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Chemical and Cytotoxic Investigation of Non-Polar Extract from *Ceiba Pentandra* (L.) Gaertn.: A Study Supported by Computer Based Screening

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ABSTRACT

Nowadays, the discovery of new drugs from natural sources is increasing dramatically. Recently, the sight is directed toward ornamental plants due to their sustainability. In this study, we are reporting on the gas chromatography profile of fatty acids, other related lipids, and hydrocarbons of the non-polar methylene chloride (MC) fraction of the extract of the ornamental tree *Ceiba pentandra* (L.) Gaertn. Chromatographic purification of the same fraction furnished eight pure known constituents, 3*β*-taraxerol (**1**), 3*β*-taraxerol acetate (**2**), all-trans-squalene (**3**), oleic acid (**4**), 1-hexacosanol (**5**), *β*-amyrin (**6**), *β*-sitosterol (7) and *β*-sitosterol-3-O-*β*-D-glucopyranoside (**8**), which are relatively abundant in the fraction, six of them are firstly isolated from the plant (**1**–**6**). A computer-based bio-activity study indicated cancer treatment and/or prevention of the isolated compounds (**1**–**3** and **6**) with pa values higher than for the other predicted effects. The experimental cytotoxicity assessment of the MC fraction demonstrated prominent cytotoxic effect against the cancer cell lines HepG2 (IC_{50} = 14.895 µg/mL) and MCF-7 (IC_{50} = 18.859 µg/mL).

INTRODUCTION

Cancer is one of the major causes of mortality with higher levels in developing countries (Houghton *et al.*, 2007). In Egypt, hepatocellular carcinoma (HCC) and breast cancer are the most serious cancer problems with elevated incidence rates [liver cancer (23.8%), breast cancer (15.4%)] (Ibrahim *et al.*, 2014). Despite the successes in oncology chemotherapeutics, the impulse to develop novel, alternative or synergistic anti-cancer agents is becoming popular among the herbal medicine researchers to avoid the side effects imposed by recently used chemotherapeutics (Yin *et al.*, 2013). Approximately 60% of drugs currently in clinical use for cancer treatment are obtained from natural sources as paclitaxel (Taxol) from yew (*Taxus*) species (Houghton *et al.*, 2007).

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Ceiba. pentandra (L.) Gaertn. (*C. pentandra*), also called Kapok and Silk-Cotton tree, is a fast-growing gigantic tree belongs to family Bombacaceae. It is widely distributed in the tropical, intertropical and subtropical regions of the world and planted as wayside and shade trees (Alvarado *et al.*, 2002). The plant is used in folklore medicine as an emetic, diuretic, and antispasmodic (Lim, 2012). It is also recommended for the treatment of intestinal disorders (diarrhoea and dysentery), hormonal disease (diabetes) bone disease (arthritis), skin diseases, painful eye diseases, bronchitis, insect bites and chronic fever (Elumalai *et al.*, 2012). Recent pharmacological studies revealed that solvent extracts of various parts of the plant have hypoglycaemic (Djomeni *et al.*, 2006), hypolipidemic (Aloke *et al.*, 2010), hepatoprotective (Bairwa *et al.*, 2010), anti-inflammatory (Alagawadi and Shah, 2011), and anti-ulcerogenic effects.

In this study, a gas-chromatographic investigation of the non-polar fraction of the aerial parts extract of *C. pentandra* coupled with actual isolation by chromatography from the MC fraction led to the purification of eight major constituents (**1-8**).

Moreover, employing prediction of activity spectra for substances (PASS) computer program revealed tumor treatment and/or prevention activities of these compounds. The experimental study on hepatocellular carcinoma (HepG2) and breast cancer (MCF-7) cell lines using SRB assay was also conducted.

EXPERIMENTAL

General procedures

Authentic *β*-sitosterol, *β*-amyrin and ceryl alcohol were obtained from the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt. Silica gel (70–230 mesh, Merck, Germany) was used for column chromatography. Precoated silica gel (G_{60} F_{254}) plates (E. Merck, Darmstadt, Germany) were used for TLC analyses and spots were visualized by spraying with sulfuric acid (10% in MeOH) and heating at 110°C on a hot plate (Bolliger *et al.*, 1970). The solvents petroleum ether, *n*-hexane, MC, EtOAc, *n*-butanol, acetone, ethanol, MeOH and DMSO and others were purchased from El-Nasr Pharmaceutical and Chemical Co., Egypt (ADWIC). ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance III 400 MHz for ^{1}H and 100 MHz for ^{13}C (Bruker AG, Switzerland) with BBFO smart probe and Bruker 400 AEON nitrogen-free magnet. Agilent 7890A network gas chromatograph with an Agilent 5975B inert XL EI/C/MSD mass selective detector with J&W 122−5532 DB-5ms (30 m, 0.25 mm, 0.25 um Film), (5% Phenyl-methylpolysiloxane) capillary column (USA) was used for gas chromatography. The data were analyzed using Topspin 3.1 software.

Plant material

The fresh aerial parts, composed of leaves, petioles and young stems, of *C. pentandra* were collected at March 2017 from the field of ornamental plants, Faculty of Agriculture, Assiut University, Egypt. The plant was kindly identified by Dr. Essam Youssef, Associate Professor of Horticulture, Faculty of Agriculture, Assiut University, Egypt. A voucher specimen (No. Cpp1) was kept in Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Assuit-branch, Egypt. The plant materials were dried under shade, finely powdered and the powder was used for the extraction procedure.

Cell lines

MCF-7 and HepG-2 cancer cell lines were supplied by VACSERA, Egypt. The media (Dulbecco's modified Eagle's medium; DMEM) were sterilized by 0.22 µm microbiological filters and kept at 4°C before use.

A computer program for prediction of biological activity

Prediction of Activity Spectra for Substances (PASS) program (ver. 4.20) was used for prediction of biological activity (Ramakrishnan, 2010).

Extraction and isolation

The air-dried powdered aerial parts (2 kg) of *C. pentandra* were extracted by maceration in MeOH–H_2O (8:2, *v/v*) at room temperature (5 L × 3). The alcoholic extract was concentrated at 45°C under reduced pressure till constant weight (250 g). The dry methanolic extract was digested with distilled H_2O (500 mL). The suspension was transferred to a separating funnel and the phytoconstituents were successively partitioned between the aqueous layer and MC (500 mL × 3), EtOAc (500 mL × 3), *n*-butanol (500 mL × 3) till exhaustion. Solvents of each fraction were distilled off then the fractions were dried under reduced pressure to give MC (52 g), EtOAc (28.06 g), *n*-butanol (19.23 g) and aqueous fractions (149 g), respectively.

Investigation of lipids content

Preparation of the unsaponifiable matter

A 0.2 g of the MC fraction was saponified by reaction with 40 mL of 20% ethanolic KOH overnight at room temperature. The alcohol was distilled off and the aqueous liquid was diluted with H_2O then extracted with several portions of petroleum ether (40–60°C) till exhaustion. The combined ethereal extract was washed with H_2O and tested with litmus paper till the washings became free from any alkalinity then washed with 10% NaCl solution. The ethereal extract was dehydrated over anhydrous sodium sulfate and then the ether was distilled off under reduced pressure to produce a viscous yellowish residue (unsaponifiable fraction). For detection of the hydrocarbons a 1 µL of the unsaponifiable fraction was subjected to GC-MS analysis at 40°C for 3 min then temperature increased by a rate of 10°C/min till 150°C with holding time 3 min. then temperature increased by a rate of 10°C/min to 220°C with holding time 6 min. finally, temperature increased by a rate of 15°C/min till 260°C with holding time 13 min. GC-MS analysis for detection of steroids and terpenes was performed at 100°C for 1 min then temperature increased by a rate of 10°C/min to 260°C with holding time for 29 min (El-Saied *et al.*, 1981).

Fatty acids analysis

The alkaline aqueous solution (soap) left after extraction of the unsaponifiable matter was acidified with a dilute sulphuric acid, and the liberated fatty acids were extracted with petroleum ether (40–60°C) (50 mL × 3). The combined ethereal extracts were washed several times with distilled H_2O and tested with litmus paper until the washings were free from acidity then washed by 10% NaCl solution. The ether extract was dried over anhydrous sodium sulfate. The solvent was distilled off under reduced pressure to give a viscous residue of the free fatty acids.

The free fatty acids were dissolved in little absolute MeOH in a glass tube and esterified by cooling in ice and adding an ethereal solution of diazomethane in small portions until gas evolution ceases and the solution acquires a pale yellow color. The solvent was evaporated under reduced pressure, the residue was digested with little H_2O, and then extracted with ether several times (each of 10 mL). The combined ethereal extracts were dehydrated over anhydrous sodium sulfate, and the solvent was then concentrated to yield an oily residue (methyl esters). A 1 µL of the obtained methyl esters was subjected to GC-MS analysis at 80°C for 2 min then temperature increased by a rate of 3°C/min till 230 with holding time 5 min for each 40 min (El-Sayeda *et al.*, 2013).

Chromatographic isolation of major constituents of MC fraction

A part of the MC fraction (30 g) was chromatographed over silica gel (70–230 mesh, 600 g), eluted with *n*-hexane and *n*-hexane–EtOAc gradients with a collection of 100 mL aliquot.

Similar fractions were grouped together, concentrated under reduced pressure to give six fractions (M-I–M-VI). The fraction M-II (2.576 g), eluted with *n*-hexane–EtOAc (97:3, *v/v*), was crystallized from MC to give 3*β*-taraxerol acetate (**2**, 21 mg). The fraction M-III (200 mg), eluted with *n*-hexane–EtOAc (95:5, *v/v*), was re-chromatographed on silica gel column chromatography (10 g) and eluted with *n*-hexane–EtOAc (97:3, *v/v*) isocratically and afforded *β*-amyrin (**6**, 9 mg). The fraction M-IV (650 mg), eluted with *n*-hexane–EtOAc (9:1, *v/v*), was re-chromatographed on silica gel column chromatography (25 g) and eluted with *n*-hexane–EtOAc (9:1, *v/v*) isocratically and afforded 3 sub-fractions (M-IV-1–M-IV-3). Both M-IV-2 (200 mg) and M-IV-3 (120 mg) sub-fractions were recrystallized from MC and furnished 3*β*-taraxerol (**1**, 90 mg) and *β*-sitosterol (**7**, 70 mg), respectively. The sub-fraction M-IV-1 (162 mg) was re-chromatographed on silica gel (10 g) and eluted with *n*-hexane–MC–EtOAc (60:20:2.5, *v/v/v*) isocratically and yielded another two sub-fractions M-IV-1-A (98 mg) and M-IV-1-B (18 mg). The sub-fraction M-IV-1-B was dissolved in MC and left overnight where hexacosanol (**5,** 12 mg) gave an amorphous precipitate. The M-IV-1-A sub-fraction was re-chromatographed on silica gel (162 mg) and eluted with *n*-hexane–EtOAc (95:5, *v/v*) isocratically to give all-trans-squalene (**3,** 9 mg) and oleic acid (**4,** 15 mg) in subsequent elutes. A part of fraction M-V (200 mg) eluted with *n*-hexane–EtOAc (80:20, *v/v*) was re-chromatographed on silica gel (10 g) and eluted with MC–MeOH (90:10, *v/v*) isocratically and afforded *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**8,** 29 mg).

Cytotoxic activity of MC fraction of *C. pentandra* (L.) Garten. var. *pentandra*

The cytotoxic activity of the MC fraction was conducted against two cancer cell lines HepG-2 and MCF-7 using sulforhodamine B cytotoxicity assay method (Priya *et al.*, 2015; Ul-Haq *et al.*, 2012). All experiments were carried out in triplicate (El-Hawary *et al.*, 2015).

Data are expressed as the percentage of relative viability, which was calculated using the following equation:

$$\text{Relative viability} = \frac{\text{treated cells absorbance}}{\text{control cells absorbance}} \times 100$$

Cell survival was measured as the percentage of absorbance compared with the control. The positive cytotoxic activity is indicated by less than 100% relative viability. The relation between surviving fraction and extract concentration is plotted to get the survival curve of the liver, and breast cell lines. Also, the IC_{50} (dose of the extract which reduces survival to 50%) for active extracts was calculated using Sigma plot 12 computer software.

RESULTS AND DISCUSSION

Investigation of unsaponifiable matter composition

Fatty acids, steroids, and other unsaponifiable substances are widely distributed in non-polar fractions of plant extracts. The characterization of their profiles and relative proportions can serve as a fingerprint for identification of plants and as a parameter in identifying and detecting adulterant (Bezerra and Antoniosi Filho, 2014). Unsaturated fatty acids play an essential role in stabilization and function of biological membranes of living organisms (Zhang *et al.*, 2004). Moreover, they have hypolipidemic properties against triglycerides and may also reduce the developing of colon, breast, and prostate cancers (Lunn and Theobald, 2006). In addition, several chemotherapeutic agents-unsaturated fatty acids conjugates prodrugs and prodrug-based nanoparticulate drug delivery systems are talented prospects for developing potential chemotherapeutic applications in cancer treatment (Sun *et al.*, 2017). Plant-derived sterols are also important natural products since they have known for their potential treatment and prevention of cardiovascular diseases (Maeorg *et al.*, 2007).

The unsaponifiable matters were identified by matching their retention times and fragmentation patterns with those of reference compounds analyzed under the same conditions by gas-liquid chromatography (GLC). The major known identified hydrocarbons are listed in table 1; they include 3-ethyltetracosane (4.82%), *n*-nonacosane (4.79%), *n*-docosane (4.71%) and *n*-pentacosane (4.31%). Meanwhile, the major known sterols and terpenes are phytol (14.99%), taraxerol (14.01%), *β*-sitosterol (11.87%) and squalene (9.96%) as listed in table 2.

Table 1: GLC analysis results of unsaponifiable fraction (Hydrocarbons) of *C. pentandra.*

NO.	Name	t_R (min)	Relative %
1	1-Hexadecene	21.544	1.01%
2	Cycloeicosane	23.948	1.39%
3	5-Eicosene	26.551	1.24%
4	*n*-Hexadecane	26.631	0.94%
5	2,6,11-Trimethyl-dodecane	28.525	1.72%
6	Cyclotetracosane	30.745	0.67%
7	*n*-Tetracosane	30.843	3.15%
8	*n*-Pentacosane	32.342	4.31%
9	*n*-Docosane	33.498	4.71%
10	5,9-Dimethyl-4,10-octadecadiene	34.282	0.89%
11	3-Ethyltetracosane	34.802	4.82%
12	*n*-Nonadecane	36.382	3.87%
13	*n*-Nonacosane	38.316	4.79%
14	(5E)-2,3,5,8-Tetramethyl-1,5,9-decatriene	38.67	1.19%
15	2-Methyl-7-nonadecene	39.792	3.25%
16	1,19-Eicosadiene	40.524	0.92%
17	17-Pentatriacontene	43.826	2.78%
18	9-Tricosene	44.038	0.98%

Investigation of the fatty acids composition

The fatty acids methyl esters were identified by matching their retention times and fragmentation patterns with those of reference compounds analyzed under the same conditions. The identified fatty acids with their relative abundance in the MC fraction of aerial parts of *C. pentandra* are linoleic acid (34.38%), *α*-linolenic acid (17.47%), palmitic acid (17.30%), stearic acid (14.22%), myristic acid (12.26%) and oleic acid (4.37%).

Identification of isolated compounds

The MC fraction of *C. pentandra* was subjected to several chromatographic techniques, where eight (**1**–**8**)

compounds were purified, six of them (**1**–**6**), are firstly isolated from the plant. The compounds were identified by comparison of their chromatographic, physical, chemical and spectroscopic data with literature data as 3*β*-taraxerol (**1**), 3*β*-taraxerol acetate (**2**), all-trans-squalene (**3**), oleic acid (**4**), 1-hexacosanol (**5**), *β*-amyrin, (**6**), *β*-sitosterol (**7**) and *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (**8**) (Fig. 1). The physico-chemical and spectroscopic data of the isolated compounds are as follow:

3*β*-taraxerol (**1**). MP 281–284°C. *Rf*: 0.51 (*n*-hexane–EtOAc, 80:20). ^{1}H NMR (400 MHz, $CDCl_3$): 0.80 (3H, s, H-24), 0.82 (3H, s, H-28), 0.88 (1H, m, H-5), 0.91 (3H, s, H-27), 0.91 (3H, s, H-30), 0.92 (3H, s, H-25), 0.95 (3H, s, H-29), 0.97 (3H, s, H-23), 0.99 (1H, m, H-1), 0.99 (1H, m, H-18), 1.00 (1H, m, H-22), 1.02 (1H, m, H-19), 1.10 (3H, s, H-26), 1.25 (2H, m, H-21), 1.33 (1H, m, H-19), 1.36 (1H, m, H-7), 1.38 (1H, m, H-21), 1.38 (1H, m, H-22), 1.43 (1H, m, H-9), 1.45 (1H, m, H-6), 1.49 (1H, m, H-11), 1.53 (1H, m, H-12), 1.59 (1H, m, H-16), 1.62 (1H, m, H-6), 1.62 (2H, m, H-2), 1.63 (1H, m, H-11), 1.63 (1H, m, H-1), 1.64 (1H, m, H-12), 1.92 (1H, dd, J = 14.8, 3.2 Hz, H-16), 2.03 (1H, dt, J = 12.5, 3.0 Hz, H-7), 3.19 (1H, dd, J = 11.2, 4.8 Hz, H-3), 5.53 (1H, dd, J = 8, 3.2 Hz, H-15).

Table 2: GLC analysis results of unsaponifiable fraction (sterols and terpenes) of *C. pentandra.*

No.	Name	t_R (min)	Relative %
1	3*β*-Hydroxy-androst-5-ene-17-one	10.632	0.15%
2	6,10,14-Trimethyl-2-pentadecanone	11.654	0.76%
3	*γ* cis-Sesquicyclo geraniol	12.385	1.04%
4	Phytol	14.286	14.99%
5	Stearyl alcohol	15.027	0.90%
6	Phytane	15.943	1.69%
7	6,10,14-Trimethyl-5,9,13-pentadeca-trien-2-one	16.589	0.96%
8	Squalene	21.456	9.96%
9	Farnesol	23.505	1.04%
10	*β*-Sitosterol	36.166	11.87%
11	Taraxerol	36.701	14.01%

^{13}C NMR (100 MHz, $CDCl_3$): 15.6 (C-24), 15.6 (C-25), 17.7 (C-11), 18.9 (C-6), 21.5 (C-27), 26.1 (C-26), 27.3 (C-2), 28.2 (C-23), 28.9 (C-20), 29.9 (C-28), 30.1 (C-30), 33.2 (C-12), 33.5 (C-29), 33.8 (C-21), 35.3 (C-17), 35.9 (C-22), 36.8 (C-16), 37.7 (C-19), 37.9 (C-13), 37.9 (C-10), 38.1 (C-1), 38.9 (C-4), 39.1 (C-8), 41.5 (C-7), 48.9 (C-9), 49.4 (C-18), 55.7 (C-5), 79.2 (C-3), 117.0 (C-15), 158.2 (C-14).

EIMS: $[M]^+$ ion at *m/z* = 426.30.

3*β*-taraxerol acetate (**2**). MP 304–305°C. *Rf*: 0.3 (*n*-hexane–EtOAc, 90:10). ^{1}H NMR (400 MHz, $CDCl_3$): 0.81 (3H, s, H-30), 0.85 (3H, s, H-29), 0.87 (3H, s, H-28), δ_H 0.90 (3H, s, H-27), 0.91 (3H, s, H-25), 0.94 (1H, m, H-18), 0.94 (3H, s, H-23), 0.94 (3H, s, H-24), 0.97 (1H, m, H-5), 0.98 (1H, m, H-1), 1.09 (3H, s, H-26), 1.35 (1H, m, H-1), 1.36 (1H, m, H-7), 1.36 (2H, m, H-21), 1.45 (1H, m, H-6), 1.45 (2H, m, H-22), 1.53 (1H, m, H-9), 1.53 (2H, m, H-11), 1.59 (2H, m, H-2), 1.61 (1H, m, H-6), 1.61 (2H, m, H-12), 1.61 (2H, m, H-19), 1.92 (2H, m, H-16), 2.01 (1H, m, H-7), 2.04 (3H, s, $C\underline{H}_3COO$), 4.46 (1H, dd, *J* = 9.6, 5. 2 Hz, H-3), 5.53 (1H, dd, *J* = 7.6, 2.4 Hz, H-15).

^{13}C NMR (100 MHz, $CDCl_3$): 15.7 (C-25), 16.7 (C-24), 17.7 (C-11), 18.8 (C-6), 21.4 ($\underline{C}H_3COO$), 21.5 (C-30), 23.6 (C-2), 26.1 (C-26), 28.1 (C-23), 28.9 (C-20), 30.0 (C-28), 30.1 (C-27), 33.2 (C-7), 33.5 (C-29), 33.8 (C-16), 35.3 (C-21), 35.9 (C-17), 36.8 (C-12), 37.5 (C-22), 37.7 (C-10), 37.8 (C-1), 37.8 (C-13), 38.0 (C-4), 39.1 (C-8), 41.4 (C-19), 48.9 (C-18), 49.3 (C-9), 55.8 (C-5), 81.2 (C-3), 117.1 (C-15), 158.1 (C-14), 171.1 ($\underline{C}OO$).

All-trans-squalene (**3**). *Rf*: 0.9 (*n*-hexane–MC, 70:30).

^{1}H NMR (400 MHz, $CDCl_3$): 1.60 (18H, s, H-24-29), 1.68 (6H, s, H-1, 30), 2.10–1.95 (20H, m, H-4, 5, 8, 9, 12, 13, 16, 17, 20, 21), 5.15–5.08 (6H, m, H-2, 6, 10, 14, 18, 22).

EIMS: $[M]^+$ ion at *m/z* = 410.

Oleic acid (**4**) *Rf*: 0.35 (*n*-hexane–MC, 40:60). ^{1}H NMR (400 MHz, $CDCl_3$): 0.88 (3H, t, *J* = 7.2 Hz, H-18), 1.29 (8H, m, H-4–H-7), 1.29 (12H, m, H-12–H-17), 1.63 (2H, m, H-3), 2.00 (4H, m, H-8, H-11), 2.34 (2H, t, *J* = 7.6 Hz, H-2), 5.34 (1H, m, H-9, H-10), 11.22 (1H, br. s., COO$\underline{H}$).

^{13}C NMR (100 MHz, $CDCl_3$):14.2 (C-18), 22.8 (C-17), 24.8 (C-16), 27.3 (C-11), 27.4 (C-8), 29.2 (C-4), 29.2 (C-6), 29.3 (C-5), 29.5 (C-13), 29.5 (C-15), 29.7 (C-14), 29.8 (C-7), 29.9 (C-12), 32.1 (C-3), 34.3 (C-2), 129.8 (C-9), 130.1 (C-10), 180.7 (C-1). EIMS: $[M]^+$ ion at *m/z* = 282.

1-Hexacosanol (**5**). EIMS: $[M]^+$ ion at m/z = 364. ^{1}H NMR (400 MHz, $CDCl_3$): 0.91 (3H, t, H-26), 1.59 (2H, m, H-3), 1.80 (46H, br. s., H-4–H-25), 3.67 (2H, t, H-1).

***β*-Amyrin** (**6**). MP 200–203°C. *Rf*: 0.43 (*n*-hexane–MC, 40:60). EIMS: *m/z* = 426.

***β*-Sitosterol** (**7**). MP 134–136°C. *Rf*: 0.43 (*n*-hexane–EtOAc, 85:15).

^{1}H NMR (400 MHz, $CDCl_3$): 0.68 (3H, s, H-18), 0.81 (3H, d, *J* = 7.2 Hz, H-26), 0.83 (3H, d, *J* = 7.2 Hz, H-27), 0.84 (3H, t, *J* = 7.0 Hz, H-29), 0.92 (3H, d, *J* = 6.4 Hz, H-21), 1.00 (3H, s, H-19), 3.51 (1H, m, H-3), 5.35 (1H, d, *J* = 5.2 Hz, H-6).

^{13}C NMR (100 MHz, $CDCl_3$): 12.0 (C-18), 12.1 (C-29), 18.9 (C-27), 19.2 (C-21), 19.6 (C-19), 20.0 (C-26), 21.2 (C-11), 23.2 (C-28), 24.5 (C-15), 26.2 (C-23), 28.4 (C-16), 29.3 (C-25), 31.8 (C-7), 32.1 (C-2), 32.1 (C-8), 34.1 (C-22), 36.3 (C-20), 36.7 (C-10), 37.4 (C-1), 39.9 (C-12), 42.4 (C-4), 42.5 (C-13), 46.0 (C-24), 50.3 (C-9), 56.2 (C-17), 56.9 (C-14), 71.9 (C-3), 121.9 (C-6), 140.9 (C-5).

***β*-Sitosterol-3-*O*-*β*-D-glucopyranoside** (**8**). *Rf*: 0.54 (MC–MeOH–H_2O, 80:20:0.1).

^{1}H NMR (400 MHz, DMSO-d_6): 0.66 (3H, s, H-18), 0.79 (3H, d, *J* = 7.2 Hz, H-27), 0.81 (3H, d, *J* = 6.8 Hz, H-26), 0.83 (3H, t, *J* = 7 Hz, H-29), 0.88 (1H, m, H-9), 0.91 (3H, d, *J* = 6.4 Hz, H-21), 0.91 (1H, m, H-24), 0.96 (3H, s, H-19), 0.99 (lH, m, H-17), 1.01 (2H, m, H-1, H-22), 1.05 (1H, m, H-15), 1.09 (lH, m, H-14), 1.14 (1H, m, H-12), 1.17 (2H, m, H-23), 1.32 (1H, m, H-22), 1.34 (lH, m, H-20), 1.39 (2H, m, H-8, H-11), 1.49 (3H, m, H-2, H-28), 1.50 (3H, m H-11, H-15, H-16), 1.63 (lH, m, H-25), 1.80 (3H, m, H-1, H-16, H-28), 1.93 (2H, m, H-7), 1.95 (1H, m, H-12), 2.12 (1H, m, H-4), 2.36 (1H, m, H-4), 3.48 (1H, m, H-3), 5.33 (1H, d, *J* = 4.8 Hz, H-6); glucose; 2.89 (lH, m, H-2′), 3.03 (lH, m, H-4′), 3.06 (lH, m, H-5′), 3.12 (lH, m, H-3′), 3.40 (1H, dd, *J* = 11.6, 5.6 Hz, H-6′), 3.64 (1H, dd, *J* = 11.6, 1.6 Hz, H-6′), 4.22 (1H, d, *J* = 7.6 Hz, H-1′).

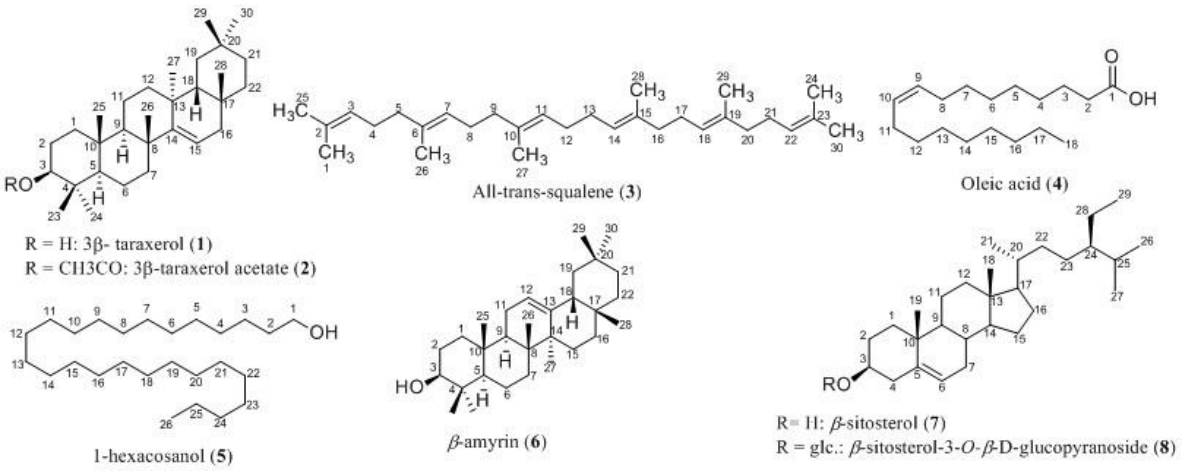

Fig. 1: Structures of isolated compounds.

^{13}C NMR (100 MHz, DMSO-d_6): 11.7 (C-26), 11.8 (C-18), 18.6 (C-21), 18.9 (C-27), 19.1 (C-19), 19.7 (C-29), 20.6 (C-11), 22.6 (C-15), 23.8 (C-23), 25.5 (C-16), 27.8 (C-25), 28.7 (C-2), 29.3 (C-28), 31.4 (C-7), 31.4 (C-8), 33.4 (C-22), 35.5 (C-20), 36.2 (C-10), 36.8 (C-1), 38.3 (C-4), 41.8 (C-12), 41.8 (C-13), 45.2 (C-24), 49.6 (C-9), 55.4 (C-14), 56.2 (C-17), 76.9 (C-3), 121.2 (C-6), 140.4 (C-5); glucose; 61.1 (C-6′), 70.1 (C-4′), 73.4 (C-2′), 76.7 (C-5′), 76.9 (C-3′), 100.8 (C-1′).

3*β*-Taraxerol (**1**, Fig. 1) was obtained from MC fraction as cubic crystals (CH_2Cl_2) (21 mg). The ^{1}H NMR data of **1** revealed the presence of a double doublet at δ_H 5.53 (1H, dd, J = 8, 3.2 Hz) attributable to an olefenic proton. The signal at δ_H 3.18 (1H, dd, J = 11.2, 4.8 Hz) is attributable to an oxymethine proton. Six 3H singlets at δ_H 1.08, 0.98, 0.94, 0.92, 0.82 and 0.80 are assignable to six methyl protons. Another singlet integrating for six protons at δ_H 0.90 is due to protons of two equivalent methyl moieties.

The ^{13}C NMR spectrum showed carbon peaks at δ_C 28.15, 15.58, 15.61, 26.06, 21.47, 29.98, 33.5, 30.08 assignable to eight methyl carbons. The carbon peaks at δ_C 158.22 and 117.02, C-14 and C-15, respectively, agree with the presence of double bond involving quaternary carbon atom. The chemical shift at δ_C 79.22 due to an sp^3 hybridized carbon attached to an OH group is assignable for the C-3. The remaining chemical shifts (experimental section) were consistent with the other carbon atoms of 3*β*-taraxerol in the published data (Ha, 2014).

The EI mass spectrum (supplementary data) showed a molecular ion peak at m/z = 426.30 with fragmentation pattern consistent with the reported fragmentation pattern of pentacyclic triterpes (Shiojima *et al.*, 1992). Compound **1** was identified as 3*β*-taraxerol by comparison of the chromatographic and spectroscopic (^{1}H and ^{13}C NMR and EIMS) with the reported data (Ha, 2014; Koay *et al.*, 2013). This compound is firstly reported from the family Bombacaceae.

3*β*-Taraxerol acetate (**2**, Fig. 1) was obtained from MC fraction as colorless needle crystals, (21 mg). The ^{1}H NMR spectrum of **2** (supplementary data) revealed an olefinic proton signal at δ_H 5.53 (1H, dd, J = 7.6, 2.4 Hz), an oxymethine proton signal (δ_H 4.48, dd, J = 9.6, 5.2 Hz), and eight 3H-singlets (δ_H 1.08, 0.94, 0.94, 0.90, 0.89, 0.87, 0.85, 0.81) closely related to those of 3*β*-taraxerol (**1**). A characteristics signal at δ_H 2.04 (3H, s) indicates the presence of acetyl moiety, which is placed at C-3 because of the resonance of H-3 at more downfield shift (δ_H 4.46) lower than that (δ_H 3.19) of the free hydroxylated analog (3*β*-taraxerol, **1**). The ^{13}C NMR spectrum (supplementary data) showed thirty-two carbon peaks. Among them, the signal at δ_C 171.08 was attributed to the carbonyl of the acetate function (Mahato and Kundu, 1994). Placement of the acetate group at C-3 was also supported by the downfield shift of the C-3 carbon (δ_C 81.17) instead of (δ_C 79.1) of taraxerol (Muithya, 2010). The ^{13}C NMR spectrum also showed signals at δ_C 158.12 and δ_C 117.08 due to double bond functionality, C-14, and C-15, respectively. The spectrum also showed eight carbon peaks at δ_C 15.65, 16.74, 21.47, 26.07, 28.13, 29.98, 30.07 and 33.3, diagnostic for the eight methyl carbons of the pentacyclic triterpenoidal skeleton.

The ^{1}H and ^{13}C NMR data of **2** are identical to the published data of 3*β*-taraxerol acetate (Sasaki *et al.*, 1965). It is reported from the genus *Ceiba* for the first time.

All-trans-squalene (**3**, Fig. 1) was obtained as pale yellow oily residue, (9 mg). The EIMS spectrum (supplementary data) showed a molecular ion peak at m/z 410 [M$^+$] and diagnostic ion peaks at m/z = 367 [M$^+$- C_3H_7], 341[M$^+$- C_5H_9] and a base peak at m/z = 69. Other significant fragment ions observed were at m/z 81, 83, 95, 107, 121, 136. In general, this mass ionization pattern indicates a 410 molecular mass compound of $C_{30}H_{50}$ formula which is the same as those for all-trans-squalene (**3**) (Dagan and Amirav, 1995). The ^{1}H NMR spectrum of **3** (supplementary data) showed the presence of signals of 6 olefinic protons at δ_H 5.15–5.08 (6H, m), cluster of 10 methylene proton signals at δ_H 2.09–1.97 (20H, m), two equivalent methyl signals 1.68 (6H, s), and 6 equivalent methyl signals 1.60 (18H, s) which are the same as those reported in literature for all-trans-squalene (Pogliani *et al.*, 1994). It is reported from the genus *Ceiba* for the first time.

Oleic acid (**4**, Fig. 1) was obtained as a colorless liquid, (15 mg). The EIMS spectrum (supplementary data) of **4** showed weak [M]$^+$ ion at m/z = 282 and an ion representing the loss of the elements of H_2O from a carboxyl group ([M−18]$^+$, m/z = 264). The ^{1}H NMR data (experimental section) indicated the presence of a methyl moiety (δ_H 0.88, 3H, t, J = 7.2 Hz), cluster of CH_2 groups (δ_H

1.26–1.32, 20H, br. s.), a CH_2 group neighbor to carboxylic moiety (δ_H 2.34, 2H, t, J = 7.6 Hz), two olefinic protons δ_H 5.34 (2H, m) and a carboxylic group (δ_H 11.22, 1H, br. s.). These were further confirmed from the ^{13}C NMR spectrum (supplementary data) by the presence of corresponding carbon peaks at δ_C 14.23, δ_C 22.83–34.27, δ_C 129.83, 130.13 and δ_C 180.72, respectively. From the above-mentioned data, which agree with the previously published data (Sahu *et al.*, 2013), the compound was identified as oleic acid. It was previously reported from *C. pentandra* species (Anwar *et al.*, 2014).

1-Hexacosanol (= ceryl alcohol, **5**, Fig. 1), was obtained as white flakes (MeOH), (12 mg). The EIMS spectrum (supplementary data) of **5** exhibited fragment ion peaks (*m/z* = 364 and 336) consistent with the loss of H_2O and $[CH_2{=}CH_2]^+$ from a long chain alcohol (Dass, 2007). The examination of the rest of the fragmentation pattern showed an even cascade of [M-14] (CH_2^+) characteristic for saturated linear hydrocarbons. The fragments observed are consistent with the expected fragmentation of a long chain aliphatic alcohol with the molecular weight of 382 and by comparison with the reported data it found to be identical for ceryl alcohol (Joshi and Poudel, 2013). The ^1H NMR spectrum of **5** (supplementary data) showed: A triplet signal at δ_H 0.91 (3H) accounted for one terminal methyl protons; a big broad singlet signal at δ_H 1.80 (46H) accounts for protons of CH_2 units; a triplet signal at δ_H 3.67 (2H) accounts for the protons of CH_2 unit attached with electronegative hydroxyl group; a multiplet signal at δ_H 1.59 (2H) assignable for the CH_2 beta for the hydroxyl group. It thus could be concluded that compound **5** is 1-hexacosanol (ceryl alcohol) which is first time reported from the genus (Refaat *et al.*, 2013).

***β*-Amyrin** (**6**, Fig. 1) was obtained as white fine needle crystals, (9 mg). Upon co-chromatography with authentic triterpenes it showed *Rf* value of 0.43 in *n*-hexane-MC (40:60, *v/v*) and 0.56 in *n*-hexane-EtOAc (95:5, *v/v*), and attained reddish brown color with spray reagent A, and violet color with Liebermann-Burchard's test (Harborne, 1998) which are the same as *β*-amyrin. The EI mass spectrum of **6** (supplementary data) showed a molecular ion peak at *m/z* = 426 corresponds to molecular formula $C_{30}H_{20}O$, agree with *β*-amyrin (Ercil *et al.*, 2004). The fragment ion peaks *m/z* = 218.2, 203, 189, 175.1, 135.1, 95.1 and 55.1 are also typical that of *β*-amyrin. It is the first report for the isolation of *β*-amyrin from the species.

***β*-Sitosterol** (**7**, Fig. 1) was obtained as white needle-like crystals (ether), (70 mg). The co-chromatography with authentic sterols suggests that **7** is *β*-sitosterol. The EIMS spectrum of **7** (supplementary data) showed a molecular ion at *m/z* 414, corresponding to molecular formula ($C_{29}H_{50}O$) of *β*-sitosterol. The ^1H NMR data (experimental section) revealed the characteristic signals of *β*-sitosterol as follow: A doublet signal at δ_H 5.35 (d, J = 5.2 Hz) due to the olefinic proton at C-6. A multiplet 1H signal at δ_H 3.52 assignable for the oxymethine proton of C-3. Two singlets at δ_H 1.00 and 0.68 assignable for methyl groups of C-19 and C-18, respectively. The ^{13}C NMR spectrum (supplementary data) showed the presence of 29 carbons (experimental section) as follow, six methyls (δ_C 19.97, 19.55, 19.18, 18.93, 12.13 and 12.01), eleven methylenes (δ_C 42.44, 39.92, 37.4, 34.09, 32.05, 31.8, 28.4, 26.22, 24.45, 23.21 and 21.23), nine methines (δ_C 121.85, 71.94, 56.91, 56.2, 50.27, 45.97, 36.29, 32.06 and 29.29) and three quaternary carbons (δ_C 140.89, 42.46 and 36.65). Based on these spectroscopic data and co-chromatography with the authentic sample, the compound **7** identified as *β*-sitosterol (Patra *et al.*, 2010). It was previously isolated from the species (Ngounou *et al.*, 2000).

***β*-Sitosterol-3-*O*-*β*-D-glucopyranoside** (**8**, Fig. 1) was obtained as a white granular powder (MeOH), (29 mg). The ^1H NMR data showed six methyl groups at δ_H 0.66 (3H, s), 0.83 (3H, t, J = 7 Hz), 0.81 (3H, s), 0.79 (3H, s), 0.96 (3H, s) and 0.91 (3H, d, J = 6.4 Hz) indicating a phytosterol skeleton (Zhao, 2014). The presence of a distinctive olefinic signal at δ_H 5.33 (1H, d, J = 4.8 Hz) and olefinic carbons at δ_C 121.15 (C-6) and 140.43 (C-5) suggests the presence of double bond. A sugar unit was evidenced from an anomeric proton at δ_H 4.22 (1H, d, J = 7.6 Hz) with large coupling constant which pointed out the *β*-configuration of the glycosidic bond (Bezerra de Sá de Sousa Nogueira *et al.*, 2013). This was confirmed by appearing of anomeric sugar carbon at δ_C 100.79 in its ^{13}C NMR.

^{13}C NMR spectrum of compound **8** indicated the presence of 35 carbons among them 6 signals (δ_C 100.79, 76.94, 76.71, 73.44, 70.08, 61.08) are attributable to the glucose moiety. From the above-mentioned data, were in good agreement with published literature and co-chromatography with the authentic sample, the compound **8** was identified for *β*-sitosterol-3-*O*-*β*-D-glucopyranoside (Saeidnia *et al.*, 2011). Finally, acid hydrolysis of compound **8** gave *β*-sitosterol (direct authentication and co-chromatography; *Rf* value of 0.43 in *n*-hexane-EtOAc (85:15, *v/v*) and glucose (P.C. alongside authentic sugars; *Rf* value of 0.19 in *n*-Butanol–Acetic acid–H_2O (4:1:5, *v/v/v*). Compound **8** previously isolated from the species (Ngounou *et al.*, 2000).

Computer-based prediction of biological activities of isolated compounds

Prediction of Activity Spectra for Substances (PASS ver. 4.20) predicts over 4000 kinds of biological activities, including pharmacological effects, mechanisms of action, toxic and adverse effects, interaction with metabolic enzymes and transporters,…. etc. To predict biological activity profile for a compound, only structural formula is necessary thus, the prediction is possible even for compounds identified by hyphenated techniques as GC-MS or LC-MS without the need for its separation or isolation. It is reasonable that only those types of activities of compounds whose Pa > Pi and Pa > 0.700 are very likely to have the selected activity in experiments (Ramakrishnan, 2010).

Herein, we investigated the pharmacological activities of the isolated compounds from MC fraction by PASS (ver. 4.20) computer program. The results (Table S1, Supplementary data) revealed antitumor treatment and/or prevention of some (**1**–**3** and **6**) of the isolated compounds **1**–**8**. The main predicted activities with great pa values of the **1**–**3** and **6** compounds are antineoplastic, apoptosis agonist and proliferative diseases treatment. This provoked us to investigate MC fraction of *C. pentandra* aerial parts for its cytotoxic activity as one of the possible antineoplastic mechanisms.

Cytotoxic activity of *C. pentandra* (L.) Garten. var. *pentandra*:

The MC fraction of *C. pentandra* was tested for its

cytotoxic activity against HepG2 and MCF-7 cell lines using SRB method assay. The 50% inhibitory concentration (IC_{50}) was determined from the dose-response curve (Fig. 2). The results revealed that the MC fraction of *C. pentandra* exhibited clear cytotoxic activity against the two tested cell lines. The IC_{50} values against the cancer cell lines (HepG2 and MCF-7) were 14.895 µg/mL and 18.859 µg/mL, respectively, which is acceptable level of activity according to the national cancer institute (NCI) statement; plant extracts with cytotoxic values $IC_{50} \leq 30$ µg/mL are considered active (Alonso-Castro *et al.*, 2011). The experimental results agreed with the results of the in silico study (Table S1, supplementary data) and worthy need further experimental investigation to explore their mechanism of action.

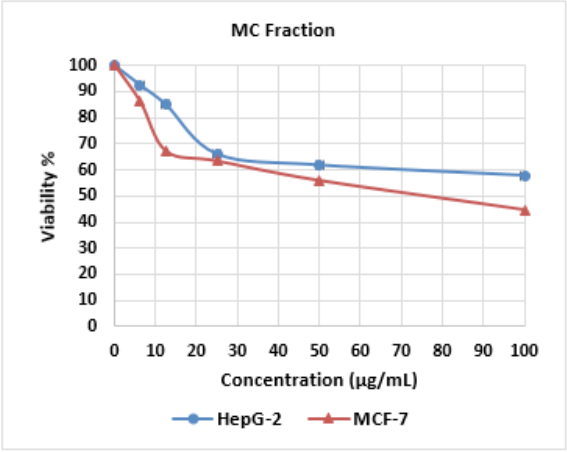

Fig. 2: Dose-cytotoxicity curves of MC fraction of *C. pentandra* aerial parts on HepG-2 and MCF-7 cell lines.

Previous studies concerning the antitumor activity of the *Ceiba pentandra* extracts, and many of our identified compounds corroborate our findings as follows: The bark extracts, namely petroleum ether, acetone, and ethanol extracts have prominent short-term cytotoxic effect on Ehrlich ascites carcinoma (EAC) cells and long-term cytotoxic effect on human breast cancer cell line (MCF-7) and melanoma cell line (B16F10) (Kumar *et al.*, 2016). The diterpene alcohol phytol which represents about 14.99% of the identified terpene compounds in our examined extract was previously showed a promising cytotoxic activity against MCF-7 (Pejin *et al.*, 2014) and enhanced the apoptotic mechanism (Komiya *et al.*, 1999). Moreover, the conjugated dienoic fatty acid linoleic acid and the trienoic fatty acids α-linolenic acid which are among the major fatty acids identified here have also reported producing cytotoxic effects on human tumor cells (Igarashi and Miyazawa, 2000).

Taraxerol and taraxerol acetate have potent anti-tumor effects through the induction of apoptosis, autophagy, inhibition of cell migration and cell cycle arrest (Hong *et al.*, 2016; Yao *et al.*, 2017). Moreover, squalene was reported to exhibit anti-tumor, cytotoxic properties against colon and breast cancer (Mariquit *et al.*, 2017) and chemopreventive activities against colon carcinogenesis. β-sitosterol also was reported to inhibit tumor promotion in two stages skin carcinogenesis in a mouse model (Zakaria *et al.*, 2017).

CONCLUSIONS

The current study shed the light on the probability of developing a new antitumor drug from *C. pentandra* or implementing its constituents in pro-drug formulations in nanoparticles. The pure isolated compounds (**1**–**3** and **6**) showed predicted antineoplastic, apoptosis agonist and proliferative diseases treatment. Other predicted biological activities of the compounds are worthy need further experimental investigation.

SUPPLEMENTARY DATA

Electronic Supplementary Material associated with this article can be found in the online version of this paper.

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