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Unveiling the anti-cancer potential of *Euphorbia greenwayi*: cytotoxicity, cell migration, and identification of its chemical constituents

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Abstract

Background Different herbal phytochemicals have potential in cancer treatment, Euphorbia genus has valuable secondary metabolites and is used in traditional medicine to treat various ailments. However, the specific constituents and biological activity of *Euphorbia greenwayi* remain largely unexplored.

Results *Euphorbia greenwayi* aerial parts were extracted using methanol. Consequently, the methanol extract was then fractionated with solvents of different polarities viz., *n*-hexane, chloroform, and ethyl acetate. All were screened for their cytotoxic activity against different cell lines; MCF-7, HepG-2, and SW620. The *n*-hexane (HF) and chloroform (CF) fractions showed considerable activity against all tested cell lines especially MCF-7 with IC_{50} values at 18.6 ± 0.2 and 17.5 ± 0.6 µg/ml respectively. Therefore, a cell migration assay on the MCF-7 cell line was applied to both fractions as well as investigation and isolation of the main active constituents. Lupeol, *β*-sitosterol, and cycloartenol were isolated from the nonpolar fractions of *E. greenwayi* for the first time.

Conclusions *Euphorbia greenwayi* aerial parts exhibit considerable anti-cancer effects via cytotoxicity. Three chemical constituents with promising cytotoxic activity are identified.

Keywords *Euphorbia greenwayi*, Euphorbiaceae, Cytotoxicity, Cell migration, Lupeol, *β*-Sitosterol, Cycloartenol

Background

Worldwide, cancer ranks as the second leading cause of death. The most prevalent cancers are colon, liver, and breast cancers. Cancer is characterized by its widespread occurrence globally. It exhibits notably high mortality rates according to statistical data. Lifestyle and genetic predisposition are commonly acknowledged as the primary factors contributing to its development. Phytochemicals derived from herbs and traditional medicine are becoming more widely recognized as effective cancer treatments. Recent clinical trials have demonstrated the beneficial effects of herbal medications on cancer patients' quality of life, survival rates, and immune system control when used in conjunction with traditional treatments. Numerous phytochemicals, including phenolic compounds, terpenoids, lignans, tannins, alkaloids, and others, have been studied from herbal sources and show potent antioxidant qualities that can suppress cell division and boost the immune system, improving prevention [1–4].

The ornamental medicinal plant species, *Euphorbia greenwayi* P.R.O. Bally & S. Carter is a member of the Euphorbiaceae (Spurge) family [5]. Euphorbia is considered the third biggest genus of flowering plants having

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milky poisonous latex. It consists of many species that are used in traditional medicine to cure a wide range of illnesses. This might be related to the wealth of their secondary metabolites [6, 7]. Different studies reported that Euphorbiaceae members consist mainly of terpenes, flavonoids, and tannins, which are known for their antioxidant, hepatoprotective, and anti-tumour properties [8–10]. As a member of the Euphorbia genus, *E. greenwayi* possesses succulent quality produces milky latex and may grow up to 1.2 m tall [11]. It was introduced to Egypt a short time ago, but it is native to Tanzania and East Africa. Upon reviewing the available literature, little information was reported on *E. greenwayi*; one study compared the immune-boosting capabilities of fifteen plant extracts from the Euphorbiaceae family, demonstrating *E. greenwayi*'s mild antiviral activity. [10]. Moreover, another report proved that the hydroalcoholic extract of *E. greenwayi* has significant antimicrobial potential [12]. Our recently published work demonstrated its anti-inflammatory and antioxidant potential [13].

The current study intends to assess the cytotoxic activity of the *E. greenwayi* methanol extract and its fractions. Additionally, an in vitro migration assay (wound healing activity) for the most active fractions is conducted to determine the tumour cell migration capacity of cell lines and, consequently, their invasiveness and potential to generate metastases. The chemical components of the most active fractions are determined using spectroscopic and chromatographic techniques.

Results

Phytochemical screening

A phytochemical screening is essential to determine the active substances responsible for the biological activity that plants are known to display and to evaluate a plant's potential medicinal usefulness. It also provides the foundation for more precise compound identification and investigation. Tannins, flavonoids, unsaturated sterols, and/or terpenes were found, together with carbohydrates and/or glycosides. As shown in Table 1, there were no volatile oils, alkaloids, nitrogenous bases, anthraquinones, or saponins.

Table 1 Preliminary phytochemical screening

Constituents	Presence
Carbohydrates and/or glycosides	+
Flavonoids	+
Tannins	+
Unsaturated sterols and/or terpenes	+
Volatile oils	–
Alkaloids and/or nitrogenous bases	–
Anthraquinones	–
Saponins	–

(+) = present, (–) = absent

Identification of the isolated Compounds

Compound EA$_1$

Compound (EA_1) 20 mg was isolated as white amorphous powder. It is soluble in *n*-hexane, chloroform and insoluble in methanol, m.p. 215–216°C.; the R_f values were 0.59 on silica TLC using *n*-hexane:ethyl acetate (95:5 *v/v*) as developer. It gave a pink color when sprayed with 10% H_2SO_4..It also gave positive Salkowski and Liebermann-Burchard tests [14]. The ^{1}HNMR spectrum Table 2 demonstrated the characteristic deshielded proton at δH 3.3

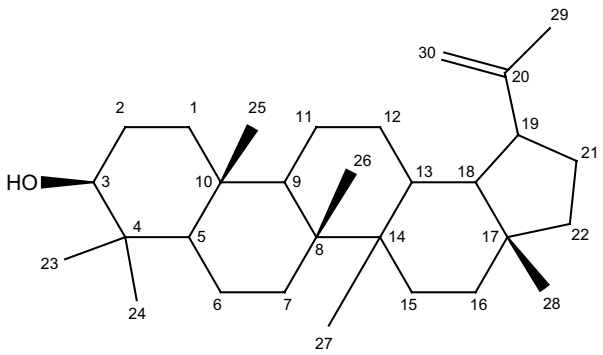
Table 2 ^{1}H NMR and ^{13}C spectral data of compounds EA 1, 2, and 3

Carbon number	(EA_1) Lupeol		(EA_2) *β*-sitosterol	(EA_3) Cycloartenol	
	δ_H (ppm)	δ_C (ppm)	δ_H (ppm)	δ_H (ppm)	δ_C (ppm)
1	–	38	–	–	32.54
2	–	25.13	–	–	30.39
3	3.2(*dd*)	78.9	3.50 (*m*)	3.12 (1H, *m*)	58.82
4	–	38.6	–	–	40.47
5	–	55.3	–	–	47.51
6	–	18.31	5.30 (*br.s*)	–	20.79
7	–	34.1	–	–	28.29
8	–	41.2	–	–	48.57
9	–	50.4	–	–	20.07
10	–	37.3	–	–	25.05
11	–	21.3	–	–	26.01
12	–	27.5	–	–	27.54
13	–	38.7	–	–	45.37
14	–	42.6	–	–	48.07
15	–	27.9	–	–	34.53
16	–	35.6	–	–	27.09
17	–	43.2	–	–	53.30
18	–	48.3	0.87(*s*)	0.8(*d*)	18.35
19	–	47.07	1.04(*s*)	0.3–0.5(*dd*)	29.89
20	–	151.6	–	–	36.53
21	–	30	0.88(*d*)	–	19.85
22	–	39.9	–	–	36.33
23	0.77(*s*)	28.7	–	–	24.95
24	0.80(s)	15.9	–	5.1 (*t*)	123
25	0.87(*s*)	16.3	–	–	129.69
26	1.12(*s*)	16.1	0.79(*d*)	1.7 (*s*)	19.77
27	0.97(*s*)	15.3	0.82(*d*)	1.78 (*s*)	25.67
28	0.71(*s*)	18	–	1 (*s*)	29.03
29	1.71(*s*)	20.9	0.84(*t*)	0.91 (*s*)	14
30	4.6(*d*)	108.6	–	0.88 (*s*)	21.64

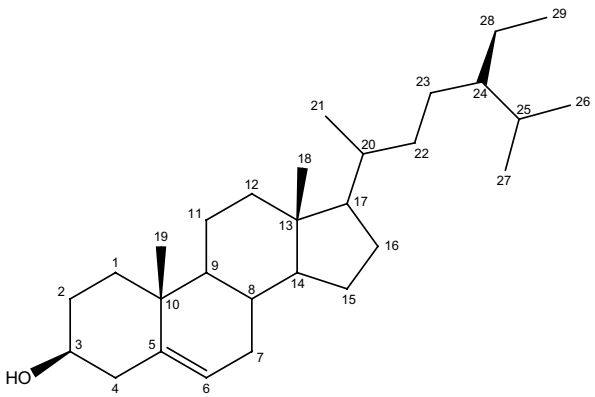
(1H, *m*) assigned to C-3 attached to hydroxyl group, the deshielded olefinic proton at δH 4.6 (H-30, *d*,2H) was assigned to C-30, the characteristic 7 methyl singlets at δ 0.77 (H-23, *s*, 3H), 0.85 (H-24, *s*, 3H), 0.87 (H-25, *s*, 3H), 1.12 (H-26, *s*, 3H), 0.97 (H-27, *s*, 3H), 0.71 (H-28, *s*, 3H), and 1.71 (H-29, *s*, 3H) [15]. The ^{13}C NMR of the compound revealed 30 distinct signals corresponding to the terpenoid of the lupane skeleton. Among these signals, a carbon bonded to the hydroxyl group at the C-3 position was observed at δ 78.9. Additionally, the olefinic carbons associated with the exocyclic double bond manifested signals at δ 151.6 and 108.6. The EI-MS spectrum showed a molecular ion peak at 426(36%) calculated for the molecular formula $C_{30}H_{50}O$. In addition to the following characteristic peaks at 316, 218, 207, 189, 149, 135, 69, 95, 109, 121, 135 compared to published data [16, 17]. Compound (EA1) was identified as Lupeol based on the data presented above, comparison to published data [13, 18] and co-chromatography with a standard sample (Fig. 1). It was separated for the first time from *E. greenwayi*.

Compound EA$_2$

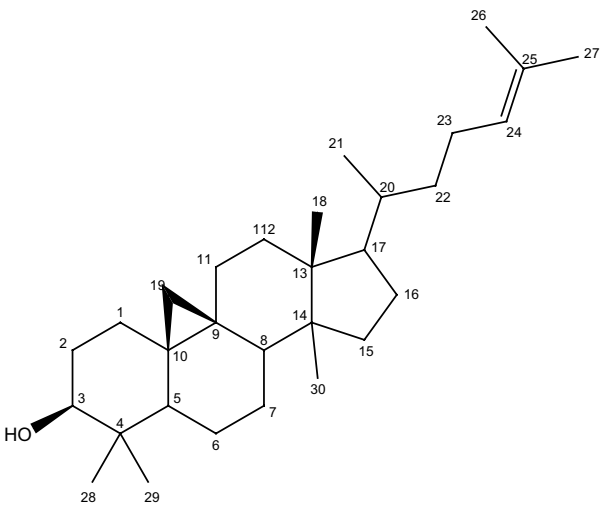
Needle crystals give a dark blue colour with 10% H_2SO_4, positive Liebermann- Burchard [19] and Salkowski [14] tests. Its molecular formula $C_{29}H_{50}O$ *m/z* 414 (86.8%). ^{1}HNMR spectrum; presented in Table 2; showed δ 5.3 (H-6, *br s*, 1H), 3.5 (H-3, *m*, 1H), the characteristic 2 methyl singlets δ 0.86 (H-18, *s*, 3H) and 1.04 (H-19, *s*, 3H), and 4 methyl doublets at δ 0.88 (H-21, *d*, *J*=9.6, 3H), 0.84 (H-29, *t*, 3H), 0.79. (H-26, *d*, *J*=6.3, 3H) and 0.82 (H-27, *d*, *J*=6.3, 3H). In addition to the following



Compound EA$_1$ (Lupeol)



Compound EA$_2$ (*β*-sitosterol)



Compound EA$_3$ (Cycloartenol)

Fig. 1 Chemical structure of the identified compounds

characteristic peaks at 414 $[M]^+$, 396 $[M\text{-}H_2O]^+$, 381 $[M\text{-}CH_3\text{-}H_2O]^+$, 329 $[M\text{-}C_6H_{13}]^+$, 303 $[M\text{-}C_7H_{11}O]^+$, 255 [M-side chain-$H_2O]^+$, 231 [M-side chain-ring D cleavage-$CH_3]^+$, 213 [M-side chain-ring side chain -$H_2O]^+$. The compound (EA2) was identified as β-sitosterol through data analysis, comparison to published data [15, 20–22] and co-chromatography with a standard sample (Fig. 1). *β*-Sitosterol was previously isolated from various Euphorbia species. [13, 23, 24]. It was isolated from *E. greenwayi* for the first time.

Compound EA$_3$

Yellowish white microcrystalline powder gives a purple colour with 10% H_2SO_4, positive Liebermann- Burchard [19] and Salkowski tests [14]. It is soluble in *n*-hexane, chloroform and insoluble in methanol, m.p. 99–110 °C. The R_f values were 0.56 on silica TLC using *n*-hexane: ethyl acetate (80:20 v/v) as developer. ^{1}HNMR spectrum; presented in Table 2; showed the following signals: δ5.1 (H-24, *t*, *J*=5.6, 1H), 3.12 (H-3, *m*, 1H), 0.3–0.5 (H-19, *dd*, *J*=3.2, 2H), 0.8 (H-18, *d*, *J*=3.2, 3H), 1.78 (H-27, *s*, 3H), 1.7 (H-26, *s*, 3H), 0.91 (H-29, *s*, 3H), 1 (H-28, *s*, 3H), 0.88 (H-30, *s*, 3H). ^{13}C NMR spectrum of compound EA_3 displayed 30 carbons corresponding to 7 methyl carbons, 11 carbenes, 5 methine carbons, 5 quaternary carbons and 2 olefinic carbons at δ 123 and 129.69 (Table 2). Mass spectrum of isolated compound showed molecular ion *m/z* 427 [M+H] corresponding to the molecular formula $C_{30}H_{51}O$. It gave MS spectra with a base peak at *m/z* 409 which resulted from loss of 1 water molecule $[M+H-H_2O]^+$. MS^2 also showed characteristic peaks at *m/z* 257, 271 and 285, 191, 203 and 217 and compared with published data [25]. Based on the data presented above and published data [26], compound (EA3) was identified as cycloartenol (Fig. 1). It was previously isolated from several Euphorbia species [26–28]. It has been isolated for the first time from *E. greenwayi*.

Biological activity

Antitumor activity (Screening)

As shown in Table 3 *n*-Hexane fraction (HF) and chloroform fraction (CF) were the most active fractions to the 3 cancerous cell lines compared to the total methanol extract (ME) and ethyl acetate fraction (EF) in both tested concentrations. (HF) showed viability percentage against MCF-7, breast adenocarcinoma, HepG-2; hepatocellular carcinoma, and SW620; colorectal adenocarcinoma at 75.1622, 48.396 and 97.3144% respectively in 10 µg/ml concentration and 1.9754, 6.8561 and 0.79856% respectively in 100 µg/ml concentration. Whereas (CF) in 10 µg/ml concentration showed viability percentage at 80.0813, 78.9734 and 99.4913% against MCF-7, HEPG-2 and SW620 respectively and in 100 µg/ml it showed viability % at 2.42084, 4.98625 and 0.38287% respectively.

Antitumor activity (IC$_{50}$*)*

According to the cell viability assay (Table 3) HF and CF were the most cytotoxic fractions of the 3 tested cancerous cell lines. Accordingly, those 2 fractions were further tested to find their (IC_{50}) using Sulforhodamine B (SRB) analysis in comparison with doxorubicin as a reference antitumor drug. As shown in Table 4 both HF and CF have a moderate to low activity against all tested cell lines. In specific the breast adenocarcinoma (MCF-7) cells were the most susceptible cell line against both fractions with IC_{50} values at 18.6±0.2 µg/ml against HF and 17.5±0.6 µg/ml against CF.

It can be deduced that the aerial parts of *E. greenwayi* have a moderate antitumor activity especially against MCF-7 cell line. The United States National Cancer Institute (NCI) stated that any crude extract with IC_{50} value ≤ 20 µg/ml is considered an active cytotoxic agent [29]. The

Table 4 IC_{50} of HF and CF against MCF-7, HepG-2, and SW620

	IC_{50} (µg/ml)		
Cell line	**MCF-7**	**HEPG-2**	**SW-620**
HF	18.6 ± 0.2	35.6 ± 1.2	28.4 ± 0.8
CF	17.5 ± 0.6	36.3 ± 0.9	24.1 ± 1.3
Doxorubicin	3.3 ± 0.10	4.8 ± 0.14	4.2 ± 0.18

Table 3 Cell viability % of ME, HF, CF, and EF against MCF-7, HEPG-2, and SW620

Cell line	**MCF-7 viability %**		**HEPG-2 viability %**		**SW620 viability %**	
Conc.	**10 µg/ml**	**100 µg/ml**	**10 µg/ml**	**100 µg/ml**	**10 µg/ml**	**100 µg/ml**
ME	83.52 ± 0.59	54.16 ± 1.04	82.19 ± 0.15	46.30 ± 1.89	95.99 ± 0.5	74.40 ± 0.66
HF	75.16 ± 0.71	1.97 ± 0.17	48.39 ± 1.37	6.85 ± 0.35	97.31 ± 0.37	0.79 ± 0.22
CF	80.08 ± 0.54	2.42 ± 0.22	78.97 ± 1.5	4.98 ± 1.32	99.49 ± 0.22	0.38 ± 0.02
EF	97.72 ± 0.52	84.03 ± 0.41	96.42 ± 1.21	94.70 ± 0.28	97.041 ± 1.3	93.34 ± 0.14
Negative control	100		100		100	

highest cytotoxic activity was observed in the nonpolar fractions (*n*-hexane and chloroform) of *E. greenwayi.* This was confirmed after tracing the anticancer potential of the 3 isolated compounds (lupeol, *β*-sitosterol, and cycloartenol) that was already proven in previous studies [30–34]. This reveals a good correlation between antitumor potential and nonpolar constituents of *E. greenwayi* like sterols and terpenes.

Anti-Migration Activity of MCF-7 Cell Line

A wound healing assay, conducted *in vitro*, aims to assess the migratory potential of cell lines treated with the most potent fractions. This assay helps evaluate the cells' ability to migrate, thereby indicating their invasiveness and the likelihood of generating metastases. Based on the antitumor activity results, only HF and CF were continued in this study, because those fractions showed cytotoxic activity superior to ME and EF against MCF-7 which was the most susceptible cell line among the 3 cell lines tested.

HF and CF antimigration assay was performed using 2 doses (subtoxic and a lethal dose (IC_{50})), 1.9 and 19 µg/ml respectively for HF and 1.7 and 17 µg/ml respectively for CF. Figures 2, 3 and 4 demonstrated the MCF-7 monolayer which was scratched and treated with selected fractions. The wound area was monitored and imaged every 24 hours for 72 hours. Finally, the migration rate was calculated and compared with the negative control Figs. 5 and 6.

As shown in Figs. 5 and 6 both the subtoxic and lethal doses of both HF and CF don't exhibit an anti-migratory effect.

Discussion

The exploration of medicinal plants has garnered increased attention as a means to discover more effective treatments for various cancer types. Presently, a substantial portion of pharmaceutical agents, especially in cancer therapy, comprises natural products. Taxol, vinblastine, and camptothecin are illustrative examples, distinguished by their unique structures and mechanisms of action,

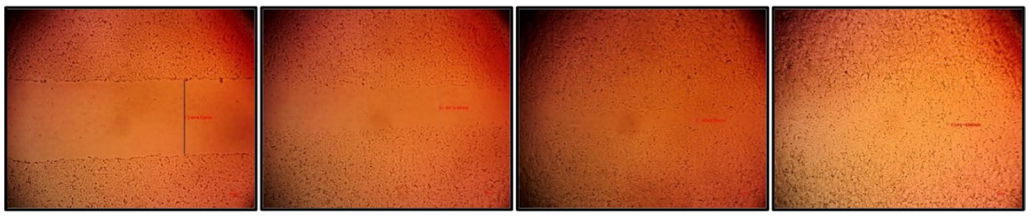


Fig. 2 Migration rate of MCF-7 cells without any drug applied (Negative control) at A = 0, B = 24, C = 48, D = 72 h. respectively

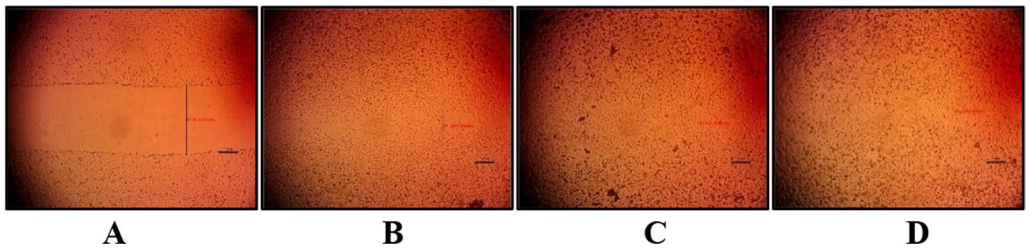


Fig. 3 Migration rate of MCF-7 cells using HF (n-hexane fraction) lethal dose (19 µg/ml) at A = 0, B = 24, C = 48, D = 72 h. respectively

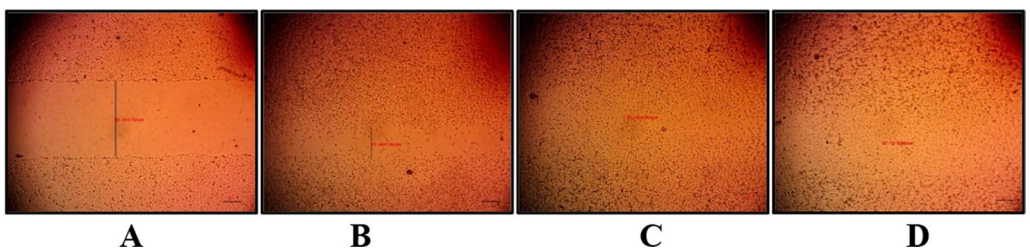


Fig. 4 Migration rate of MCF-7 cells using CF (chloroform fraction) lethal dose (17 µg/ml) at A = 0, B = 24, C = 48, D = 72 h. respectively

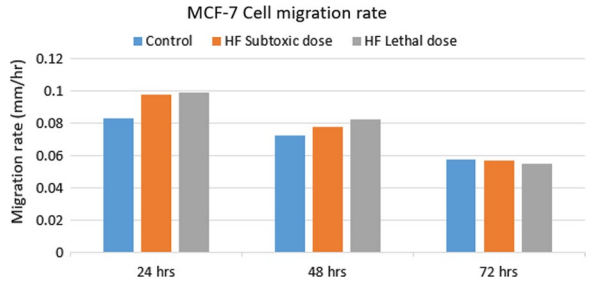


Fig. 5 Rate of MCF-7 cells migration with subtoxic and lethal dose of HF (n-hexane fraction)

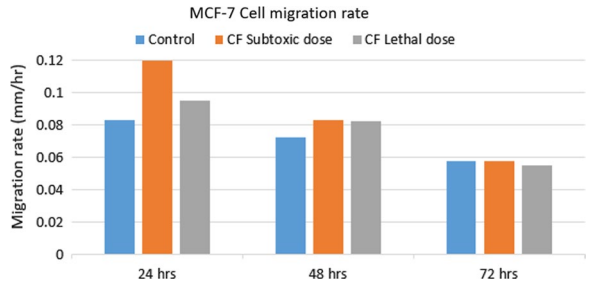


Fig. 6 Rate of MCF-7 cells migration with subtoxic and lethal dose of CF (chloroform fraction)

with their discovery primarily attributed to isolation from natural sources. In this sense, the Euphorbia genus is distinguished by its richness in biologically active phytoconstituents with promising cytotoxic activity [35–37]. In this regard, *E. greenwayi* was chosen to be the subject of our study because of the little-known information regarding its primary components and its biological activity.

E. greenwayi showed positive presence of sterols, triterpenes, and phenolic compounds; hence its methanol extract (ME) was fractionated using *n*-hexane (HF), chloroform (CF), and ethyl acetate (EF) to test these fractions for cytotoxic activity against MCF-7, HepG-2, and SW-620 cell lines. (HF) and (CF) showed significant cytotoxic activity against MCF-7 with IC_{50} values at 18.6 ± 0.2 and 17.5 ± 0.6 µg/ml respectively. However, they showed significant cytotoxic effect on HepG-2 and SW-620 at higher doses. This confirms the susceptibility of MCF-7 against (HF) and (CF). On the other hand, ME and EF didn't exhibit any cytotoxic activity against the 3 cell lines at all tested concentrations. Wound healing assay for cancer metastasis is highly reproducible method to study cancer cell in vitro. By this method we can develop an additive treatment combined with the main drugs in order to decrease migration of cancer cell to another organs. Based on the previous findings (HF) and (EF) were tested against MCF-7 cell migration yet they didn't display a significant antimigratory effect. These findings don't contradict the results of antitumor assay, but rather suggest that both (HF) and (CF) are cytotoxic to MCF-7 cells in a non-apoptotic cell death mechanisms other than decreasing cell migration or inhibiting the cell motility [38]. Also, it was already established that some of the most effective anticancer drugs such as carboplatin and paclitaxel were observed to induce the migration of cancer cells in different kinds of cancers [39].

Driven by the antitumor assay findings we decided to explore the constituents of the nonpolar fractions (HF) and (CF) of *E. greenwayi*. Phytochemical analysis reveals the separation of three compounds from (HF) and (CF): lupeol, β-sitosterol, and cycloartenol. The 3 compounds are isolated from *E. greenwayi* for the first time. Based on previous data and the pharmacological effects of the three compounds, we realised that they all exhibited cytotoxic activity against various cancer cell lines [30, 32–34, 40, 41]. This supports the antitumor effects of *E. greenwayi*'s nonpolar fractions. In addition, in our recently published work through LC–MS we identified several nonpolar compounds in *E. greenwayi* [13] with reported cytotoxic activity such as taraxasterol [42], ingenol dibenzoate [43], and ingenol mebutate [44].

Methods

Plant material

Collection, handling, and authentication of plant material was previously discussed in our recently published work [13].

Extraction and fractionation

Twelve Kg of *E. greenwayi* fresh plant was macerated with absolute methanol till exhaustion (12 L × 3). The methanol extract was evaporated under a vacuum at 40°C. The crude extract (40 g) was suspended in 200 ml distilled water. The aqueous suspension was successively fractionated by partition with *n*-hexane, chloroform, and ethyl acetate. The results of the fractionation are summarized in (Fig. 7).

Phytochemical screening

Dried aerial parts of *E. greenwayi* (40 g) underwent a phytochemical screening to identify the different phytochemical components that were found in it. These components included volatiles, carbohydrates and/or glycosides, alkaloids and/or nitrogenous bases, saponins, anthraquinones, unsaturated sterols and/or triterpenes, tannins, and flavonoids [45, 46]. The Pio-chem corporation in Cairo, Egypt provided all the chemicals, which were of high purity. Among the substances utilized following instructions were glacial acetic acid, concentrated ammonia, alcoholic KOH, $FeCl_3$, HCl, Dragendorff's reagent, methanol, chloroform, and H_2SO_4 [47, 48].

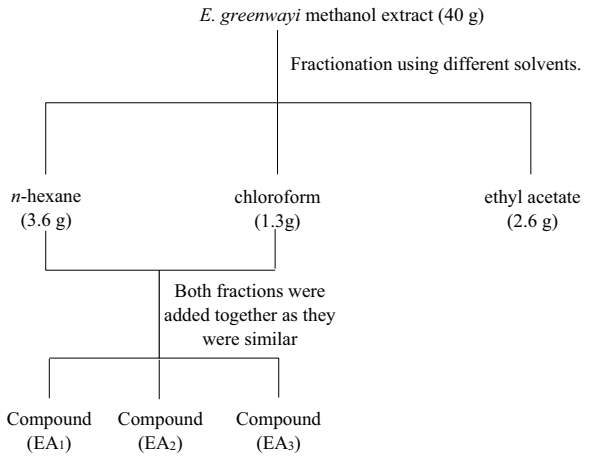


Fig. 7 Scheme for compound isolation

Compound isolation

N-hexane (3.6 g) and chloroform (1.3 g) fractions showed similar spots, so both fractions were added together. Fractionation was done through column chromatography using a silica gel (Merck) (200 g, 100 cm X 5 cm). Gradient elution started with 100% *n*-hexane then 5% increments of ethyl acetate, till the elution reaches 100% ethyl acetate. Fractions, each of 15 ml, were collected, concentrated under reduced pressure, and monitored using thin liquid chromatography (TLC). A system consisting of *n*-hexane: ethyl acetate with a different ratio was used as a developer. 10% H_2SO_4 was used as a spraying reagent for spot visualization. Similar fractions were pooled together. Fraction (I) was fractionated through column silica gel using *n*-hexane/methylene chloride gradient elution resulting in compound (EA_1) separation.

Fraction (VI) was found to have 2 major compounds. The fraction was further chromatographed on a silica gel column (30 × 1 cm). Gradient elution was performed using *n*-hexane followed by 5% increments of ethyl acetate. Fractions of 10 ml were collected and run on TLC. subfraction (35–45) yielded 3 mg of needle crystals compound (EA_2) and subfraction (98–106) yielded 5 mg of needle crystals compound (EA_3).

Structure elucidation of the purified compound

NMR spectroscopic analysis used a Bruker spectrometer at 400 MHz for (^{1}H NMR) and 100 MHz (^{13}C NMR) according to [49]. The UPLC-ESI–MS/MS negative and positive ion modes were executed on a Waters Corporation, Milford, MA01757, USA, XEVO TQD triple quadrupole mass spectrometer.

Biological activity

Antitumor activity

Cell viability test was done for the *E. greenwayi* total methanol extract (ME), and its fractions; n-hexane fraction (HF), chloroform fraction (CF), and ethyl acetate fraction (EF) according to [50]. It was assessed by Sulforhodamine B (SRB) assay against 3 human tumor cell lines (MCF-7, breast adenocarcinoma, HepG-2; hepatocellular carcinoma, and SW620; colorectal adenocarcinoma) using 2 concentrations of each tested sample (10 and 100 µg/ml), to identify fractions with the most powerful anti-tumor properties.

In this experimental procedure, 100 µL aliquots of a cell suspension containing 5×10^3 cells were dispensed into individual wells of 96-well plates and incubated in complete media for a duration of 24 h. Subsequently, the cells were subjected to treatment with another 100 µL of media containing various concentrations of drugs. Following 72 h of exposure to the drugs, the cells were fixed by replacing the media with 150 µL of a 10% trichloroacetic acid (TCA) solution and incubated at 4 °C for 1 h. After removal of the TCA solution, the cells underwent five washes with distilled water. Subsequently, 70 µL aliquots of a sulforhodamine B (SRB) solution at a concentration of 0.4% w/v were added to each well, and the plates were incubated in darkness at room temperature for 10 min. Following this incubation period, the plates underwent three washes with 1% acetic acid and were then allowed to air-dry overnight. To dissolve the protein-bound SRB stain, 150 µL of a tris(hydroxymethyl) aminomethane (TRIS) solution was added at a concentration of 10 mM. The absorbance of the resulting solution was measured at 540 nm using a BMG LABTECH®-FLUOstar Omega microplate reader (Ortenberg, Germany).

Cell migration (wound healing) assay

N-hexane and chloroform fractions were evaluated for their potential to inhibit wound healing in cancerous cell lines according to [51, 52]. Since MCF-7 (Breast Adenocarcinoma) was the most susceptible cell line, it is chosen to be used in this assay. Both fractions were evaluated in 2 concentrations, lethal dose (IC_{50}) and Subtoxic dose.

Cells were seeded at a density of 2×10^5 cells per well on a 12-well plate that had been pre-coated for scratch wound assay. They were cultured overnight in a medium consisting of 5% fetal bovine serum (FBS) in Dulbecco's Modified Eagle Medium (DMEM) at 37°C and 5% CO_2. The following day, horizontal scratches were carefully introduced into the confluent cell monolayer. Subsequently, the plate underwent thorough washing with phosphate-buffered saline (PBS). Control wells were replenished with fresh medium, while wells designated for drug treatment were supplied with fresh medium containing the specified drug. Images were captured at designated time intervals using an inverted microscope. The plate was maintained at 37°C and 5% CO_2 between

these time points. Analysis of the acquired images was conducted using MII Image View software version 3.7.

Wound width is the distance between the edges of the scratches in average; as cell migration is induced the wound width decreases.

Migration rate is determined according to the formula below: MR = IW − FW / t where MR is the rate of cell migration, IW is the average wound width at 0 h, FW is the average final wound width, and t is duration of migration (in hours).

Conclusion

In conclusion, the investigation into the anti-cancer properties of *E. greenwayi* has revealed promising findings. The study encompassed cytotoxicity assays, evaluations of cell migration, and identification of its chemical constituents.

The cytotoxicity assessments demonstrated considerable potency within specific fractions (*n*-hexane and chloroform fractions) of *E. greenwayi*, notably highlighting considerable toxicity against cancerous cell lines.

Furthermore, the identification of chemical constituents within *E. greenwayi* provides valuable insights into potential bioactive compounds responsible for its anti-cancer effects. These constituents may serve as a foundation for further research and development of novel anti-cancer agents.

The collective findings underscore the significance of *E. greenwayi* as a potential source of compounds with anti-cancer properties. Continued exploration and elucidation of its mechanisms and active compounds could pave the way for the development of new therapeutic strategies in combating cancer.

Abbreviations

M.p.	Melting Point
R_f	Retention Factor
TLC	Thin Layer Chromatography
NMR	Nuclear Magnetic Resonance
H^1NMR	Proton nuclear magnetic resonance
C^{13}NMR	Carbon nuclear magnetic resonance
EI-MS	Electron-impact ionization Mass
Ms	Mass Spectroscopy
ME	Methanolic Extract
HF	*n*-Hexane Fraction
CF	Chloroform Fraction
EF	Ethyl acetate Fraction
MCF-7	Breast Adenocarcinoma
HepG-2	Hepatocellular Carcinoma
SW-620	Colorectal Adenocarcinoma
SRB	Sulforhodamine B
IC_{50}	Half Maximal Inhibitory Concentration
NCI	National Cancer Institute
TQD	Triple Quadrupole
TCA	Trichloroacetic Acid
TRIS	Tris(hydroxymethyl) aminomethane
FBS	Fetal Bovine Serum
DMEM	Dulbecco's Modified Eagle Medium
PBS	Phosphate-buffered Saline
MR	Migration Rate
IW	Initial Width
FW	Final Width

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Author contributions

Conceptualization, A.Z., Z.K., M.Y., R.S.E. and W.E.; Data curation, A.Z.; Investigation, A.Z.; Methodology, R.S.E., and W.E.; Supervision, Z.K., M.Y., R.S.E., and W.E.; Visualization, A.Z.; Writing – original draft, W.E.; Writing – review & editing, Z.K., M.Y., R.S.E. and W.E.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from the research ethics committee, Faculty of Pharmacy, Cairo University; serial number MP (2448).

Consent for publication

Not applicable.

Plant authentication

Aerial shoots of *E. greenwayi* were collected in March 2019 at the Helal Cactus farm in Al Mansoureyah, Giza Governorate, Egypt (30.10812667354337, 31.105346915336153). Professor Dr. Reem Samir Hamdy, a botany professor at Cairo University's Faculty of Science, kindly verified and recognized the plant. The collection and handling of the plant material were in accordance with all the relevant guidelines.

Competing interests

The authors declare that they have no competing interests.

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