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Bio-guided Isolation, Purification and Chemical Characterization of Epigallocatechin; Epicatechin, Stigmasterol, Phytosterol from of Ethyl Acetate Stem Bark Fraction of *Spondias mombin* (Linn.)

Oludare Temitope Osuntokun[1*], T.O Idowu[2] and Gamberini Maria Cristina[3]

[1]*Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, P.M.B 001, Ondo State, Nigeria*
[2]*Department of Pharmaceutical Chemistry, Obafemi Awolowo University, Nigeria*
[3]*Department of Life Sciences, University of Modena and Reggio Emilia, via G. Campi 103, 41125 Modena, Italy*

Abstract

Spondias mombin (Linn.) is a widely cultivated edible plant used in folkloric medicine for the treatment of severe infection and health disorders. This research work was carried out to isolation, purification and chemical characterization the bioactive constituents of the ethyl acetate stem bark fraction of *Spondias mombin* (Linn.), a medicinally important plant of the *Anacardiaceae* family. This study revealed the presence of flavonoid and steroids, which have been found to be important hormone regulators which possess antimicrobial, anti-inflammatory, antioxidant properties. The chemical investigation resulted in the isolation of ($C_{15}H_{14}O_{6.}$) *5, 7, 3', 4'-pentahydroxy flavanol (*Epicatechin), ($C_{15}H_{14}O_{7.}$) Epigallocatechin ($C_{29}H_{48}O.$), Stigmasterol phytosterol. It is here reported isolated from *Spondias mombin* for the first time, this makes the *Spondias mombin* very important medicinal plant in Nigeria and west Africa. EGC and EC arts as a strong inhibitor of HIV replication in cultured peripheral blood cells and inhibition of HIV-1 reverse transcriptase *in vitro*. EGC binds directly to CD4 molecules with consequent inhibition of Gp 120 binding and inactivate viruses *in-vitro* by deformation of phospholipids. Stigmasterol phytosterol have been shown to lower/reduce blood cholesterol and this lowering may reduce the risk of coronary heart disease. The structure was elucidated using two dimensional NMR spectroscopy, NMR (^{1}H, ^{13}C) spectroscopy in combination with Infra-red (IR) and Mass spectrometer (MS) spectra data.

Keywords: Epigallocatechin; Epicatechin; Stigmasterol; Phytosterol; Isolation; Purification; Chemical Characterization Ethyl acetate; *Spondias mombin* (Linn.)

Introduction

Plants are the basis of traditional medicine in Africa and have been used for thousands of years. These plants often exhibit a wide range of pharmacological activities [1]. Due to the need for development of new drugs with better pharmacological activities, dependence on plants grew increasingly as scientists continuously exploited them for isolation of bioactive compound.

Spondias mombin is a small tree that grows up to 20 m (60 ft.) high and 1.5 m (5 ft) in girth, moderately buttressed; bark thick, corky, deeply fissured, slash pale pink, darkening rapidly, branches low, branchlets glabrous; leaves pinnate, leaflets 5-8 opposite pairs with a terminal leaflet. It belongs to the family Anacardiaceae. It flowers between January to May and fruits between July to September. The fruits have a sharp, somewhat acid taste and are edible. The matured fruit has a leathery skin and a thin layer of pulp. The fruit pulp is either eaten fresh, or made into juice, concentrate, jellies, and sherbets. The fruit-juice is used as a febrifuge and diuretic. The roots are also used as febrifuge in Ivory Coast. The stem bark is used as a purgative and in local applications for leprosy. The stem bark decoction is also used in the treatment of severe cough. It serves as an emetic, a remedy for diarrhea, dysentery, haemorrhoids and a treatment for gonorrhoea and leucorrhea [2].

A report showed that the bark contains a certain amount of tannin and this explains the reason why the dry pulverized bark is applied as a dressing to a wound [2]. In Belize, a decoction of the young leaves is a remedy for diarrhea and dysentery. The juice of crushed leaves and the powder of dried leaves are used as poultices on wounds and inflammations. The gum is employed as an expectorant and to expel tapeworms [3].

A decoction of the mashed leaves is used by the Ibos (Nigeria) for washing a swollen face. A leaf infusion is a common cough remedy or used as a laxative for fever with constipation. A leaf decoction is used in treatment of gonorrhea. The leaves are used in Ivory Coast for fresh wounds to prevent inflammation. A decoction of pounded leaves of *S. mombin* is used as an eye lotion and the juice pressed from young, warm leaves is given to children for stomach troubles. The extract has shown anti-inflammatory activity in Wistar rats [4]. A tea made from the flowers and leaves is taken to relieve stomach ache, biliousness, and urethritis, cystitis and eye and throat inflammations. A decoction of the root is used as purgative [4].

Material and Methods

Collection of plant materials

The stem bark of *Spondias mombin* (Linn.) was collected from a local farm at Owo (710'59.998N and 534'59.988E), Ondo State, Nigeria at WAT UTC+1 time zone on 26th and 27th of February, 2016. Fresh and healthy plants were also collected during its fruiting season, between April and July 2017, from the same geographical locations.

***Corresponding author:** Osuntokun OT, Department of Microbiology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, P.M.B 001, Ondo State, Nigeria, Tel: 08063813635; E-mail: osuntokun4m@gmail.com

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Authentication of *Spondias mombin* (Linn.)

The plants were authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba- Akoko, Ondo state, Nigeria.

Preparation and extraction of *Spondias mombin* (Linn.) Plant

The **s**tem-bark of *Spondias mombin* plant were harvested and air-dried. The dried stem bark were milled into powdered form using manual grinder. Powdered plant material (1 kg) each of the different plant parts was extracted with 3 L of 70% (v/v) ethanol, ethyl acetate and distilled water for 72 h at room temperature. The extraction process was repeated four times until the extract became clear. The filtrates were combined and concentrated under reduced pressure rotatory evaporator at 35°C to give, SMSBEA for the stem-bark part. The dry extracts were kept in tightly stoppered bottles in a refrigerator at -4°C for further analysis

Fractionation of SMSBEAEA on column Chromatography

The ethyl acetate fraction SMSBEAEA (11.0 g) was adsorbed unto silica and allowed to dry before packing on to column. The column was wetted with *n*-hexane and gradient elution effected with the following solvent/ solvent mixtures (Table 1).

Fractions collected were analysed by TLC (Thin layer chromatography) in Hex - EtOAc - AcOH (1: 9: 0.5). The resulting spots on TLC plates 1-3 were visualized under UV light (254 nm) and detected by the use of vanillin/sulfuric acid and the antioxidant compound(s) were detected using DPPH spray reagents. Fractions having the same TLC patterns were bulked, concentrated in vacuo to dryness and weighed; resulting in three fractions coded SMSBEAEA1, SMSBEAEA2 and SMSBEAEA3 (Table 2).

Fractionation of SMSBEAEA1 on Sephadex LH-20

Fraction SMSBEAEA1 (1.6 g) was dissolved in a small amount of $CHCl_3$-MeOH (70:30) solvent mixture and loaded on a Sephadex LH-20 column previously equilibrated with the same solvent mixture and elution was isocratically effected. Fractions (about 20 ml each) collected were analysed by TLC in CH_2Cl_2 - MeOH (10: 1.0) and fractions having the similar TLC patterns were bulked together, concentrated in vacuo to dryness and weighed; resulting in eight fractions coded SMSBEAEA1a, SMSBEAEA1b, SMSBEAEA1c (compound **1**), SMSBEAEA1d, SMSBEAEA1e (compound **2**), SMSBEAEA1f, SMSBEAEA1g and SMSBEAEA1h (Table 3).

Hexane	100%	200 ml	1-7
Hexane - ethyl acetate	(80:20)	100 ml	8-12
Hexane - ethyl acetate	(70:30)	200 ml	13-22
Hexane - ethyl acetate	(60:40)	100 ml	23-27
Hexane - ethyl acetate	(50:50)	200 ml	28-37
Hexane - ethyl acetate	(30:70)	100 ml	38-42
Hexane - ethyl acetate	(20:80)	200 ml	43-52
Hexane - ethyl acetate	(10:90)	100 ml	53-57
Ethyl acetate	100%	200 ml	58-67
Ethyl acetate - Methanol	(95:5)	200 ml	68-77
Ethyl acetate - Methanol	(90:10)	200 ml	78-87
Ethyl acetate - Methanol	80-20	200 ml	88-97
Ethyl acetate - Methanol	75-25	100 ml	98-102
Ethyl acetate - Methanol	70-30	200 ml	103-112
Ethyl acetate - Methanol	65-35	200 ml	113-122

Table 1. Fractionation of SMSBEAEA on Column Chromatography with Hexane/ ethyl acetate/ Methanol solvent.

Fractions	Codes	Weight
1-67	SMSBEAEA1	1.6 g
68-77	SMSBEAEA2	0.1 g
78-122	SMSBEAEA3	0.2 g

Table 2: Three fractions coded SMSBEAEA1, SMSBEAEA2 and SMSBEAEA3 final Weight after Column Chromatography.

Fractionation of SMSBEAEA1a on column Chromatography

Fraction SMSBEAEA1a (600 g) was adsorbed unto silica and allowed to dry before packing on to column. The column was wetted with *n*-hexane and gradient elution effected with the following solvent/ solvent mixtures (Table 4):

Fractions (about 20 ml each) collected were analysed by TLC in hexane - CH_2Cl_2 (8:4) and hexane - CH_2Cl_2 (2:8) and fractions having the similar TLC patterns were bulked together, concentrated in vacuo to dryness and weighed resulting in six fractions coded SMSBEAEA1a1, SMSBEAEA1a2, SMSBEAEA1a3, SMSBEAEA1a4 **(3)**, SMSBEAEA1a5 and SMSBEAEA1a6 (Table 5 and Figure 1).

Result

The physical and spectroscopic data on the isolated compounds are as recorded below.

Fractions	Codes	Weight
1-7	SMSBEAEA1a	623 mg
8-9	SMSBEAEA1b	68 mg
10-14	SMSBEAEA1c (**1**)	230 mg
14-15	SMSBEAEA1d	97 mg
16	SMSBEAEA1e (**2**)	15 mg
17-21	SMSBEAEA1f	155 mg
22-26	SMSBEAEA1g	180 mg
27-31	SMSBEAEA1h	62 mg

Table 3. Eight fractions coded SMSBEAEA1a, SMSBEAEA1b, SMSBEAEA1c (compound 1), SMSBEAEA1d, SMSBEAEA1e (compound 2), SMSBEAEA1f, SMSBEAEA1g and SMSBEAEA1h final Weight on Sephadex LH-20.

Hexane	100%	100 ml	1-3
Hexane - dichloromethane	(95:5)	100 ml	4-8
Hexane - dichloromethane	(90:10)	500 ml	9-31
Hexane - dichloromethane	(85:15)	500 ml	32-52
Hexane - dichloromethane	(80:20)	300 ml	53-66
Hexane - dichloromethane	(70:30)	300 ml	67-79
Hexane - dichloromethane	(50:50)	200 ml	80-89
Hexane - dichloromethane	(30:70)	200 ml	90-98
Hexane - dichloromethane	(10:90)	200 ml	99-107
Dichloromethane	100%	200 ml	108-117

Table 4: Fractionation of SMSBEAEA on Column Chromatography with Hexane/ dichloromethane solvent

Fractions	Codes	Weight.
1-52	SMSBEAEA1a1	196 mg
53-75	SMSBEAEA1a2	121 mg
76-87	SMSBEAEA1a3	95 mg
88-91	SMSBEAEA1a4 **(3)**	23 mg
98-105	SMSBEAEA1a5	60 mg
106-117	SMSBEAEA1a6	35 mg

Table 5: Six fractions coded SMSBEAEA1a1, SMSBEAEA1a2, SMSBEAEA1a3, SMSBEAEA1a4 (3), SMSBEAEA1a5 and SMSBEAEA1a6 final Weight on Sephadex LH-20

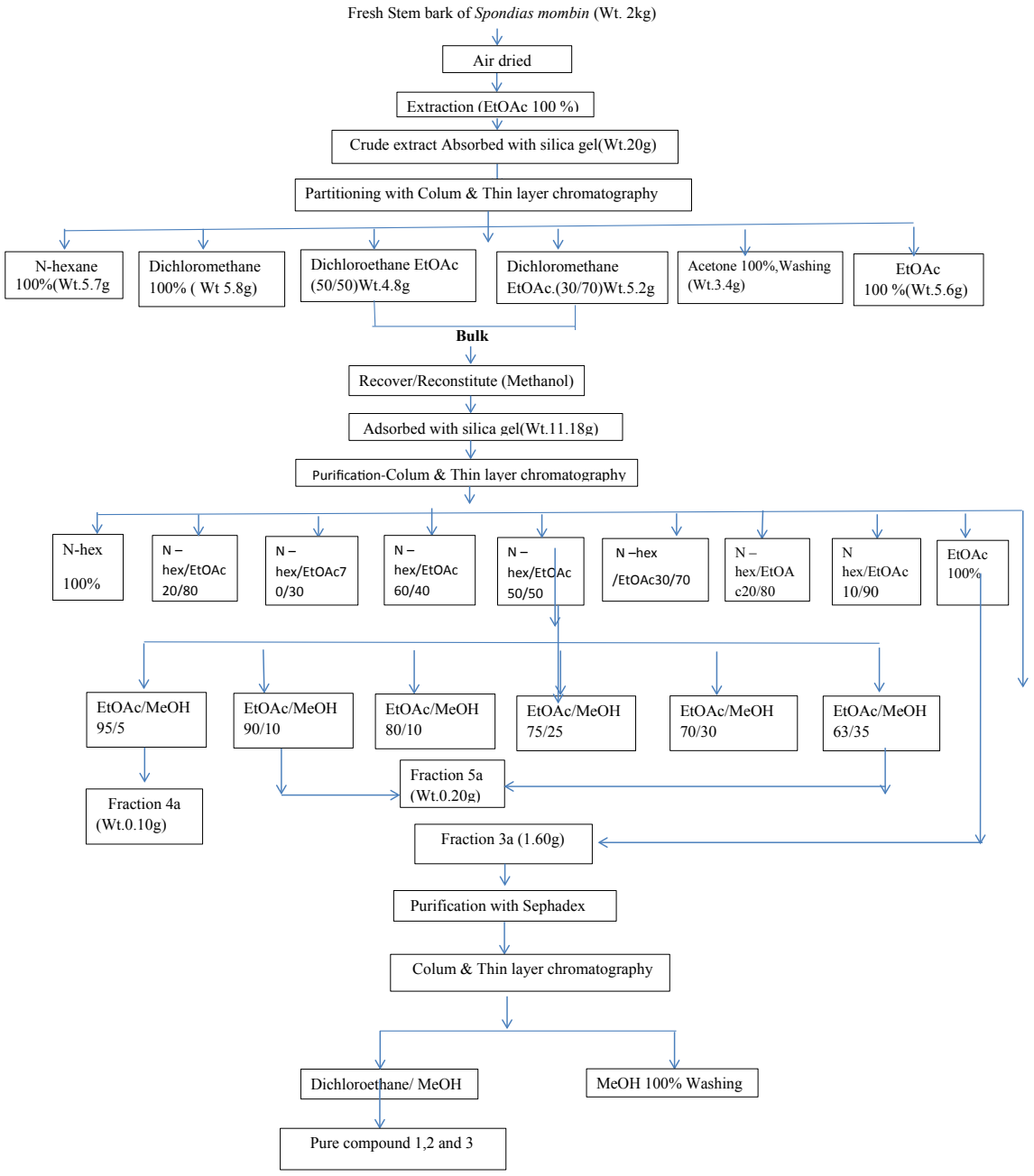


Figure 1: Isolation and purification of active compounds from the bioactive fractions (Pure compound) Key: 1- Epigallocatechin; 2- Epicatechin and 3-Stigmasterol Phytosterol.

Characterization of compound 1

Physical characteristics: Pale yellow powder, m.p. 240-242^{0}C (decomp); $[\alpha]_D^{20}$ -60 (CD_3OD, c=1.00); ESI-MS m/z 291 $[M+H]^+$, 139 $[M+H-152]^+$; IR *V*max KBr cm^{-1}: 3456.2, 1620.10, 1521.70, 1450.40, 1265.2 1143.7; UV λmax MeOH nm 213.00, 280; ^{1}H- and ^{13}C-NMR (CD_3OD).

Spectroscopic data 1: ^{1}Hnmr (300 MHz, CD_3OD) δ ppm: 4.89 (1H, bs, H-2), 4.01 (1H, bs, H-3), 2.88 ($1H_a$, *dd, J*=4.1, 12.15 Hz, H-4a), 2.84, $1H_b$, *dd*, (4.5, 12.09 Hz, H-4b), 5.89 (1H, *d, J*=2.2 Hz, H-6), 5.97 (1H, *d, J*=2.2 Hz, H-8), 7.09 (1H, d, *J*= 2.2 Hz, H- 2′), 6.74 (1H, d, *J*= 6.00 Hz, H- 5′), 6.79 (1H, dd, *J*= 6.2, 1.9 Hz, H-6′).

Spectroscopic data 2: ^{13}Cnmr (75 MHz, CD_3OD) δ ppm: 82.8 (C-2), 68.8 (C-3), 28.5 (C-4), 157.5 (C-5), 95.6 (C-6), 157.7 (C-7), 96.5 (C-8), 156.8 (C-9), 101.0 (C-10), 132.2 (C-1′), 115.4 (C-2′), 146.2 (C-3′), 146.4 (C-4′), 116.3 (C-5′), 120.2 (C-6′).

The ^{1}Hnmr, ^{13}Cnmr, DEPT, HMQC, HMBC, MS, IR and UV spectra are provided in Figure 1.

Characterization of compound 2

Physical characteristics: Brown amorphous powder, $[_D^{20}$ 110^0 (Me_2CO, c=1.00); EIMS m/z ESI-MS m/z 345 $[M+K]^+$, 139 [M+H-

168]$^{+}$; IR V_{max} KBr cm^{-1}: 3345, 2923.45.9, 1614.3, 1519.8, 1463.9, 1353.9, 1282.6, 1195.8; ^{1}H- and 13C-NMR (CD3OD).

Spectroscopic data 1: **^{1}Hnmr** (300 MHz, CD_3OD) δ ppm: 4.89 (1H, bs, H-2), 4.01 (1H, bs, H-3), 2.88 (1H_a, *dd, J*=4.1, 12.15 Hz, H-4a), 2.84, 1H_b, *dd*, (4.5, 12.09 Hz, H-4b), 5.89 (1H, *d, J*=2.2 Hz, H-6), 5.97 (1H, *d, J*=2.2 Hz, H-8), 6.9 (2H, s, H- 2′ / H-6′).

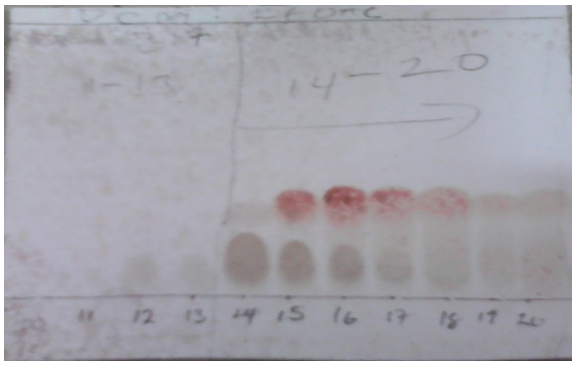
Spectroscopic data 2: **^{13}Cnmr** (75 MHz, CD_3OD) δ ppm: 82.8 (C-2), 68.8 (C-3), 28.5 (C-4), 157.5 (C-5), 95.6 (C-6), 157.7 (C-7), 96.5 (C-8), 156.8 (C-9), 101.0 (C-10), 132.2 (C-1′), 110.5 (C-2′), 146.2 (C-3′), 132.6 (C-4′), 146.4 (C-5′), 110.5 (C-6′).

The ^{1}Hnmr, ^{13}Cnmr, COSY, DEPT, HMQC, HMBC, MS, IR and UV spectra are provided as Figure 2.

Characterization of compound 3

Physical characteristics: White crystal, ESI-MS *m/z:* [M + H] 413, ^{1}H- and ^{13}C-NMR ($CDCl_3$)

Spectroscopic data 1: ^{13}Cnmr (75 MHz, $CDCl_3$) d ppm: 30.79 (C-1), 30.18 (C-2), 69.26 (C-3), 38.42 (C-4), 146.29 (C-5), 117.74 (C-6), 37.56 (C-7), 41.12 (C-8), 48.78 (C-9), 31.66 (C-10), 21.65 (C-11), 39.34 (C-12), 42.63 (C-13), 56.51 (C-14), 24.08 (C-15), 25.00 (C-16), 54.52 (C-17), 12.87 (C-18), 14.56 (C-19), 40. 80 (C-20), 21.49 (C-21), 132.46 (C-22), 129.94 (C-23), 51.35 (C-24), **30.99** (C-25), 21.29 (C-26), 19.14 (C-27), 25.23 (C-28), 11.57 (C29) (Figures 3-5).



TLC Plate 1: Isolation, Identification and Characterization of Epigallocatechin, and Epicatechin.

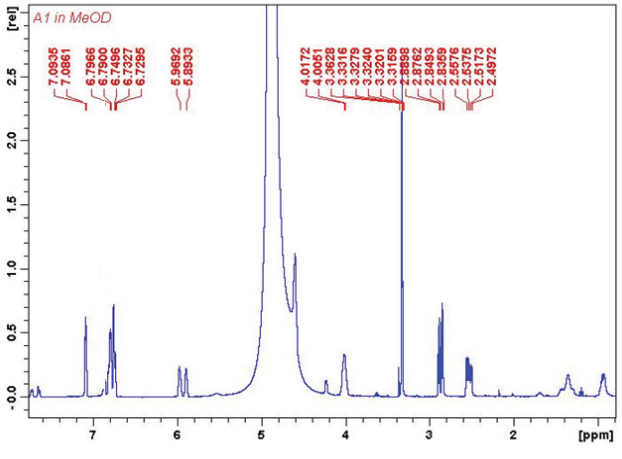


Figure 2: MeOD of Epicatechin.

Spectroscopic data 1: The ^{13}Cnmr, MS, spectra (fig 2). **^{13}Cnmr** (75 MHz, CD_3OD) δ ppm: 82.8 (C-2), 68.8 (C-3), 28.5 (C-4), 157.5 (C-5), 95.6 (C-6), 157.7 (C-7), 96.5 (C-8), 156.8 (C-9), 101.0 (C-10), 132.2 (C-1′), 110.5 (C-2′), 146.2 (C-3′), 132.6 (C-4′), 146.4 (C-5′), 110.5 (C-6′).

Discussion

This research work is based on the isolation, purification and elucidation of structure (Chemical characterization) of the isolated constituents from *Spondias mombin* extract. It was observed that, three different bioactive *constituents* were isolated, purified and structurally

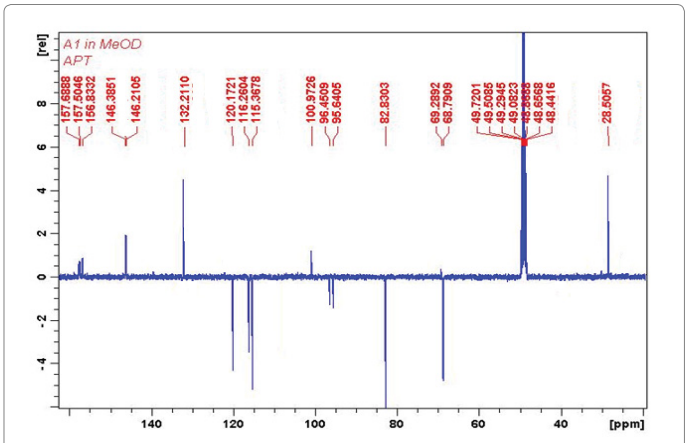


Figure 3: APT of Epicatechin.

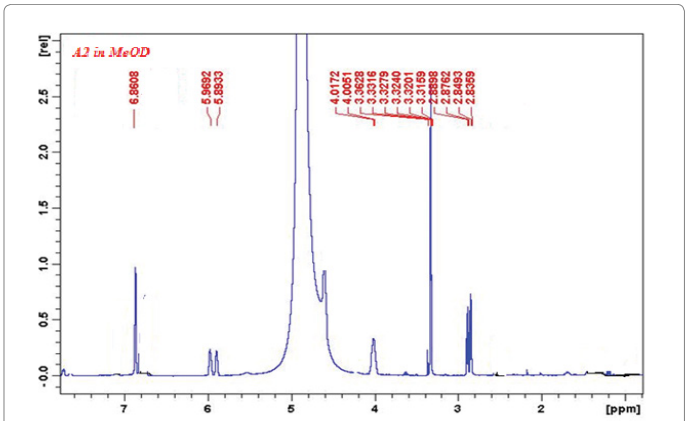


Figure 4: MeOD of Epigallocatechin.

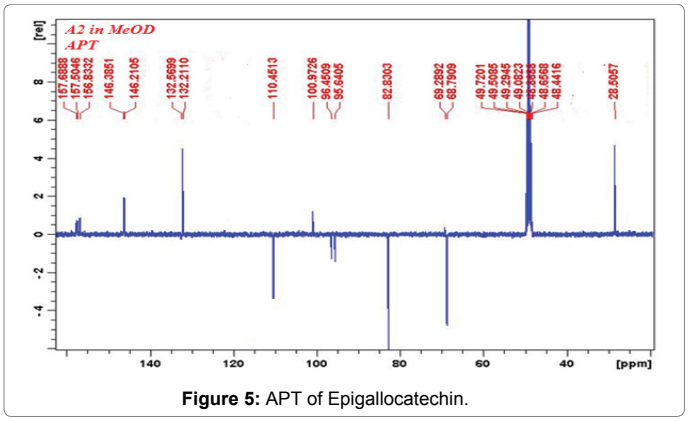
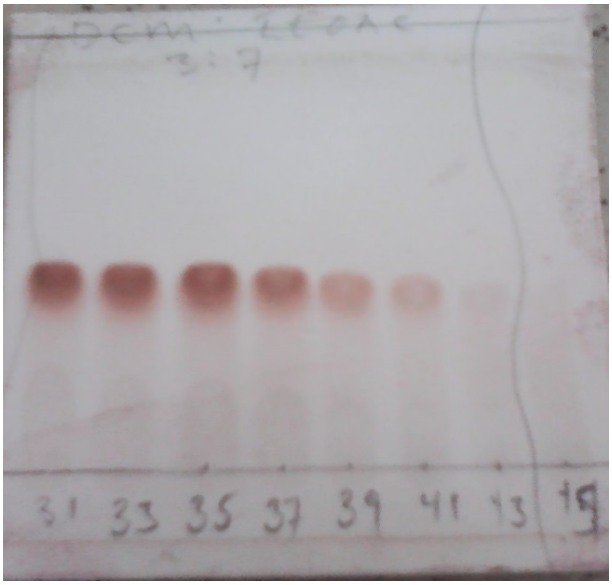


Figure 5: APT of Epigallocatechin.

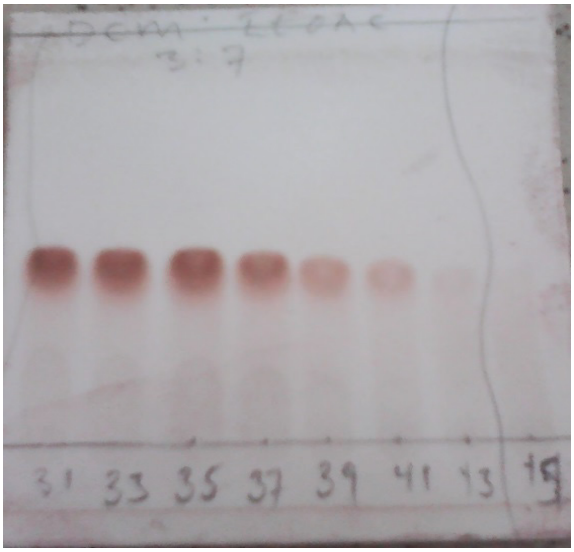
elucidated from the stem bark extract of *Spondias mombin.* They are Epigallocatechin, Epicatechin and Stigmasterol Phyto sterol**.**

Identification of Compound 1 as 3,5,7,3',4'-pentahydroxyflavanol (Epicatechin)

From the ethyl acetate fraction of the stem bark, compound **1** was isolated as yellow solid. The negative ESI –MS spectrum of compound **1** showed ion peak at *m/z* 291 $[M + H]^+$ corresponding to the molecular formula $C_{15}H_{14}O_6$. In the same MS spectrum, fragment ion found at *m/z* 139 resulted from a retro-diels-Alder (RDA) fragmentation. Analyzing the ^{13}C NMR and ^1H NMR spectral data revealed presence of two aromatic systems one exhibiting an ABD proton spin system with protons resonating at δ_H 7.09 (1H, *d, J* = 1.8 Hz), δ_H 6.74 (1H, *d, J* = 6.0 Hz) and 6.79 (1H, *dd, J* = 6.2, 1.9 Hz) assignable to H-2', H-5' and H-6' protons attached on carbons C-2' ($_C$ 115.4), C-5' (δ_C 116.3) and C-6' (δ_C 120.2) on ring B respectively. The HMBC spectrum showed that H-2' proton correlated with carbons C-2 (δ_C 78.9), C-3' (δ_C 146.2), C-4' (δ_C 146.4), C-5' and C-6' whereas H-6' proton showed correlations with carbons C-1' (δ_C 132.2), C-2, C- 3', C- 4', C-5' and C-6' (Figures 6-13).



TLC Plate 2: Isolation, Identification and Characterization of Stigmasterol Phytosterol.



TLC Plate 3: Isolation, Identification and Characterization of Stigma sterol Phytosterol.

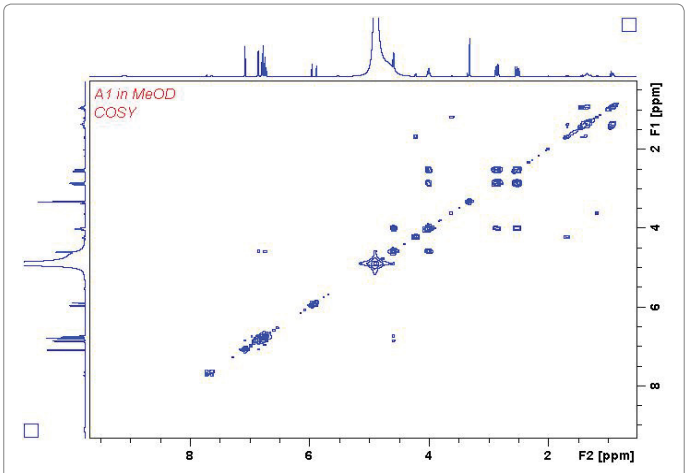


Figure 6: COSY analysis of compounds isolated from Stem bark extract of *Spondias mombin.*

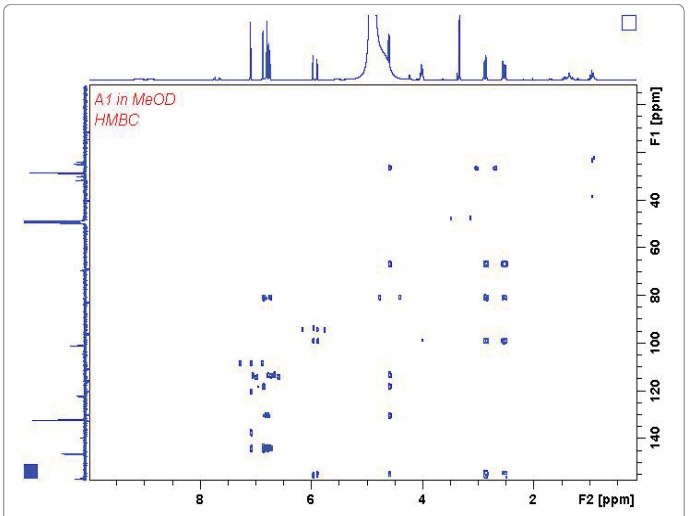


Figure 7: HMBC analysis of compounds isolated from Stem bark extract of *Spondias mombin.*

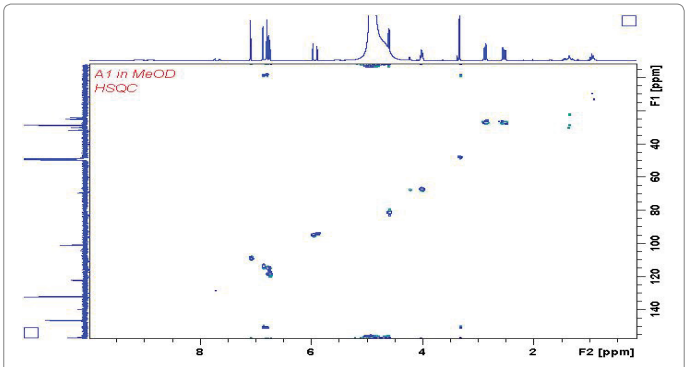


Figure 8: HSQC analysis of compounds isolated from Stem bark extract of *Spondias mombin.*

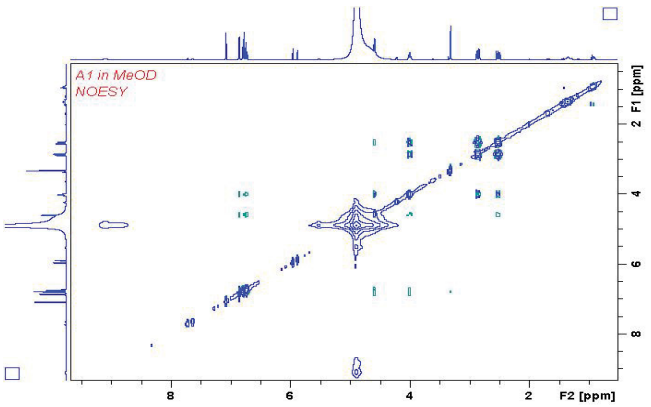


Figure 9: Noesy analysis of compounds isolated from Stem bark extract of *Spondias mombin*.

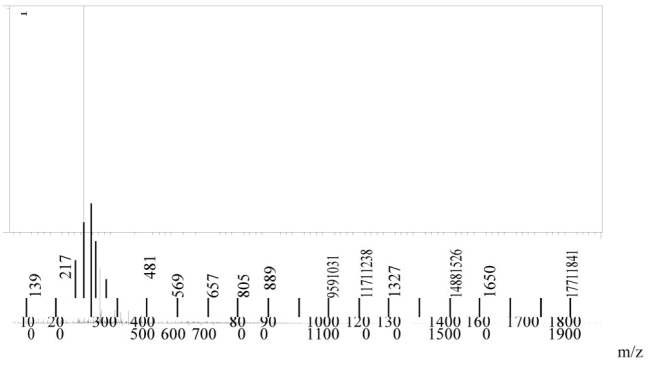


Figure 10: MS analysis of compound A1 isolated from Stem bark extract of *Spondias mombin* (SpectrumR.Time:1.067(Scan#:65) MassPeaks:2005).

Furthermore the HMBC spectrum showed correlations of H-5' proton with carbons C-1', C-3' and C-4'. Thus the ABD proton spin system was placed on ring B. Ring C on the other hand displayed a saturated system with two germinal protons resonating at δ_H 2.88 ($1H_a$, *dd, J* = 4.1, 12.15 Hz) and 2.84 ($1H_b$, *dd, J* = 4.5, 12.09 Hz) and they were placed at C-4 (δ_C 28.5) based on DEPT, HMQC and HMBC experiments. The negative ESI –MS fragment ion at *m/z* 139 resulting from a *retro* Diels – Alder fragmentation (Scheme 1) confirmed that carbon C-4 consists of two protons consistent with the flavan skeleton. The absence of carbonyl absorption peaks in the ^{13}C NMR spectrum (Figures 6-9) on the region 170 – 210 ppm (Agrawal,1989) and IR around 1700 – 1750 cm^{-1} confirmed the proposed flavanol skeleton. The second aromatic ring exhibited a proton spin system comprised of *meta*-coupled protons with resonances observed at δ_H 5.89 (1H, *d, J* = 2.2 Hz) and 5.97 (1H, *d, J* = 2.2 Hz) which were assigned to H-6 and H-8 protons placed on carbons C-6 ($_C$ 95.6) and C-8 (δ_C 96.5) respectively based on HMBC, DEPT and HMQC experiments, this proton spin system was thus placed on ring A.

From the above information compound **1** was identified as 3, 5, 7, 3', 4'–pentahydroxyflavanol, commonly known as (-) – epicatechin, a widely distributed compound in the plant kingdom and has been reported to be responsible for anti-inflammatory and antioxidant properties in green teas [5] and other plant extracts. However this is the first time we report its occurrence from *Spondias mombin* (Scheme 1).

^1H and ^{13}C NMR Data of Compound 1 were listed as follows - Position 2 (δ_H- 4.89, 1H, br s)(δ_C-82.8), Position 3(δ_H-4.01, 1H, *br s*)(δ_C-68.8), Position 4((δ_H- 2.88, $1H_a$, *dd,* (4.1, 12.15 Hz, 2.84, $1H_b$, *dd,* (4.5, 12.09 Hz)(δ_C-28.5), Position 5 (δ_C-157.5), Position 6 (δ_H-5.89, 1H, *d,* (2.2 Hz)(δ_C-95.6), Position 7(δ_C-157.7), Position 8 (δ_H-5.97, 1H, d, (2.2 Hz))($_C$-96.5), Position 9 (δ_H-)(δ_C--156.8), Position 10 ($_H$-)(δ_C-101.0), Position 1' (δ_H-)(δ_C-132.2), Position 2' (δ_H-7.09, 1H, *d,* (2.2 Hz)(δ_C-115.4), Position 3' (δ_H-)

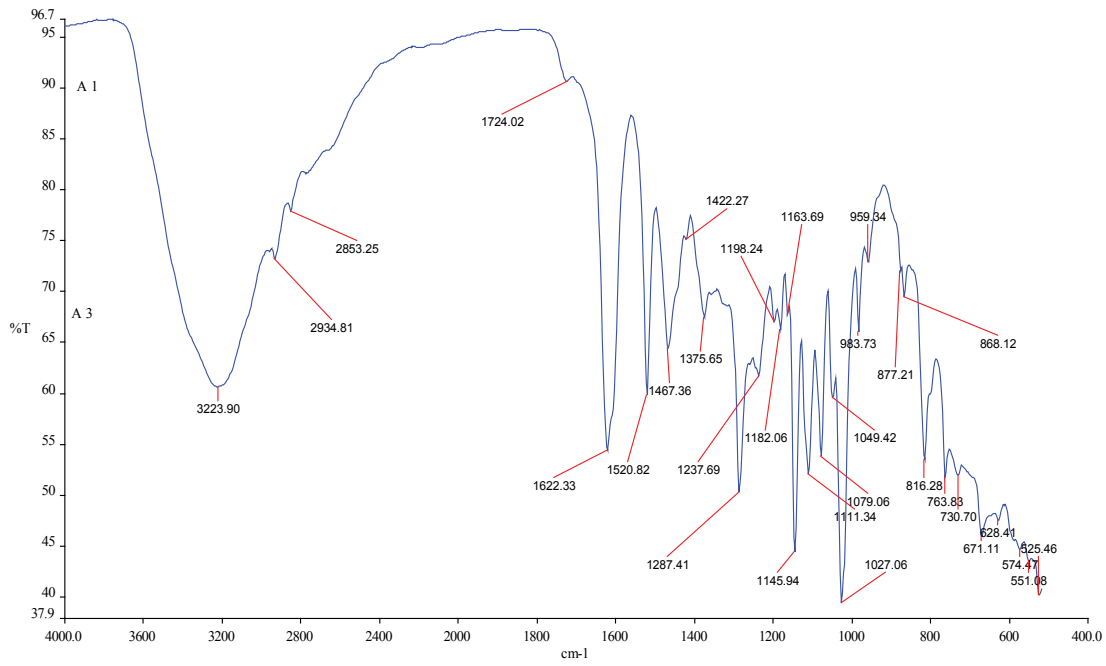


Figure 11: FTIR of Epicatechin.

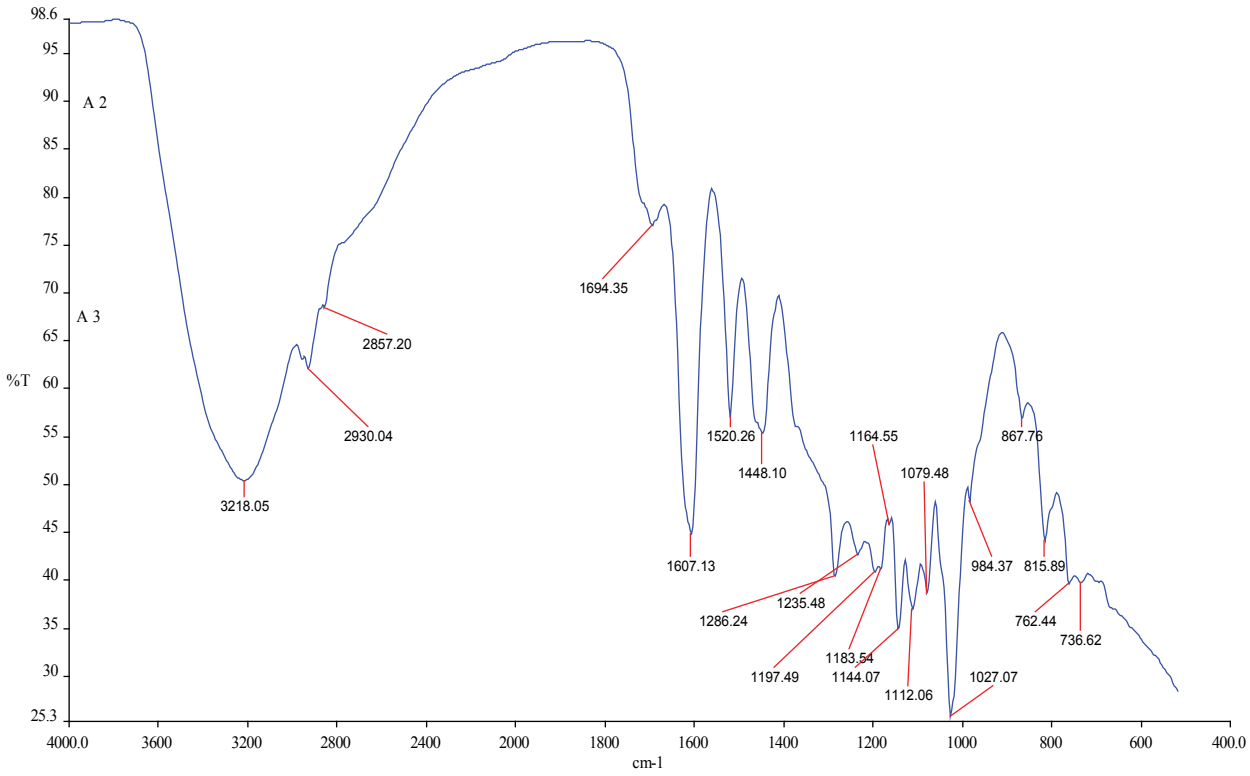


Figure 12: FTIR of Epigallocatechin.

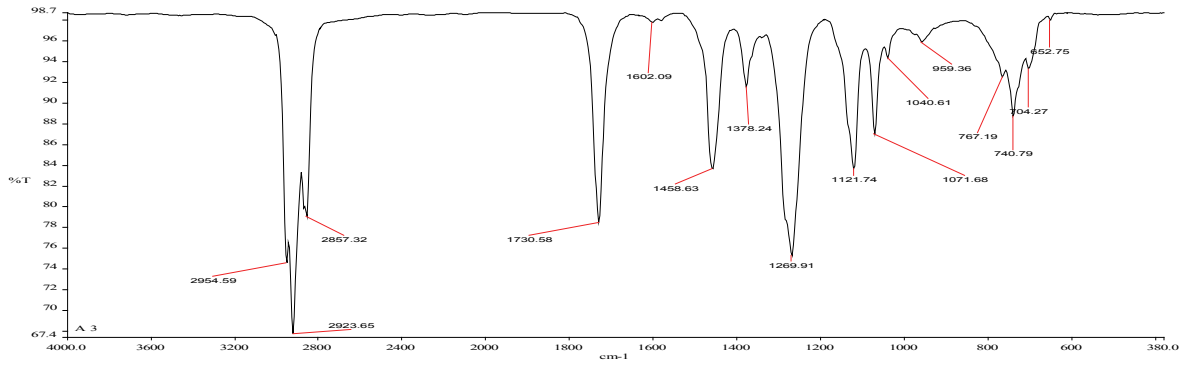


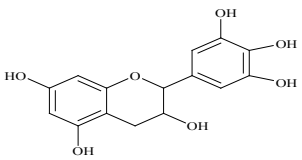
Figure 13: FTIR of Stigmasterol Phytosterol.

(δ_C-146.2), Position 4' (δ_H-)(δ_C-146.4), Position 5' (δ_H-6.74, 1H, *d*, (6.0 Hz)(δ_C-116.3), Position 6' (δ_H-6.79, 1H, *dd*, (6.2, 1.9 Hz)) (δ_C-120.2), Assignments were confirmed by HMBC, HMQC, DEPT and COSY experiments

Identification of Compound 2 as Epigallocatechin

This compound was obtained as orange powder and gave a molecular ion peak at m/z at 306.07 in EIMS which is compatible with the molecular formula $C_{15}H_{14}O_7$. Another fragment ion was found at m/z 139.04 which resulted from a retro-Diels–Alder (RDA) fragmentation.

The ^{1}H-NMR spectrum of **2** showed signals due to the presence of a pyrogallol ring at 6.53, 2H, s) which was assigned to protons H-2′ /H-6′ placed on carbons C-2′ / C-6′ (δ_C 105.59) in ring B, a phloroglucinol ring comprising of two meta - coupled protons observed at δ_H 5.96 (1H, d, J = 2.4 Hz) and 5.93 (1H, d, J = 2.4 Hz) which were assigned to protons H-6 and H-8 placed on carbons C-6 (δ_C 94.98) and C-8 ($_C$ 94.50) respectively, two non –aromatic oxygen bearing methane 4.76, and 4.18 which were



Compound 2

assigned to protons H-2 and H-3, a methylene signal at 2.86 and 2.74 which was assigned to H-4. By the combination of ^{1}H-NMR, ^{13}C-NMR, DEPT, HMQC and HMBC spectral analysis, all carbon and proton signals could be definitely assigned.

Based on the above evidence, compound **2** were concluded to be epigallocatechin which was in agreement with the reported literature values [6]. It is a broadly distributed chemical constituent in the plant kingdom and is reported to possess a powerful antioxidant activity [7].

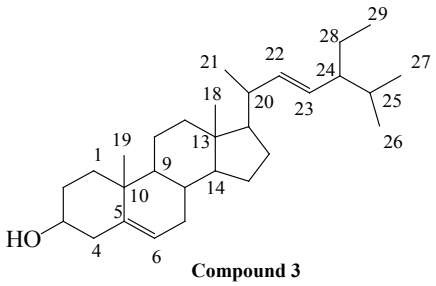
^{1}H- and ^{13}C-NMR Spectral Data for compound **2** in CD_3OD at 300 MHz were listed below.

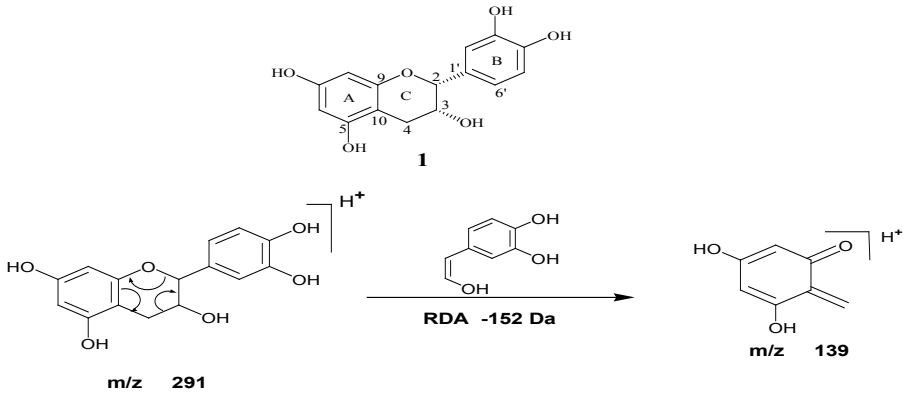
Ring C- C-2 (δ_H-4.89, 1H, br s)(δ_C-82.8), ^{13}C NMR(in acetone-d_6)- 79.4, C-3 (δ_H-4.01, 1H, *br s*)) δ_C-68.8), ^{13}C NMR(in acetone-d_6)- 67.0, C-4 (δ_H-2.88, 1H$_a$, *dd*, (4.1, 12.15 Hz, 2.84, 1H$_b$, *dd*, (4.5, 12.09 Hz)(δ_C-28.5), ^{13}C NMR(in acetone-d_6)- 28.8, Ring A- C-5 (δ_H-)(δ_C-157.5), ^{13}C NMR(in acetone-d_6)- 157.1, C-6 (δ_H-5.89, 1H, *d*, (2.2 Hz)) δ_C-95.6, ^{13}C NMR(in acetone-d_6)- 96.3, C-7 (δ_H-)(δ_C-157.7), ^{13}C NMR(in acetone-d_6)- 157.5, C-8 (δ_H-5.97, 1H, *d*, (2.2 Hz))(δ_C-96.5), ^{13}C NMR(in acetone-d_6)- 95.7, C-9 (δ_H-)(δ_C-156.8), ^{13}C NMR(in acetone-d_6)- 157.5, C-10(δ_H-)(δ_C-101.0), ^{13}C NMR(in acetone-d_6)- 99.9, Ring B- C-1′ (δ_H-)(δ_C-132.2), ^{13}C NMR(in acetone-d_6)- 131.5, C-2′ (δ_H- 6.9, 1H, s)(δ_C-110.5), ^{13}C NMR(in acetone-d_6)- 107.0, C-3′ (δ_H-)(δ_C-146.2), ^{13}C NMR(in acetone-d_6)- 146.1, C-4′ (δ_H-)(δ_C-132.60), ^{13}C NMR(in acetone-d_6)- 132.9, C-5′ (δ_H-)(δ_C-146.4), ^{13}C NMR(in acetone-d_6)- 146.1, C-6′ ($_H$-6.9, 1H, s)(δ_C-110.5), ^{13}C NMR(in acetone-d_6)- 107.0, (Scheme 2).

Identification of compound 3 as Stigmasterol phytosterol**.** Compound **3** was obtained as a white crystal. The positive- ion ESI-MS exhibited $[M + H]^+$ at m/z 413 compatible with the molecular formula $C_{29}H_{48}O$. Its ^{1}H-nmr and ^{13}C-nmr spectrum showed three olefinic protons as one triplet at 5.23 (C-6, 117.74), and two doublets of doublets at 5.16 (C-22, 132.46) and 5.10 (C-23, 129.94), a proton germinal with hydroxyl group as a multiplet at 3.60 (C-3, 71.45). The ^{13}Cnmr spectra showed 29 carbon signals composed of 6 methyl, 6 methylene, 3 olefinic methines, 8 non-olefinic methines and 3 quaternary carbons. The identity of compound **3** as stigmasterol was confirmed by comparison of its physical and spectra data with those reported in the literature [8-10]. Stigmasterol is a ubiquitous phytosterol, occurring naturally in a wide variety of plants. However, it is here reported isolated from the plant for the first time.

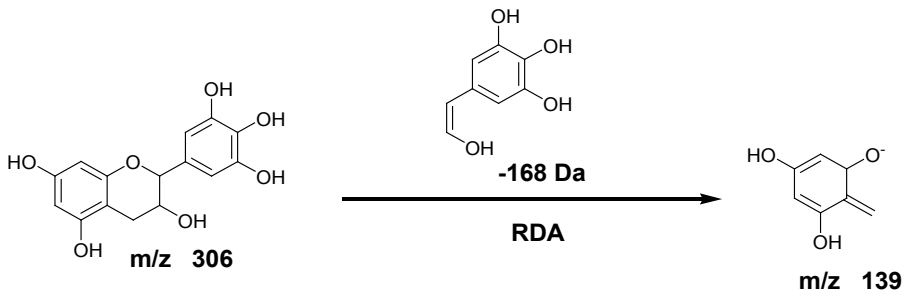
^{1}H- and ^{13}C-NMR Spectral Data for compound **3** in CD_3Cl at 300 MHz were listed below.

Position 1-^{13}C (30.18)(^{13}C NMR-in CD_3Cl-31.90), Position 2-^{13}C (69.26)(^{13}C NMR-in CD_3Cl-71.45), Position 4-^{13}C (38.40)(^{13}C NMR-in CD_3Cl-38.42), Position 5-^{13}C (146.29)(^{13}C NMR-in CD_3Cl-139.96),





Scheme 1: Fragmentation pathways for compound 1 (**RDA** = retro-Diels-Alder).



Scheme 2: ESI–MS fragmentation pattern for compound XII (RDA = retro-Diels-Alder fission (–)-epigallocatechin).

), Position 7-^{13}C (37.66)(^{13}C NMR-in CD_3Cl-37.56), Position 8-^{13}C (41.12) (^{13}C NMR-in CD_3Cl-41.20), Position 9-^{13}C (48.78) (^{13}C NMR-in CD_3Cl-49.88), Position 10-^{13}C (31.66) (^{13}C NMR-in CD_3Cl-34.63), Position 11-^{13}C (21.65) (^{13}C NMR-in CD_3Cl-21.96), Position 12-^{13}C (39.34) (^{13}C NMR-in CD_3Cl-39.88), Position 13-^{13}C (42.63)(^{13}C NMR-in CD_3Cl-43.70), Position 14-^{13}C (56.51) (^{13}C NMR-in CD_3Cl-56.33), Position 15-^{13}C (24.08) (^{13}C NMR-in CD_3Cl-23.41), Position 16-^{13}C (25.00) (^{13}C NMR-in CD_3Cl-28.88), Position 17-^{13}C (54.52) (^{13}C NMR-in CD_3Cl-55.54), Position 18-^{13}C (12.87) (^{13}C NMR-in CD_3Cl-12.62), Position 19-^{13}C (14.56) (^{13}C NMR-in CD_3Cl-13.42), Position 20-^{13}C (40.18) (^{13}C NMR-in CD_3Cl-40.68), Position 21-^{13}C (21.49) (^{13}C NMR-in CD_3Cl-21.46), Position 22-^{13}C (132.46) (^{13}C NMR-in CD_3Cl-138.55), Position 23-^{13}C (129.94) (^{13}C NMR-in CD_3Cl-129.88), Position 24-^{13}C (51.35) (^{13}C NMR-in CD_3Cl-51.65), Position 25-^{13}C (30.99) (^{13}C NMR-in CD_3Cl-32.27), Position 26-13C (21.29) (^{13}C NMR-in CD_3Cl-21.76), Position 27-13C (19.14) (^{13}C NMR-in CD_3Cl-19.37), Position 28-^{13}C (25.23) (^{13}C NMR-in CD_3Cl-25.78), Position 29-^{13}C (11.57) (^{13}C NMR-in CD_3Cl-12.44), (Idowu, 2016). All the three pure compounds are well known but reported for the first time in *Spondias mombin*.

Some authors classified epicatechin as a type of flavonoid which is found in *Spondias mombin* extract, polyphenols comprises of 30-40% of extract solids in the herbaceous tree, there are different classes of epicatechin, epigallocatechin [11]. Epicatechin have been proven to have diverse benefits to human health. It reduces the risks of diabetes mellitus and cardiovascular diseases this shows the basic important aspect of *Spondias mombin* tree, which have pharmalogical effects such as anti hyporlipidemic, anti-inflammatory, antioxidative effect, anticarcinogenic and cytoprotective [7].

EGC arts as a strong inhibitor of HIV replication in cultured peripheral blood cells and inhibition of HIV-1 reverse transcriptase *in vitro*. EGC binds directly to CD4 molecules with consequent inhibition of Gp 120 binding and inactivate viruses *in vitro* by deformation of phospholipids [12-14]. It should be mentioned that EC are antioxidant, they are effective scavengers and free radical such a reactive oxygen species (ROS), Reactive nitrogen species and superoxide [15].

Epicatechin and other polyphenols decreases the susceptibility of low density lipoprotein to oxidation which prevents the initiation of artherosclerosis, HIV protein (Tat) and gp120 is known to cause neurotoxicity in human via mechanisms that activate macrophages and glial cells and finally, oxidative stress it can be suggested that epicatechin are neuroprotective by blocking the neurotoxic effects of the HIV protein which cause oxidative stress [16].

However, it should be mentioned that Catechins have an anti-prohferative effect on turmor cells as well as inhibiting metastasis and it ability to modulate antioxidant enzymes *in vivo*. It has been shown to increase the activity of super oxide dismutase and catalase. It also suppresses lipid peroxidation of turmor cells [17].

It must be clearly stated that stigmasterol phytosterol have been shown to lower/reduce blood cholesterol and this lowering may reduce the risk of coronary heart disease. Phytosterols are under preliminary research for their potential to inhibit lung, stomach, ovarian and breast cancers as well as colon and prostate cancers [15].

Apart from lipid peroxidation, epicatechin binds secondary bile acid (Tauro-deoxycholic acid), thus secondary bile has been associated with increased risk of developing colorectal cancer thence these epicatechin from *Spondias mombin* polyphenolic compound may reduce the risk factor for the developing colorectal cancer [18].

Conclusion

In conclusion, epicatechin, epigallocatechin and stigmasterol phytosterol isolated from stem bark of *Spondias mombim* extract has proven itself to be nature's extraordinary therapeutic agent and it has also been proved during the course of this project work. It has also been proved that, *Spordias mombin* is a potent medicinal plant that its uses and application should be encouraged. More time and attention should be spent on developing it as a sustainable drug for prophylaxis and treatment of complications and diseases.

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