 52-5.

Ibrahim, M.A., Koorbanally, N.A., and Islam, M.S. (2013). In vitro anti-oxidative activities and GC-MS analysis of various solvent extracts of *Cassia singueana* parts. *Acta Pol Pharm*, 70, 709-719.

Ifeanyi, I.M., and Ode, O.J. (2012). *In vitro* and *in vivo* antioxidant potential of the methanolic extract of *Cassia singueana* Delile (Fabaceae) Lock leaves. *Comp. Clin Pathol, 21*, 1565-1569.

Lim, T.K. (2012). Edible Medicinal and Non-Medicinal Plants. Springer, London, 109.

Luz A.A., Cecilia, V.N., Graciela, N.P., María, E.A., Cristina, B.C. (2016). Biological activity of 1-heneicosanol isolated from *Senecio coluhuapiensis*, an endemic species from Patagonia, Argentina. *Pharm Chem. J., 3*(4), 73-77.

Mebrahtom, G., Kaleab, A. and Ciddi, V. (2007). In-vitro radical scavenging activity of the leaf and bark extracts of *Senna singueana* (Del). Lock. *Ethiop. Pharm. J.*, 25, 77-84.

Niko S.R., and Dragan B.Z. (2014). *n*-Octyl ester of long chain fatty acids are not anthropogenic markers. *Environ. Chem. Lett.*, 12, 303-312.

Ode, O.J., and Asuzu, O.V. (2011). Investigation of *Cassia singueana* leaf extract for antiulcer effects using ethanol-induced gastric ulcer model in rats. *Int. J. plant, anim environ. sci.*, 1, 1-7.

Ode, O.J., and Asuzu, I.U. (2014). Luteolin isolate from the methanol extract identified as the single-carbon compound responsible for broad antiulcer activities of *Cassia singueana* leaves. *IOSR Journal of Pharmacy*, 4, 17-23.

Ode, J.O., and Asuzu, I.U. (2011). The protective effects of the methanol extract of *Cassia singueana* leaves against histamine-induced stomach ulcers in albino rats. *IJPAES*, 1, 53-60.

Ode, O.J., Asuzu, I.U. and Ajayi, I.E. (2011). Bioassay-guided fractionation of the crude methanol extract of *Cassia singueana* leaves. *J. Adv. Sci. Res., 2*(4), 81-86.

Ode, O.J., and Onakpa, M.M. (2010). Evaluation of *Cassia singueana* extract on stomach HCl production and gastric emptying in rats. *Int. J. Appl. Biol. Pharm. Technol.*, 1, 1352-1358.

Saidu, J., Adoum O.A. and Mukhtar, M.D. (2011). Screening of *Cassia singuaena*, *Commiphora kerstingii*, *Khaya senegalensis* for brine shrimp lethality and antiplasmodium activity. *Chemsearch Journal*, I, 50 - 52.

Schmelzer, G.H., Gurib-Fakim, A., Arroo, R., Bosch, C.H., Ruijter, A., Simmonds, M.S.J. (2008). Plant Resources of Tropical Africa II (1)-Medicinal palnts 1. Netherlands, Backhuys publisher, 49-60.