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Highlights

- In this study seven previously reported compounds (**1-7**) were isolated and identified from the root extract of *Euphorbia grandicornis* for the first time.
- Isolated compounds were evaluated for cytotoxic activities against MCF-7, HCC70 and MCF-12A cell lines.
- Of the pure compounds isolated from the root extract, only hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**) exhibited significant toxicity against MCF-7 (IC_{50}=23.41 µM), HCC70 (29.45 µM) and MCF-12A (27.01 µM).

In vitro cytotoxic effects of chemical constituents of *Euphorbia grandicornis* Blanc against breast cancer cells

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Abstract

Euphorbia grandicornis Blanc is widely utilized in traditional medicine for a variety of ailments including body pains associated with skin irritations, inflammation, and snake or scorpion bites. Compounds from *E. grandicornis* were characterized using spectroscopic techniques, NMR, IR, MS, and melting points and alongside the extracts were evaluated for *in vitro* anticancer activity against several cancer cell lines. The root extract afforded known, β-glutinol (**1**), β-amyrin (**2**), 24-methylenetirucalla-8-en-3β-ol (**3**), tirucalla-8,25-diene-3β,24*R*-diol (**4**), stigmasterol (**5**), sitosterol (**6**), and hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**) based on their NMR spectroscopic data for the first report in *E. grandicornis*. The extracts and isolated compounds were evaluated for anticancer activities against hormone receptor-positive breast cancer (MCF-7), triple-negative breast cancer (HCC70), and non-tumorigenic mammary epithelial (MCF-12A) cell lines. The CH_2Cl_2 extract exhibited potent, cytotoxicity against MCF-7, HCC70, and MCF-12A cells. The aerial extract exhibited IC_{50} values of 1.03, 0.301, and 1.68 µg/mL, and root extract displayed IC_{50} values of 0.83, 0.83 and 3.98 µg/mL against MCF-7, HCC70, and MCF-12A cells respectively. The root extract thus showed selectivity for the cancer cell lines over the non-cancerous control cell line (SI = 4.80). Hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**) showed significant activity with IC_{50} values of 23.41, 29.45 and 27.01 µM against MCF-7, HCC70 and MCF-12A cells respectively, suggesting non-specific cytotoxicity.

Keywords

Euphorbia grandicornis; chemical constituents; cytotoxic activities; MCF-7; HCC70; MCF-12A

Introduction

Cancer is a global health burden affecting every region and social-economic group [1]. Among females, breast cancer is the most frequently diagnosed cancer and the leading cause of cancer-related deaths [1]. The 2018 estimates from Global Cancer statistics (GLOBOCAN) show that there were over 18 million incidences of cancer and over 9.2 million deaths. These numbers were predicted to increase to over 21 million cases by 2030, with less developed countries accounting for approximately 57% of new cases and 65% of cancer-related deaths [1].

Breast cancers are characterized by a high degree of molecular, functional, and morphological heterogeneity [2]. Breast cancers are classified according to the presence or absence of key hormonal receptors which include estrogen (ER), progesterone (PR), or human epidermal growth factor-2, (HER2) receptor. Approximately 10-24% of breast cancers lack all three of these receptors and are termed triple-negative breast cancer (TNBC) [3-5]. Interestingly, the TNBC subtype appears to be common among women of African descent compared to Caucasians [3]. TNBC represents a major challenge for clinicians because these cancers do not respond to available targeted hormonal therapies due to lack of expression of PR, ER, and HER2, which are used as predictive and prognostic markers [3]. Positive expression of these receptors in breast cancers are the basis of many targeted therapies, such as trastuzumab and tamoxifen, which improve prognosis and outcome and reduces the adverse side effects that are consistent with nonspecific chemotherapy [3].

Due to the lack of specific therapeutic agents for the management of TNBCs, this form of cancer is treated with comparatively less effective generic cytotoxic agents [3, 6]. In addition, while non-specific chemotherapy remains the most widely used treatment option for cancers, the available drugs are frequently limited with toxicity and increased frequency of tumor recurrence. When the TNBCs patients do not achieve complete recovery from these carcinomas, there is a higher risk of deterioration or recurrence as compared to patients having positive receptor cancers [3]. There is therefore an increasing need to discover lead compounds from medicinal plants that can be potential candidates for TNBCs therapeutics.

Euphorbia plant species are used in traditional medicine for relieving body pains associated with skin irritations, inflammation, and snake or scorpion bites. *Euphorbia* species are reported to exhibit anti-microbial and anti-inflammatory effects [7-9]. Reports demonstrated that extracts possess a wide range of pharmacological activities including anticancer properties *in vitro* due to the presence of various bioactive compounds [10-13]. Among these constituents, macrocyclic diterpenes and triterpenoids are the major secondary metabolites.

Triterpenoids including tirucallane [11], euphane [12], cycloartane [13-14], and oleanane [13, 15] subclasses were evaluated for anticancer activities against estrogen-sensitive cells (MCF-7) and against TNBC cells (MDA-MB 468) cells. In particular, 3β,25-dihydroxycycloart-23-(*E*)-ene isolated from *E. macrostegia* showed significant activities with LD_{50} value of 2.05 µg/mL and 5.40 µg/mL against MDA-MB-468 and MCF-7 cells respectively, while 3β,25-dihydroxycycloart-23-(*Z*)-ene exhibited anticancer activity against MCF-7 and MDA-MB-468 cells with LD_{50} of 8.90 µg/mL and 34.00 µg/mL respectively [14]. In addition, (24*R*)-tirucalla-8,25-diene-3β,24-diol isolated from *E. micractina* displayed moderate activities against MCF-7 cells with IC_{50} of 56.20 µM [11]. Lupenone from *Artocarpus integra* [15] and 11β-hydroperoxyeupha-8, 25-diene-3β-ol from *E. resinifera* [12] showed moderate anticancer activities with IC_{50} of 37.36 and 8.07 µg/mL respectively against MDA-MB-468 and MCF-7 cells.

However, despite the reported evidence indicating that TNBC is prevalent among women of African descent and continued utilization of *E. grandicornis* in traditional medicine in Africa, no anticancer activities of *Euphorbia* triterpenoids have been evaluated against triple-negative breast cancer cells derived from women of African origin. Furthermore, there is no report on the phytochemistry of root extract of *E. grandicornis* previously. In search of new lead compounds for drug discovery, the current research focused on the evaluation of cytotoxic activities of chemical constituents isolated from root and aerial extracts of *E. grandicornis* against MCF-7, HCC70, and MCF-12A.

Methodology

General

Column chromatography was performed with SiO_2 (Kieselgel-60 GF_{254}, 15 μm, 100−200 mesh Merck, Germany) on polyamide columns (5 x 60 cm, 200 g) (Germany GmbH). While Thin Layer Chromatography (TLC) was carried out on Kieselgel-60 F_{254} (Merck). Analytical thin-layer chromatography (TLC) was done on silica gel plates (Kieselgel-60 F_{254} (Merck) and was visualized by exposure to short and long ultraviolet light at 245 nm and 336 nm respectively and stained using concentrated sulphuric acid-anisaldehyde spray mixture followed by heating at 105 °C for 2 minutes. Solvents used for column chromatographic analysis were of an analytical grade and purchased from Merck and Sigma.

Varian Unity-Inova 400 MHz (400.13 MHz; 100.62 MHz) spectrometer at 25 °C was used to record ^{1}H, and ^{13}C NMR. Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane. Spin-spin coupling constants (*J*) were expressed in Hz and other data were reported as follows: s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet, and br s = broad singlet. Deuterated chloroform ($CDCl_3$) was used to dissolve all the compounds. Melting points were determined on an Ernst-LeitzWetziar melting point apparatus. Infrared Spectroscopy (IR) spectra were measured using Perkin-Elmer spectrometer, version 10.54. The infrared absorptions were recorded in wavenumbers (cm^{-1}) to determine functional groups at the highest frequencies.

Specific optical rotations $[\alpha]_D$ were analyzed on a Jasco P-2000 Polarimeter (JASCO, Germany). The angle of rotation α was measured at 200 ± 0.50° in a solution ($CDCl_3$) of the samples and expressed in the degree of the plane of polarization at the wavelength of the D-line of sodium (λ = 546.3 nm).

The high-resolution LC-MS data were acquired using a Bruker Daltonics Compact QTOF Mass Spectrometer with an electrospray ionization probe in positive mode (ESI^{+}). The Mass Spectrometer was coupled to a Thermo Scientific Ultimate 3000 Dionex UHPLC system consisting of an RS Auto Sampler WPS-3000, Pump HPG-3400 RS and detector DAD-3000 RS, using an Acclaim RSLC 120, C18, 2.2 μm, 2.1 x 100 mm (P/N 068982) column at 40 °C, flow rate 0.2 mL/min, solvent: Water-Acetonitrile (10:90, v/v) each solvent containing 0.1% of formic acid, isocratic condition, 5 min run.

Plant Material

The whole fresh plants (15.0 kg) of *E. grandicornis* were purchased from wildflower wholesale nursery, Limpopo province, South Africa (S 05°04.579′ E 043°35.035′) in November 2017 and identified at South African National Biodiversity Institute (SANBI) where the voucher specimen was deposited (voucher number 18044).

Extraction and isolation

The fresh aerial portion (969.72 g) and root (800.34 g) of *E. grandicornis* were cut, dried, grounded, and separately percolated onto 2.5 L of CH_2Cl_2 at 25°C to give 25.13 g and 33.85 g of crude extracts of the aerial and root, respectively. The crude CH_2Cl_2 extracts were then concentrated under reduced pressure on a rotary evaporator and the residue was soaked in ethanol for successive extraction to obtain 5.14 g (aerial) and 4.6 g (roots) ethanol extracts. CH_2Cl_2 extracts of roots and aerial were separated on a SiO_2 gel column and eluted gradually with an *n*-hexane–EtOAc solvent system (9:1 v/v) to remove fatty acid. Based on TLC analysis, the root fractions were purified using SiO_2 gel column and eluted with a step gradient employing *n*-hexane–EtOAc (9:1-0:5 v/v) to obtain ten different fractions (FA-FJ). Fraction FB contained two major compounds with Rf values of 0.34 and 0.35 alongside other minor compounds when developed with *n*–hexane- CH_2Cl_2 (1:1 v/v). This fraction was re-crystallized out in ethanol to give a white amorphous powder, which was purified on a SiO_2 gel column and eluted with *n*-hexane–EtOAc (8:2 v/v) to afford white crystals of glutinol, **(1)**, 11 mg and β-amyrin **(2),** 7 mg. The mother liquor was evaporated and purified by repeated column chromatographic analysis over SiO_2 gel column (3 x 60 cm, 50 g) using *n*-hexane–EtOAc (9:1, 7:3 and 5:5 v/v) to yield white colourless needles of tirucalla-8,25-diene-3β,24*R*-diol **(4)**, 10 mg, white powder of stigmasterol (**5**), 13 mg, and white crystals of sitosterol (**6**), 18 mg. Further purification of this fraction afforded 24-methylenetirucalla-8-en-3β-ol, 8 mg (**3**). Fraction FA was purified on SiO_2 gel eluted with *n*-hexane–EtOAc (9:1 v/v) to yield a colourless oil, 7 mg of hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**).

Physico-chemical properties of isolated compounds

Glutinol **(1),** white crystals, mp: 212-214 °C, $[\alpha]_D^{25}$ +53.85 ($CHCl_3$, c: 0.85). HR-ESI-MS; at *m/z* 426.3862, calculated for $C_{30}H_{50}O$, $[M+Na]^+$ 449.1478, IR; 2936 cm^{-1}, 2862 cm^{-1}, 1646 cm^{-1}, 3459 cm^{-1}.

β-*amyrin* (**2**), white crystals, mp: 194-197 °C, $[\alpha]_D^{25}$ +80.3 ($CHCl_3$, c: 0.52). HR-ESI-MS; *m/z* 426.3862, calculated for $C_{30}H_{50}O$, $[M+Na]^+$ 449.1426, IR; 3434 cm^{-1} (OH), 2920 cm^{-1}, 2858 cm^{-1}, 1706 cm^{-1}.

*24-Methylenetirucalla-8-en-3*β*-ol* (**3**), colourless needles, $[\alpha]_D^{25}$-1.12 ($CHCl_3$ *c*, 0.05), mp; 125-127 °C. HR-ESI-MS; at *m/z* 440.4018, calculated for $C_{31}H_{52}O$, $[M+H]^+$ 441.1732, IR 3372 cm^{-1}, 2931 cm^{-1}, 2854 cm^{-1}, 1643 cm^{-1}.

*Tirucalla-8,25-diene-3*β*,24R-diol* (**4**): Colourless needles, mp; 120-124°C, $[\alpha]_D$ -1.12 ($CHCl_3$ *c*, 0.9), IR; 3360 cm^{-1}, 2921 cm^{-1}, 2854 cm^{-1}, 1716 cm^{-1}. HR-ESI-MS; at *m/z* 442.3811 calculated for $C_{30}H_{50}O_2$, $[M+H]^+$ 443.1825.

Stigmasterol (**5**), white powder, mp: 165-168 °C, $[\alpha]_D^{25}$ 0.24 ($CHCl_3$, c: 0.05), HR-ESI-MS; *m/z* 412.3705, calculated for $C_{29}H_{48}O$, $[M+H]^+$ 413.2688, IR; 3426 cm^{-1}, 1464 cm^{-1}, 2929 cm^{-1}.

Sitosterol (**6**), white crystals, mp: 130-133 °C, $[\alpha]_D^{25}$ +59.4 ($CHCl_3$, c: 0.22). HR-ESI-MS; *m/z* 414.3862, calculated for $C_{29}H_{50}O$, $[M+Na]^+$ 437.2388, IR; 3453 cm^{-1}, 2921 cm^{-1}, 1660 cm^{-1}.

Hexyl (E)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**), colourless oil, $[\alpha]_D^{25}$-1.23 ($CHCl_3$ *c*, 0.05), mp; 470-473°C, HR-ESI-MS; *m/z* 278.1518, calculated for $C_{29}H_{48}O$, $[M+H]^+$ 279.3121, IR 1710 cm^{-1}, 1096 cm^{-1}, 2991 cm^{-1}, 1428 cm^{-1}.

Cytotoxic Assay

The MTT assay

The cell viability of HCC70 TNBC cells (ATCC: CRL-2315) and non-tumorigenic breast epithelial MCF12A cells (ATCC: CRL-10782) after treatment with the extract and compounds from the root of *E. grandicornis* were assessed using the (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay [16]. The cells were seeded at 5000 cells/well in a 96

well plate and were left overnight in a 9 % CO_2 incubator at 37 °C to adhere. Cells were then treated with either the isolated pure compounds or crude extracts at a concentration ranging from 15.625 to 500 µM, and 0.032-100 µg/mL, respectively, or a 2% v/v DMSO vehicle control for 9 hours at 37 °C in a 9 % CO_2 incubator. Thereafter, into each well, a solution of 10 µL of a 2.5 mg/mL MTT solution was added, followed by incubation for 4 hours. Solubilization solution (10% (w/v) SDS in 0.01 M HCl) was then added overnight. The absorbance of the formazan purple dye was then measured at 570 nm using a Power wave spectrophotometer (BioTek) referenced at a wavelength of 630-690 (nm). The experiment was repeated in technical triplicate and the data was analyzed using GraphPad Prism Inc, (USA) with half-maximal inhibitory concentrations (IC_{50} values) determined by non-linear regression.

Resazurin assay

The resazurin assay was conducted according to Mbaba, [17] to assess the effect of the above extracts and purified compounds on MCF-7 hormone receptor-positive (ER^+, PR^+, $HER\text{-}2^-$) breast cancer cells (ATCC: HTB-22). Cells were seeded at 5000 cells/well in a 96 well plate and were left to adhere in a 9% CO_2 incubator overnight at 37 °C. Thereafter, the cells were treated with the pure compounds at a concentration ranging from 15.625 to 500 µM or a 2% v/v DMSO vehicle control for 96 hours at 37 °C in a 9% CO_2 incubator. Following treatment, 0.54 nM resazurin solution was added and the plate was incubated as before for 2-4 hours. The fluorescence was then measured using a Spectramax spectrophotometer set at an excitation wavelength of 560 nm and emission wavelength of 590 nm. The experiment was done in technical triplicate and the data was analyzed using GraphPad Prism Inc, (USA) with IC_{50} values determined by non-linear regression.

Results and discussion

As part of our research findings, repeated column chromatography of *Euphorbia grandicornis* root extract afforded seven compounds isolated from this species for the first time (Figure **1**). The ^1H, ^{13}C, FTIR and HR-MS data for the isolated compounds; β-glutinol (**1**), β-amyrin (**2**), 24-methylenetirucalla-8-en-3β-ol (**3**), tirucalla-8,25-diene-3β,24*R*-diol (**4**), stigmasterol (**5**), sitosterol (**6**), and hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**) are given in Tables S1 and S2.

β-glutinol (**1**) was obtained as a white powder, with a molecular ion $[M+Na]^+$ peak at *m/z* 449.1478 corresponding to a molecular formula $C_{30}H_{50}O$. Analysis of the IR spectrum displayed absorption bands at 2936 cm^{-1} and 2862 cm^{-1} assigned to C-H stretches, and bands at 3459 cm^{-1} were attributed to hydroxyl stretches. The ^1H NMR spectrum displayed characteristics peaks of a triterpenoid with eight methyl signals at δ_H 0.83 (3H, *s*), 0.89 (3H, *s*), 0.97 (3H, *s*), 0.97 (3H, *s*), 1.02 (3H, *s*), 1.09 (3H, *s*), 1.11 (3H, *s*) and 1.15 (3H, *s*) ppm. The spectrum showed partially overlapped signals between δ_H 1.22-2.01 attributed to methine and methylene groups (23H). This was supported by ^{13}C NMR which exhibited the resonances of 30 carbons atoms that were resolved by DEPT into seven quaternary carbons, five methine, ten methylene, and eight methyl carbons as in Table S1 and S2. The stereochemistry at C-3 was determined by the presence of cross-peaks between H-24 (1.11 ppm) and H-3 (3.49 ppm). The assignment confirmed the hydroxyl group as β-OH. The structure of compound (**1)** was further confirmed by comparison of the NMR experimental data with the literature values for glutinol (**1**) [13] previously isolated from *E. chamaesyce* and *E. alatavica* [18].

Likewise, β-amyrin (**2**), gave a molecular $[M+Na]^+$ ion peak at *m/z* 449.1426 which corresponded to a molecular formula $C_{30}H_{50}O$ consistent with the calculated degree of unsaturation of six. The ^1H NMR spectrum exhibited olefinic proton at 5.18 (1H, *t, J* =3.5 Hz, H-12) ppm and an oxymethine proton at δ_H 3.21 (1H, *dd, J* = 12.0, 3.5 Hz, H-3) ppm. The proton and carbon resonances were further assigned as shown in Table **S1** and **S2**. Analysis of the NOESY spectrum showed in space correlation between H-3 (3.22 ppm) and H-24 (0.77 ppm) which helped to confirm the β-OH orientation [13]. Also, the NOESY cross-peaks observed between H-25 (0.90 ppm) and H-26 (0.99 ppm) indicated a similar configuration. The structure was confirmed by comparison to literature values for β-amyrin (**2**), previously isolated from *E. hirta* [13].

The ^1H NMR spectrum of 24-methylenetirucalla-8-en-3β-ol (**3**) showed olefinic methylene signals at δ_H 4.63 (brd, *s*) and an oxymethine at 3.07 (*d, J* = 6.0) ppm and displayed methyl signals at δ_H 0.67 (3H, *s*), 0.97 (3H, *s*), 1.03 (3H, *s*), 0.99 (3H, *s*), 0.80 (3H, *s*), and 0.92 (3H, *s*) ppm, in addition to partially overlapped multiplets due to aliphatic methylenes and methines between δ_H 1.20 and 2.10 ppm as in Table **S2**. This was in agreement with the ^{13}C NMR and DEPT spectra which exhibited the signals of 31 carbons, including seven quaternary, five methine, twelve methylene, and seven methyl carbons as in Table **S1**. Analysis of NOESY

spectra exhibited correlation between H-3 (3.09 ppm) and H-2 (1.70 ppm). The coupling constant (10.5 Hz) between H-3 (3.09 ppm) and H-2 (1.70 ppm) suggested the β-orientation of the hydroxyl group. Based on the NMR experimental data and the reported values, the compound was identified as 24-methylenetirucalla-8-en-3β-ol (**3**), previously isolated from *E. micratina* [19].

The HR-ESI-MS molecular ion at *m*/*z* 442.3811 of compound (**4**) indicated that the compound has a molecular formula of $C_{30}H_{50}O_2$ (calculated for $[M+H]^+$ 443.1825). The ^1H NMR spectrum showed signals due to olefinic methylene at δ_H 4.72 (*brs*, H-26) and an oxymethine at δ_H 3.12 (*brs*) ppm. In addition, it displayed methyl signals at δ_H 1.24 (H_3-27), 0.88 (H-18), 0.97 (H-19), 1.01 (H-28), 0.74 (H-29), and 0.89 (H-30), and 0.96 (J=6.4 Hz, H_3-21) ppm. The ^{13}C NMR exhibited the signals of 30 carbons, including seven quaternary, five methine carbons, eleven methylene, and seven methyl carbons as in Tables **S1** and **S2**. In the NOESY experiment, irradiation of H-3 (3.12 ppm) enhanced H-2α (1.71 ppm) and H-2β (1.83 ppm). This together with the calculated coupling constants between H-3 (3.12 ppm) and H-2α (1.71 ppm, 5.2 Hz), H-2β (1.83 ppm, 9.2 Hz) suggested the equatorial position of the hydroxyl group. The experimental data compared well with the literature values and the compound was identified as (24*R*)-tirucalla-8,25-diene-3β,24-diol (**4**) previously isolated from *E. micratina* [20-21].

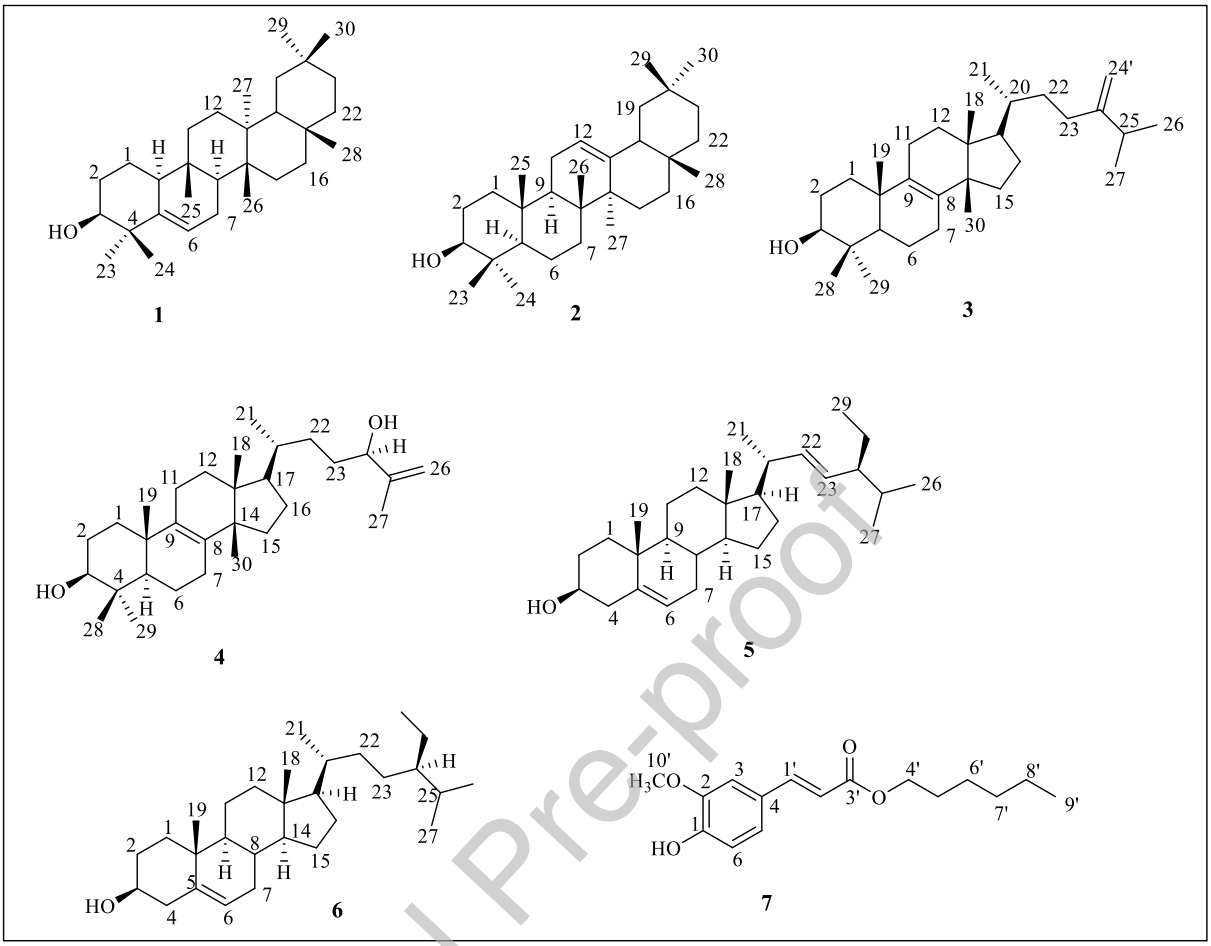

Fig. 1. Structures of *Euphorbia grandicornis* chemical constituents

Stigmasterol (**5**) exhibited a pseudo-molecular ion $[M+H]^+$ peak at *m/z* 413.2688 (calculated for *m/z* 412.2776) and corresponding to a molecular formula $C_{29}H_{48}O$. The ^1H NMR ($CDCl_3$) displayed characteristic peaks of stigmasterol, displaying three olefinic proton resonances at δ_H 5.17 (*dd*, *J*= 8.60 Hz, 15.8 Hz, H-22) ppm, 5.04 (*dd*, *J* = 8.50 Hz, 15.0 Hz, H-23) and 5.34 (b*d*, *J* = 5.40 Hz, H-6) ppm together with deshielded methine resonance at δ_H 3.51 (*m*, H-3) ppm due to the hydroxyl group. The stereochemistry was confirmed by analysis of the NOESY spectrum. NOESY cross-peaks (*J*-coupling) between H-23 (5.04 ppm) and H-24 (1.56 ppm) were confirmed. NOESY cross-peaks between H-3 (3.51 ppm), H-4 (1.56 ppm), and H-19 (1.01 ppm) helped to deduce the β-OH configuration. Based on the spectral data and reported literature values, compound **5** was identified as stigmasterol (**5**) [22]. Likewise, the structure of compound (**6)** was elucidated and identified based on analysis of 1D and 2D NMR spectroscopy and confirmed by comparison of their experimental values to the literature data for sitosterol (**6**)

[22] **(**Figure 1). Previously, compounds **5** and **6** were isolated as a mixture from the root extracts of *E. hirta* [13].

Hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**) was obtained as a colourless oil, with IR spectrum displaying absorptions bands at 1711 cm^{-1} which revealed the presence of a carbonyl group (C = O) of an ester close to the aromatic or benzene environment, and absorptions at 1170 cm^{-1} due to C-O stretches. The HR-ESI-MS showed a pseudo molecular ion $[M+H]^+$ peak at *m/z* 279.3121 (calculated for *m/z* 278.1318) and corresponding to a molecular formula $C_{16}H_{22}O_4$. The ^1H NMR further displayed the characteristic singlet signal of a methoxy with proton resonances at δ_H 3.92 (*s*, H-10') ppm downfield. The spectrum further displayed aromatic protons downfield at δ_H 7.06 (H-6), 6.92 (H-5), and 7.03 (H-3) ppm, each integrating to one proton. The ^{13}C NMR spectrum displayed 16 carbon resonances, typical of a ferulic ester but attached to an aliphatic side chain. The spectrum further showed carbon resonances at δ_C 167.4 and 64.6 ppm, four quaternary carbon resonances, five methines, five methylene, and a methoxy as shown in Table **S1** and **S2**. The NMR spectral data corresponded with literature values of a known synthetic compound named; hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate [23].

Cytotoxic activities

The crude extracts and pure compounds were evaluated for half-maximal inhibitory concentrations (IC_{50}) effect in hormone receptor-positive breast cancer (MCF-7), TNBC (HCC70), and non-tumorigenic mammary epithelial cell lines (MCF-12A). Of particular relevance, the HCC70 cell line is derived from an African-American woman and is one of very few cancer cell lines derived from individuals of African descent. The CH_2Cl_2 root extracts showed potent specific cytotoxic effects in all three cell lines, with IC_{50} values of 0.83, 0.83 and 3.98, µg/mL against MCF-7, HCC70, and MCF-12A respectively, however, the compounds were more toxic to cancerous compared to non-cancerous cells (selectivity index [IC_{50} in MCF-12A/IC_{50} in cancer lines]: 4.80). The aerial CH_2Cl_2 extract on the other hand exhibited overall greater cytotoxic effects, with a lack of selectivity, against MCF-7 HCC70, and MCF-12A cells, with IC_{50} values of 1.03, 0.31, and 1.68 µg/mL respectively, as summarized in Table **1**.

Table 1. Anticancer activities of *Euphorbia grandicornis* crude extracts against MCF-7, HCC70, and MCF12A cells *in vitro*

Plant extracts	**MCF-7**		**HCC70**		**MCF-12A**	
	IC_{50} (µg/mL) and SD	R^2	IC_{50} (µg/mL) and SD	R^2	IC_{50} (µg/mL) and SD	R^2
CH_2Cl_2 root	0.83 ± 1.14	0.9763	0.83 ± 1.14	0.9382	3.98 ± 1.26	0.8955
CH_2Cl_2 aerial	1.03 ± 1.15	0.9707	0.31 ± 1.06	0.9769	1.68 ± 1.17	0.9536

Among the pure compounds, only hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**) displayed significant cytotoxic activity with IC_{50} values of, 23.41 µM against MCF-7, 29.45 µM against the TNBC cell line (HCC70), and 27.01 µM against MCF-12A cells, indicating non-specific cytotoxic effects. Similar studies on cytotoxic effects of ferulic acid derivatives including hexyl ferulate showed significant cytotoxic activities (inhibition: 15 ± 1.25% at 75 µM) against estrogen-sensitive breast cancer cells (MCF-7) [24]. The remaining compounds (β-glutinol (**1**), β-amyrin (**2**), 24-methylenetirucalla-8-en-3β-ol (**3**), (*24R*)-tirucalla-8, 25-diene-3β-24-diol (**4**), stigmasterol (**5**) and sitosterol (**6**) demonstrated either modest toxicity (for example, sitosterol (6): IC_{50} of 95.99 against HCC70 cells) or were found non-toxic (IC_{50} > 500 µM), with none of the latter compounds displaying an IC_{50} value below 95 µM as summarized in Table 2.

In a comparable study, the anticancer activities of triterpenoids including β-glutinol (**1**), β-amyrin (**2**), 24-methylenetirucalla-8-en-3β-ol (**3**) and tirucalla-8, 25-diene-3β-24*R*-diol (**4**), isolated from *E. micratina* showed no activity against A2780 ovarian cancer cells [19]. On the other hand, triterpenoids isolated from *E. resinifera,* in particular eupha-8,25-diene-3β-24*R*-diol, exhibited moderate cytotoxic activities against the MCF-7 breast adenocarcinoma cell line with an IC_{50} value of 34.55 ± 0.95 µM [11, 19]. In addition, cycloartenol, 24-hydroperoxycycloart-25-en-3β-ol, 25-hydroperoxycycloart-23-en-3β-ol, and taraxerone from *E. hirta* were analyzed for their anticancer activities against the HCT116 colon carcinoma cell line. The mixture of triterpenoids 25-hydroperoxycycloart-23-en-3β-ol and 24-hydroperoxycycloart-25-en-3β-ol (2:1)

were cytotoxic towards HCT116 cells, with an IC_{50} value of 4.8 µg mL^{-1}, while taraxerone and cycloartenol were inactive in this cell line [25]. However, 25-hydroperoxycycloart-23-en-3β-ol, showed good activity against the A549 non-small cell lung adenocarcinoma cell line with an IC_{50} value of 4.5 µg mL^{-1} [25], while the remainder of the compounds isolated from *E. resinifera* as described above were inactive

Furthermore, Cycloartane-3β, 24, 25-triol, cycloartane-3β, 25-diol and taraxast-12-ene-3β, 20, 21(α)-triol isolated from *E. denticulate* exhibited cytotoxic effects against DU-145 prostate cancer cells with IC_{50} values of 12.1 ± 2.8, 27.4 ± 4.7 and 18.2 ± 1.3 µM respectively [26]. In another study, (−)-(24*R*)-tirucalla-8,25-diene-3β,24-diol displayed promising activities against MCF-7 breast cancer and C6 glioma cell lines, with IC_{50} values of 56.2 µM and 49.6 µM, respectively [27]. The *in vitro* cytotoxic activities of cycloschimperols B (26,27-dinor-3β-hydroxy cycloartan-25-al) and cycloart-25-en-3-one from *E. schimperi* were evaluated against MCF-7, HepG2 (liver cancer cells), and HCT116 cancer cell lines. Cycloart-25-en-3-one and 26,27-dinor-3β-hydroxy-cycloartan-25-al showed promising activities against HCT-116, HepG2, and MCF-7 cells, with IC_{50} values of 1.9 ± 0.4, 2.3 ± 0.2, 4.7 ± 0.1, 1.8 ± 0.1, 1.4 ± 0.1 and 2.1 ± 0.01 0µM respectively, when compared to an IC_{50} value of 0.20 ± 0.01, 0.6 ± 0.1, and 0.18 ± 0.01 µM for doxorubicin in the latter cell lines, respectively [28]. The findings from these studies show that *Euphorbia* species can be a potential source of bioactive constituents for discovery of anticancer drugs.

Table 2. Anticancer activities of isolated compounds from *Euphorbia grandicornis* against HCC70, MCF12A, and MCF-7 cells *in vitro*

Compound Name	**MCF-7**		**HCC70**		**MCF-12A**	
	IC_{50} (µM) and SD	R^2	IC_{50} (µM) and SD	R^2	IC_{50} (µM) and SD	R^2
β-glutinol (**1**)	260.80 ± 1.09	0.8533	Not toxic*		Not toxic*	
β-amyrin (**2**)	215.10 ± 1.05	0.9570	Not toxic		248.50 ± 1.06	0.9374
24-methylenetirucalla-8-en-3β-ol (**3**)	194.6 ± 1.09	0.9187	190.50 ± 1.10		0.8939	265.20 ± 1.08
(-)-tirucalla-8, 25-diene-3β-24*R*-diol (**4**)	140.70 ± 1.11	0.9333	198.60 ± 1.03	0.9879	235.10 ± 1.09	0.8945
Stigmasterol (**5**)	178.30 ± 1.12	0.8968	134.10 ± 1.25	0.9104	192.60 ± 1.12	0.8753
Sitosterol (**6**)	129.10 ± 1.04	0.9741	95.99 ± 1.14	0.8793	167.40 ± 1.15	0.8974
Hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**)	23.41 ± 1.25	0.8689	29.45 ± 5.69	0.8463	27.01 ± 1.32	0.9803

***NT:** IC_{50} > 500 µM

Conclusion

In this study, seven previously reported compounds (**1-7**) were isolated and identified from the root extracts of *Euphorbia grandicornis* for the first time. Isolated compounds were evaluated for cytotoxic activities against MCF-7, HCC70, and MCF-12A cell lines. The aerial plant extract exhibited potent, non-specific cytotoxic activities against all three cell lines, while the root extract was more toxic to cancer than non-cancer cell lines. Of the pure compounds isolated from the root extract, only hexyl (*E*)-3-(4-hydroxy-3-methoxyphenyl)-2-propenoate (**7**) exhibited significant toxicity against MCF-7 (IC_{50}=23.41 µM), HCC70 (29.45 µM) and MCF-12A (27.01 µM).

Supplementary materials: Supplementary materials relating to this article are available online alongside appendices **A1-A24** Table **S1, S2.**

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Declaration of Competing Interest

The authors declare no competing interest.

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Graphical abstract