IN-VITRO ANTIBACTERIAL ACTIVITY – GUIDED ISOLATION AND CHACTERIZATION OF β-SITOSTEROL FROM THE MESOCARP OF THE FRUITS OF Diospyros mespiliformis

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Abstract

A phytochemical and antibacterial study of the chloroform extract of the mesocarp of the fruits of *Diospyros mespiliformis* and its fractions led to the isolation of β-sitosterol (a phytosterol) for the first time from the fruits of the plant. The structure of the isolated compound was elucidated using physical parameters, colour reactions, spectroscopic identification and literature search. The isolated compound at 100μg/cm^3 displayed moderate inhibitory activity against tested Gram-positive and Gram-negative organisms in comparison to that exhibited by erythromycin (100 μg/cm^3) against the test organisms.

Keywords: Antibacterial, β-sitosterol, chloroform extract, *Diospyros mespiliformis*, isolation and mesocarp

1. Introduction/Literature Review

Beta-sitosterol,a 4-desmethyl sterol which structurally resembles cholesterol produced in the human body is one of the well-known bioactive plant sterols with several biological and pharmacological activities. It has been isolated and purified from non-polar fractions of diverse plant families and marine sources using different chromatographic methods (Saeidnia *et al*., 2014). *Diospyros mespiliformis* Hochst Ex A. Dc (family: Ebenaceae) isa semi-deciduous woodland, savannah plant commonly called African ebony or Jackal berry (English), Kanya (Hausa), Akawayi (Igbo) and Kanram (Yoruba). It is amedium-sized evergreen tree with dense rounded stem and bell-shaped cream-colored flowers (Belemtougri *et al*., 2006). Bark is grey-black, crown is branchy with dense foliage; leaves are alternate, 4.7 cm and 1.5-5.5 cm in length and width respectively. Fruits are spherical with a fleshy berry, greenish when immature and yellowish /orange yellow when ripe. They contain between 4-6 seeds that are bean-shaped, shiny, smooth and dark brown in colouration (Venter and Venter, 1996; Orwa *et al*., 2009). Ethnomedicinally, the plant is used in the treatment of fever, malaria, wounds, leprosy, syphilis, diarrhea, pneumonia, skin diseases and helps in facilitating delivery (Mohammed *et al*., 2009). Research carried out on various parts of the plant revealed its usefulness as an antimalarial (Etkin, 1997; Adzu and Salawu, 2009), antimicrobial (Lajubutu *et al*., 1995; Adeniyi *et al*., 1996; Esimone *et al*., 2009; Shagal *et al*., 2012; Sadiq *et al*., 2013), antitrypanosomal (Freiburghaus *et al*., 1996), anti-inflammatory, analgesic, antipyretic, sedative (Adzu *et al*., 2002a; 2002b), anticancer (Adeniyi *et al*., 2003) and antiproliferative property (Abba *et al*., 2016). Phytochemical studies of the plant revealed the presence of saponins, steroidal and triterpenoidal compounds, anthraquinones, sugars, alkaloids, tannins and flavonoids (Adeniyi *et al*., 1996; Adzu *et al*., 2009; Shagal *et al*., 2012; Abba *et al*., 2016). Triterpenes, such as, α-amyrin, β-sitosterol, lupeol, betulin, betulinic acid and lupenone were isolated from the stem bark of the plant (Zhong *et al*., 1984; Mohammed *et al*., 2009). From the roots, the isolation and antibacterial activity of diosquinone and plumbagin have been reported (Lajubutu *et al*., 1995); diosquinone was later reported to be active against cancer cell lines (Adeniyi *et al*., 2003). A high presence of C16:0 and C18:2 fatty acids were reported in the oil extracted from the fruit seeds and seeds of the plant (Chivandi *et al*., 2009; Adewuyi and Oderinde, 2014). This study was undertaken to separate, isolate and characterize a phytosterol from the chloroform extract of the mesocarp of *Diospyros mespiliformis* via antibacterial testing of the extract, its fractions and sub-fractions in comparison with a standard antibiotic, erythromycin.

2. Materials and Methods

Plant Material

Fruits of *D. mespiliformis* were collected from Angwan Biri, Bosso Local Government Area, Niger state, Nigeria in September, 2016. Fruits were identified and authenticated by Dr. (Mrs.) Jemilat Ibrahim of the Department of Medicinal Plant Research and Development (MPR&TM) of National Institute for Pharmaceutical Research and Development, Idu (NIPRD) and compared with a voucher specimen already deposited (NIPRD number 5120).

Extraction of Plant Material

The fleshy part of the fruits was separated from the seeds, air-dried and pulverized. 1kg of the dried mesocarp was extracted exhaustively by macerating with chloroform and continuously shaking using a flask shaker for 5 days. Extract was concentrated *in-vacuo* to dryness and coded crude chloroform extract of *D. mespiliformis* mesocarp (D, dark green crystalline mass, 1.72 % yield).

Phytochemical Tests for Phytosterols (Harbone, 1998)

(i) Liebermann-Burchard's test: Little quantity of extract D was dissolved in $CHCl_3$ and a few drops of conc. H_2SO_4 added, followed by addition of 3 drops of Ac_2O. A change in colour from violet-blue to green was taken as positive for the presence of a steroidal nucleus.

(ii) Salkowski's test: To a small quantity of extract D in $CHCl_3$, few drops of conc. H_2SO_4were added down the side. Appearance of a reddish-brown color in the $CHCl_3$ layer was taken as evidence of presence of steroidal nucleus.

(iii) A little quantity of extract D was dissolved in 0.5 cm^3 of dioxane and added to 0.5 cm^3 of a solution of cerric ammonium nitrate (4 g of cerric ammonium nitrate was dissolved on mild heating in 10 cm^3 of 2N HNO_3) and mixture shaken thoroughly. Emergence of a yellow color that gradually changed to red indicates the presence of an alcoholic hydroxyl group.

Antibacterial Testing of Crude Extract, D

The antibacterial activity of extract D was tested against overnight cultures of two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) and three Gram negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*) all obtained from the Department of Microbiology, Federal University of Technology, Minna, Niger State, Nigeria. The viability test for each organism was carried out by resuscitating each microorganism on nutrient agar medium and incubating at 37°C for 24h. 0.2 cm^3 of stock cultures were transferred to 20 cm^3 of Mueller Hinton broth (MHB) and incubated for 5 h to obtain $1x10^6$cfu/cm^3 and a loopful used for assay. The agar dilution method was adopted for the assay (Fadipe *et al.*, 2012). 250 mg of extract D was reconstituted in 1 cm^3 of tween-80 and 4 cm^3 of sterile distilled water was added. 1 cm^3 of reconstituted mixture was then transferred to sterile Petri dishes containing 19 cm^3 of Mueller Hinton agar (MHA) and allowed to set at room temperature. A loopful of each standardized bacterial culture was streaked unto each solidified agar plate. Plates were prepared in duplicates. Plates for standard control (erythromycin; 100 µg/cm^3), extract sterility control (ESC), organism viability control (OVC) and medium sterility control (MSC) were also prepared. All plates were incubated aerobically at 37°C for 24h and checked for growth/no growth of organisms (Table 1).

Isolation, Characterization and Antibacterial Activity of β-sitosterol

Crude extract, D (8g) was applied to the surface of a prepared flash column packed with 200 g of silica gel (60-120 mesh) by wet method and eluted sequentially with varying proportions of increasing polarity of petroleum ether: $CHCl_3$ (100:0 to 0:100). Similar fractions were pooled based on their thin layer chromatographic profile and concentrated *in-vacuo* to yield 4 major fractions, D1-D4. All chromatograms were visualized under UV light (254 and 366 nm) followed by spraying with a solution of vanillin-sulphuric acid and heating at 120°C. Fractions were tested for the presence of steroidal nucleus and also screened for their antibacterial potentials in comparison with erythromycin(Table 2).

Fraction D4 (2.5 g) obtained from solvent system, petroleum ether: $CHCl_3$, 1:1 was further purified (silica gel; mesh 230-400, 90 g, flash chromatography) and eluting with varying proportions of hexane: EtOAc gave rise

to a single spotted compound from solvent system hex: EtOAc (19:1) which concentrating *in-vacuo* and re-crystallization from EtOH afforded some cream-coloured flakes, coded D4a. The compound was subjected to physical, chemical and spectral characterization.Melting point was uncorrected and recorded by open capillary method. IR and UV were both recorded in $CHCl_3$ using FTIR 8400 spectrometer and T60 UV-Visible spectrophotometer respectively. ^{1}H-NMR, ^{13}C-NMR and DEPT-135 spectra (Table 3) were taken in $CDCl_3$ on Varian Gemini spectrometer operating at 400 MHz, while, its GC-MS was recorded using GCMS-QP 2010 plus, Shimadzu. Optical rotation was recorded using Rudolph Autopol IV automatic polarimeter. Compound D4a was further screened for its antibacterial potentials in comparison with erythromycin(Table 4).

3. Results and Discussion

The crude chloroform extract of *D. mespiliformis* gave a positive Liebermann-Burchard's, Salkowski's and cerric ammonium tests, an indication that the plant has steroidal compounds that possesses alcoholic –OH group(s). Gram positive *B. subtilis* and Gram negative *P. aeruginosa* were sensitive to the extract at 50 mg/cm^3, while only Gram positive *K. pneumoniae* was not susceptible to erythromycin at100 µg/cm^3 (Table 1). Generally, the antibacterial property of a plant extract is dependent on the quality and quantity of bioactives, such as steroidal compounds and the inter-relationship that exists between them and other bioactives (Maffei-Facino *et al*., 1990; Hili *et al*., 1997). Usually, these bioactives, even in relatively low concentrations could be responsible for the observed activity (Dall'Agnol *et al*., 2003).

Fraction D4 obtained from fractionation of extract D also gave strong positive Liebermann-Burchard's and Salkowski's test. Spraying its chromatogram (PE: EtOAc, 9:1) with a mixture of vanillin-H_2SO_4 revealed reddish-brown spots, typical of steroidal compounds (Saeidnia *et al*., 2014). Antibacterial activity of the 4 major fractions at 25 mg/cm^3 showed that the organisms were not susceptible to fractions D1 and D2, while they were moderately sensitive to fractions D3 and D4 (Table 2). Sometimes, fractionation does not improve the antibacterial potency of a medicinal plant (Okoli and Iroegbu, 2004); furthermore, the better biological activity of crude extract, D, than its fractions, probably reflects a synergistic contribution from a number of constituents present in the extract (Ndip *et al*., 2009) or could be that the activity of D was enhanced at higher concentration (Fadipe *et al*., 2012).

Table 1: Antibacterial activity of test compounds against test bacterial strains

Test bacterial strains	Extract D (50mg/cm^3)	Erythromycin (100µg/cm^3)
B. subtilis	-	+
S. aureus	+	+
E. coli	+	+
K. pneumoniae	+	-
P. aeruginosa	-	+
S. typhi	+	+

+ = activity, - = no activity

Table 2: Antibacterial activity of fractions of extract (D) against test bacterial strains

Test bacterial strains	Activity of fractions (25 mg/cm^3 each) against test bacterial strains			
	D1 (PE:$CHCl_3$ 19:1)	D2 (PE:$CHCl_3$ 9:1)	D3 (PE:$CHCl_3$ 4:1)	D4 (PE:$CHCl_3$ 1:1)
B. subtilis	-	-	+	+
S. aureus	-	-	-	+
E. coli	-	-	-	-
K. pneumoniae	-	-	+	-
P. aeruginosa	-	-	+	-

S. typhi	-	-	-	+

+ = activity, - = no activity, PE = Petroleum ether

Characterization of Compound D4a

Physical Characterization

White crystalline flakes (18 mg) re-crystallized from EtOH; melting point, 136-138°C [lit. 135-137°C] (Ahmed *et al.*, 2013); single-spotted in petroleum ether: EtOAc (9:1, R_f 0.33), petroleum ether: EtOAc (4:1, R_f 0.54) and $CHCl_3$: EtOAc (4:1, R_f 0.69). The spot gave no colour under sunlight and UV (254 and 366nm), golden brown (I_2 crystals) and reddish-brown (vanillin-H_2SO_4); colour reactions were typical of a steroidal nucleus (Saeidnia *et al.*, 2014). Compound was soluble in $CHCl_3$, EtOAc and Me_2CO, slightly soluble in hexane and petroleum ether, insoluble in MeOH, EtOH and H_2O. GC-MS revealed its molecular weight and molecular formula to be 414gmol^{-1} and $C_{29}H_{50}O$ respectively. Its observed rotation, α at 589 nm was -0.05, while, its optical rotation was calculated to be $[\alpha]^{20}_{D\,(589nm)}$= -100.0 [lit. -100.0], an indication that it is a chiral and levorotatory molecule, possessing several chiral centers (Tripathi *et al.*, 2013).

Chemical Characterization

Compound **D4a** gave a positive Salkowskii's, Liebermann-Burchard's and cerric ammonium tests indicating that the compound possesses an alcoholic group on a steroidal nucleus. This is typical of all phytosterols (Chaturvedula and Prakash 2012; Mokua, 2013; Tripathi *et al.*, 2013, Rajpoot and Singh, 2014; Saeidnia *et al.*, 2014).

Spectral Characterization

IR (ν cm^{-1}): 3431 (O-H stretching), 2945 (CH_3 stretching), 2863 (CH_2 stretching), 1632 (C=C stretching), 1451 (CH_2bending), 1072 (C-O stretching) and 1058 (cycloalkane). These absorption bands are due to energy changes arising from molecular vibrations of the bond stretching and bending (deformation) type. The strong absorption band at 3431 cm^{-1} is as a result of the polarity of an oxygen-hydrogen bond (Furniss *et al.*, 1989), while, the broad absorption band at 1072 cm^{-1} is as a result of carbon-oxygen bond stretching of a secondary alcohol (Carey, 2003).

^{1}H-NMR (δ ppm): The obtained peaks in comparison with literature (Table 3) are characteristic of a steroidal system, likely a tetracyclic skeleton (Tripathee *et al.*, 2011) A doublet at δ4.66 suggests the presence of a proton at position C-6 (olefinic bond between C-5 and C-6), while proton corresponding to H-3α of a sterol moiety appeared as a triple of doublet of doublets at δ3.35, δ3.28 and δ3.21, as earlier reported by Chaturvedula and Prakash (2012). The shielding of this signal indicates its α-orientation (Kumar *et al.*, 2014). The spectrum also displayed double of triplets at δ2.41 and δ2.35 assigned to methylene protons at positions C- 4 and C-7 (neighbors to olefinic proton at C-6). Other triplet and multiplet peaks which appeared up-field were assigned to protons appearing at δ1.67, δ1.49, δ1.53, δ1.48… representing protons on C-8, C-9, C-11 and C-12 to mention a few. Two angular protons resonating as singlet at δ0.72 and δ1.02 are due to quaternary methyl protons at C-18 and C-19 respectively, while; another singlet at δ1.01 was due to primary methyl protons at C-29. Other methyl groups resonating as doublets appearing at δ0.98, δ1.12 and δ1.08 are due to secondary methyl protons at C-21, C-26 and C-27 respectively. The peak integration ratio confirmed that the compound is made up of 50 protons.

^{13}C-NMR (δ ppm): Proton de-coupled carbon-13 NMR spectra revealed a total of 29 signals(Table 3) of which signals at δ150.5 and 109.7 ppm, were assigned to C_5 and C_6 double bonds respectively as in Δ^5spirostene (Agrawal *et al.*, 1985), while, a signal at δ78.7 was assigned to a carbon bearing an electronegative β-hydroxyl at position 3 (an oxymethine). Usually, OH at C-3 with a β orientation shows a signal of a carbinolic carbon that appears at 78-79 ppm (Kumar *et al.*, 2014). A de-shielded peak at ~46.9ppm was assigned to C-4 because it is a neighbor to C-3 (bears the OH group). Another downfield

peak at ~59.8 ppm was given to C-17 as a result of the alkyl substituents attached to it. Other low-field peaks were assigned to methine carbons, while high-field peaks were given to methyl carbons.

DEPT-135 (δ ppm): Among the 29 carbon resonances obtained, DEPT-135 (Table 3) revealed that three carbon atoms were quaternary (disappeared in the spectrum), nine were methine (above in the spectrum), eleven were methylene (below in the spectrum) and six were methyl (above in the spectrum).

GC-MS (m/z): 414[$C_{29}H_{50}O$, M]$^+$, 396[$C_{29}H_{48}$, M - H_2O]$^+$, 329[$C_{24}H_{41}$, M-C_5H_9O]$^+$, 303[$C_{22}H_{39}$, M-$C_7H_{11}O$]$^+$, 275[$C_{20}H_{35}$, M-$C_9H_{15}O$]$^+$, 273[$C_{20}H_{33}$, M-$C_9H_{13}O$]$^+$, 255 [$C_{19}H_{27}$]$^+$, 231[$C_{17}H_{27}$, M-$C_{12}H_{23}O$]$^+$, 213[$C_{16}H_{21}$]$^+$, 173[$C_{13}H_{17}$]$^+$, 145[$C_{11}H_{13}$]$^+$, 109[C_8H_{13}]$^+$, 81[C_6H_9]$^+$, 57 [C_4H_9]$^+$ and 43[base peak, C_3H_7]$^+$. The GC-MS fragmentation patterns revealed the compound to be a tetracyclic steroidal compound, with characteristic fragment ion peaks at m/z 396, 329, 303, 275 and 273. An intense peak at m/z 396 indicates loss of water from the molecular ion (M^+-18), which is characteristic for dehydration of steroidal compounds (Gangwal *et al.*, 2010). Generally, cyclic alcohols like cyclohexanol (2° alcohol) undergo fragmentation-involving dehydration by complicated pathways (Silverstein *et al.*, 1991). Usually, water is lost by losing an α-H and a β-OH (Furniss *et al.*, 1989). Peaks, especially at m/z 329, 303 and 275 are diagnostic peaks for sterols possessing Δ^5 – unsaturation. Such compounds fragment readily by a pathway in which the molecular ion loses the ring bearing OH with other rings to form various carbocations (Carey, 2003). Usually, cleavage is favoured at substituted carbon atoms helping to generate carbocations in which degree of stability is cyclic C^+> 3°C^+> 2°C^+ > 1°C^+> methyl C^+ Peak at m/z 273 is attributable to the loss of R substituents on ring at position C-17. Usually, saturated rings tend to lose alkyl side chains to form a positive charge on the ring fragment. Such cleavage is favoured at alkyl substituted carbon atoms, so that, the more substituted, the more likely is the cleavage (Silverstein *et al.*, 1991). Another peak at m/z 255 is likely as a result of loss of the side chain at C-17 and further dehydration of such fragment ion. Another peak at m/z 231 is likely as result of loss of both the ring and R groups at C-17 to form a 3° carbocation.

A comparative study of the obtained physical, chemical and spectroscopic data of compound **D4a** with those published in literature revealed it to be β-sitosterol, a tetracyclic steroidal compound (Figure 1). The assignments are in good agreement with other published data for the structure of the compound, where it has been isolated and characterized by several authors from different plants (Habib *et al.*, 2007; Pateh *et al.*, 2008; Patra *et al.*, 2010; Ahmed *et al.*, 2010; Kamboj and Saluja, 2011; Tripathee *et al.*, 2011; Trivedi and Choudrey, 2011; Chaturvedula and Prakash 2012; Sen *et al.*, 2012; Ahmed *et al.*, 2013; Mokua, 2013; Tripathi *et al.*, 2103, Isah *et al.*, 2014; Kumar *et al.*, 2014; Rajpoot and Singh, 2014; Saeidnia *et al.*, 2014; Kumar *et al.*, 2015). This is the first report of the isolation and characterization of β-sitosterol from the mesocarp of *D. mespiliformis*

Table 3: ^{1}H-NMR,^{13}C- NMR and DEPT-135° spectral data obtained for compound D4a in comparison with literature values*

Position	δ H (ppm)	δ H (ppm)*	δ C (ppm)	δ C (ppm)*	DEPT (ppm)	DEPT (ppm)*
1	1.45 (m)	1.43 (m)	29.8	30.1	29.8 (-CH_2)	29.8 (below)
2	1.55 (m)	1.57 (m)	34.5	35.8	34.5 (-CH_2)	34.5 (below)
3	3.35 (dd)	Triple dds				
	3.28 (dd)					
	3.21 (dd)					
3 (OH)	2.04 (m)	2.00 (m)	79.0	78.7	79.0 (-CH)	79.0 (above)
4	2.41 (t)	2.43 (t)	47.8	46.9	47.8 (-CH_2)	47.8 (below)
5	-	-	150.5	148.9	150.5 (-C-)	150.5 (disappeared)
6	4.66 (d)	4.70 (t)	109.7	110.9	109.7 (-CH)	109.7 (above)
7	2.35 (t)	2.34 (t)	29.7	30.0	29.7 (-CH_2)	29.7 (below)

8	1.70 (m)	1.65 (m)	37.2	37.9	37.2 (-CH)	37.2 (above)
9	1.49 (t)	1.47 (t)	50.4	50.8	50.4 (-CH)	50.4 (above)
10	-	-	40.8	39.8	40.8 (-C-)	40.8 (disappeared)
11	1.53 (t)	1.54 (t)	20.7	20.7	20.7 ($-CH_2$)	20.7 (below)
12	1.48 (t)	1.48 (t)	38.9	38.2	38.9 ($-CH_2$)	38.9 (below)
13	-	-	42.1	42.0	42.1 (-C-)	42.1 (disappeared)
14	1.37 (m)	1.40 (m)	55.2	55.5	55.2 ($-CH_2$)	55.2 (below)
15	1.60 (d)	1.61 (d)	27.4	27.7	27.4 ($-CH_2$)	27.4 (below)
16	1.80 (d)	1.78 (d)	27.1	27.3	27.1 ($-CH_2$)	27.1 (below)
17	1.51 (d)	1.49 (d)	60.2	59.8	60.2 (-CH)	60.2 (above)
18	0.72 (s)	0.71 (s)	18.3	18.7	18.3 ($-CH_3$)	18.3 (above)
19	1.02 (d)	1.06 (s)	19.1	19.0	19.1 ($-CH_3$)	19.1 (above)
20	1.63 (m)	1.64 (m)	37.3	37.1	37.3 (-CH)	37.3 (above)
21	0.98 (d)	1.02 (m)	16.1	16.4	16.1 ($-CH_3$)	16.1 (above)
22	0.97 (m)	1.01 (m)	38.7	38.9	38.7 ($-CH_2$)	38.7 (below)
23	1.08 (m)	1.05 (m)	27.9	27.9	27.9 ($-CH_2$)	27.9 (below)
24	1.60 (t)	1.61 (t)	48.6	48.1	48.6 (-CH)	48.6 (above)
25	1.42 (d)	1.42 (d)	33.8	33.7	33.8 (-CH)	33.8 (above)
26	1.12 (d)	1.11 (d)	15.9	15.6	15.9($-CH_3$)	15.9 (above)
27	1.08 (d)	1.06 (d)	15.4	15.1	15.4 ($-CH_3$)	15.4 (above)
28	1.30 (m)	1.29 (m)	25.2	25.6	25.2 ($-CH_2$)	25.2 (below)
29	1.01 (s)	0.98 (s)	12.3	12.2	12.3 ($-CH_3$)	12.3 (above)

Keys: s=singlet, d=doublet, dd=doublets of doublets, t=triplet, m=multiplet

*ACD/ChemDraw (Product version 15); Pateh *et al*., 2008; Saiednia *et al*, 2014

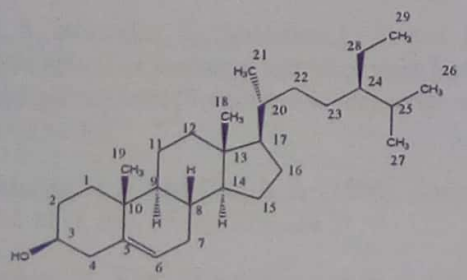

Figure 1: β-Sitosterol/ 24β-Ethylcholesterol/ 22, 23-Dihydrostigmasterol/ 5-Stigmasten-3β-ol/ 24R-Stigmast-5-en-3β-ol/ α-Dihydrofucosterol

Table 4: Antibacterial activity of isolated β-sitosterol (D4a) in comparison with erythromycin against test bacterial strains

Test bacterial strains	Activity of test compounds against test bacterial strains	
	D4a (100 µg/cm^3)	Erythromycin (100 µg/cm^3)
B. subtilis	-	+
S. aureus	+	+
E. coli	+	+

K. pneumoniae	-	-
P. aeruginosa	-	+
S. typhi	+	+

+ = activity, - = no activity

The isolated compound at 100µg/cm^3 displayed moderate antibacterial activity in comparison with erythromycin that was more active against both Gram-positive and Gram-negative organisms tested, except against Gram negative *Pseudomonas aeruginosa* (Table 4). Generally, β-sitosterol has been reported to possess low/moderate antibacterial activity against several bacterial strains (Beltrame *et al.*, 2002; Sen *et al.*, 2012; Woldeyes *et al.*, 2012; Rajpoot and Singh, 2014; Yadav *et al.*, 2014).

4. Conclusion

Extraction, fractionation, purification and *in-vitro* antibacterial testing of the chloroform extract of the mesocarp of fruits of *Diospyros mespiliformis* and its fractions led to the isolation, characterization and structural elucidation of β-sitosterol. The phytosterol in comparison with erythromycin (both100 µg/cm^3) exhibited a moderate antibacterial activity against the tested organisms. The isolated compoundprobably acted individually or synergistically with other constituents present in the mesocarp of the fruits of the plant to account for the ethnomedicinal uses of the plant. Further research will focus on the bio-assay guided isolation and characterization of more phytoconstituents from the plant.

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