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Cytotoxic activity of acyl phloroglucinols isolated from the leaves of *Eucalyptus cinerea* F. Muell. ex Benth. cultivated in Egypt

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Two acyl phloroglucinol compounds namely; Sideroxylonal B (1) and Macrocarpal A (2) were isolated from the Sideroxylonal-Rich Extract (SRE) of the juvenile leaves of *Eucalyptus cinerea*; F. Muell. ex Benth cultivated in Egypt. Identification of the isolated compounds was established on the basis of physico-chemical properties and spectral analysis (1D & 2D NMR). The two compounds were isolated for the first time from this species. The SRE alongside with the isolated compounds were tested against three human cancer cell lines; MCF7 (breast carcinoma cell line), HEP2 (laryngeal carcinoma), CaCo (colonic adenocarcinoma) and one type of normal human cell line;10 FS (fibroblast cells). The SRE, (1), and (2) showed cytotoxic activity with IC_{50} 13.6 ± 0.62, 7.2 ± 0.5, 14.8 ± 0.55 μg mL−1 against HEP2 respectively, 11.6 ± 0.47, 4 ± 0.36, 11.4 ± 0.45 μg mL−1 against CaCo, respectively, and 8.6 ± 0.29, 4.4 ± 0.25, and 7.8 ± 0.3 μg mL−1 against MCF7, respectively. Meanwhile, the (SRE) together with (1) and (2) exhibited low cytotoxicity against normal cell line 10 FS, with IC_{50} 55.4 ± 1.4, 43 ± 0.8 and 50.1 ± 1.12 μg mL−1, respectively. The antiprofilerative activity of the tested compounds was evaluated. The cell cycle profile of cells treated with Sideroxylonal-B and Macrocarpal-A indicates possible S-phase specific effects.

The problem of cancer in the developing world is so huge that it is difficult to find the right way to measure it. The complexity of cancer control increased enormously following the shift of the disease burden from wealthy to less affluent countries. According to the latest WHO statistics, cancer causes around 7.9 million deaths worldwide each year. Of these deaths, around 70%, that means 5.5 million, are now occurring in the developing world. If no action is taken, deaths from cancer in the developing world are forecast to grow to 8.9 million in 2030. Many powerful global trends contribute to the rise of cancer in the developing world: population ageing, rapid unplanned urbanization, and globalization of unhealthy lifestyles. Most developing countries do not have financial resources, facilities, equipment, technology, infrastructure, staff, or training to cope with chronic care for cancers[1,2]. The estimated new cases and deaths from breast cancer in the United States in 2014: 232,670 case (female) and 2,360 (male) and death: 40,000 (female); 430 (male); while the death rate extrapolations for larynx cancer: 3,815 per year. According to the American Cancer Society, it is estimated that in 2014, a total of 136,830 people in the United States are diagnosed diagnosed with colorectal cancer and 50,310 people will die from it[3,4].

In Egypt, carcinoma of the breast is the most prevalent cancer among Egyptian women and constitutes 29% of National Cancer Institute cases (35.1% in women and 2.2% in men) among Egypt National Cancer Institute (NCI) series of 10 556 patients during the year 2001[5] with an age-adjusted rate of 49.6 per 100 000 population[6].

A worldwide increasing interest is continuously directed towards searching for cheap and safe tumour inhibitors or cytotoxic compounds from plant origin that might help in chemotherapy and/or chemoprevention of different types of cancer. Since most of the developing countries still relay on plant-based traditional medicine for their primary health care[7], it is the main concern of the present invention to make available new compounds useful in treatment (including therapy and prophylaxis) of diseases or disorders or improvement of health

Table 1 | Results of the cytotoxic activity and selectivity of the isolated compounds from *Eucalyptus cinerea* juvenile leaves on human cancer cell lines

Tested compound	HEP2 $IC_{50}\mu g\ mL^{-1}$	CaCo $IC_{50}\mu g\ mL^{-1}$	MCF7 $IC_{50}\mu g\ mL^{-1}$	10 FS $IC_{50}\mu g\ mL^{-1}$
Sideroxylonal –Rich Extract (SRE)	13.6 ± 0.62	11.6 ± 0.47	8.6 ± 0.29	55.4 ± 1.4
Sideroxylonal B (**1**)	7.2 ± 0.5	4.0 ± 0.36	4.4 ± 0.25	43 ± 0.8
Macrocarpal A (**2**)	14.8 ± 0.55	11.4 ± 0.45	7.8 ± 0.3	50.1 ± 1.12
Doxorubicin®	0.7 ± 0.01	0.69 ± 0.01	0.8 ± 0.02	≥70

Results are expressed as mean ± S.E.

situation even in healthy persons. The isolation and structural elucidation of tumour inhibitors, during the last decades, has allowed the discovery of several new types of growth inhibitors[8].

Eucalyptus is a large genus of evergreen aromatic trees, rarely shrubs. Various species are cultivated particularly in sub-tropical and warm regions, on account of their economic value. The extracts obtained from different species revealed, anti-inflammatory, antioxidant, antibacterial and anticancer activity[9,10].

Phloroglucinol compounds (PCs) have been found almost exclusively in *Eucalyptus*. The most recent interest in phenolic compounds from *Eucalyptus* has focused on a newly identified group called the formylated phloroglucinol compounds (FPCs). This group includes the subtypes known as euglobals, macrocarpals and sideroxylonals. Naturally occurring phloroglucinol compounds have shown diverse range of biological activities including cancer chemopreventive, antitumor, antimalarial, antibacterial, HIV-RTase inhibition and antifouling[9,11–14]. Euglobals proved to be a chemoperventive agent in chemical carcinogenesis[9,15,16].

Eucalyptus cinerea F. Muell. ex Benth. (Silver Dollar Gum, Argyle Apple and Mealy Stingybark) belongs to the family Myrtaceae. It is a small to medium-sized tree. It has distinctive blue-green aromatic foliage[17].

In that respect, our study was planned to isolate and identify phloroglucinol components from the sideroxylonal -rich extract (SRE) of the juvenile leaves of *E. cinerea* F. Muell. ex Benth. cultivated in Egypt. The isolated compounds as well as the SRE were tested for their cytotoxic effect against human cancer cell lines (HEP2, CaCo and MCF7) which are most common cancer types in Egypt. In addition to, the effect on normal cell line (10 FS) to prove their selectivity. Moreover, the antiproliferative effect of the isolated compounds was tested.

Methods

Plant material. Juvenile leaves of *E. cinerea* F. Muell. ex Benth. were collected all over the years (2008–2011) from El-Salheya, Sharqia governorae, Egypt and were kindly identified by Dr. Eve Lucas, Science team leader (Myrtaceae), Kew Garden, UK. A voucher specimen (EC-2008-52) was kept in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

General experimental procedure. A Büchi melting point apparatus Model B-545(Sigma-Aldrich, Munich, Germany) was used for determination of melting points, which are uncorrected. Mass spectrometer: Varian Mat. 711, Finningan SSQ 7000 and OMM 7070 E. Tiple Quadrupole (TQD) Mass Spectrophotometer: Waters, Milford, MA, USA, for ESI-MS. ^{1}H and ^{13}CNMR analyses were operated on: Varian Mercury (^{1}H-300 MHz and ^{13}C -75 MHz: Palo Alto, CA, USA), TMS was used as internal standard and chemical shifts were given in δ value. JOEL (^{1}H-600 MHz and ^{13}C -150 MHz), TMS was used as internal standard and chemical shifts were given in δ value. Silica gel G 60 (E-Merck).RP-18 (E-Merck), Sephadex LH-20 (Pharmacia Fine Chemicals, AB Uppsala, Sweden), silica gel H (E-Merck).Thin-layer chromatography (TLC) was performed on silica gel GF254 precoated plates (Fluka, Germany). The chromatograms were visualized by spraying with natural product/PEG spray reagent. Cancer cell lines: MCF7 (breast carcinoma cell line), HEP2 (laryngeal carcinoma), CaCo (colonic adenocarcinoma) and 10 FS (normal cell lines) were obtained from National Cancer Institute, Kasr El-Aini, Cairo, Egypt. **Doxorubicin**, Sigma Company, USA. Trypsin-EDTA (Sigma-Aldrich), Tris-HCl (Changzhou Welton Chemical Co.,Ltd.)

Cell culture. Human head and neck squamous cell carcinoma (HEP2), human breast adenocarcimona cell line (MCF-7), human colorectal adenocarcinoma cells (CaCo) and normal human fibroblast cells (10 FS), were obtained from the National Cancer Institute of Egypt (Giza, Egypt). Cells were maintained in RPMI-1640 supplemented with 100 µg/ml streptomycin, 100 units/ml penicillin and 10% heat-inactivated fetal bovine serum in a humidified, 5% (v/v) CO_2 atmosphere at 37°C.

Assessment of cytotoxic activity. The potential cytotoxicity of the SRE and the isolated compounds was tested by Sulphorhodamine B assay (SRB)[18], in which the cells were placed in a 96-multi well plate (10^4 cells/well) for 24 hours before treatment with the tested extracts to allow attachment of the cells to the wall of the plate. Different concentrations of each compound/or extract under test (0, 5, 12.5, 25 and 50 µg/ml) were added to the cell monolayer. Triplicate wells were incubated with the samples for 48 hours at 37°C and in atmosphere of 5% CO_2. The cells were then fixed, washed and stained with Sulphorhodamine B stain (SRB). Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. The color intensity was measured in a microplate reader at 564 nm. The linear relation between surviving fraction and compound concentrations was plotted to get the survival curve of each tumor cell line for the specified tested compound. The curves were fitted using linear equation and IC_{50} (dose of the drug which reduces survival to 50%) was calculated and recorded in Table 1 and compared with the standard drug Doxorubicin®.

Antiproliferative assessment. The antiproliferative effect and the potential resistant fraction of cells to the treatment with SRE and its isolated compounds were further tested against MCF-7 cells by SRB assay as previously described[18]. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and plated in 96-well plates at 1000–2000 cells/well. Cells were exposed to test compound for 72 h and subsequently fixed with TCA (10%) for 1 h at 4°C. After several washing, cells were exposed to 0.4% SRB solution for 10 min in dark place and subsequently washed with 1% glacial acetic acid. After drying overnight, Tris-HCl was used to dissolve the SRB-stained cell protein and color intensity was measured at 540 nm.

The dose response curve of compounds was analyzed using logarithmic best fit equation (E_{max} model Eq. 1).

$$\%\ \text{Cell viability} = (100 - R) \times \left(1 - \frac{[D]^m}{K_d{}^m + [D]^m}\right) + R \qquad (1)$$

Where [R] is the residual unaffected fraction (resistance fraction), [D] is the drug concentration used, [K_d] is the drug concentration that produces a 50% reduction of the maximum inhibition rate and [m] is a Hill-type coefficient. IC_{50} was defined as the drug concentration required to reduce color intensity to 50% of that of the control (i.e., $K_d = IC_{50}$ when $R = 0$ and $E_{max} = 100\text{-}R$).

Analysis of cell cycle distribution. To further dissect the antiprolefirative effect of the tested compounds on the different phases of cell cycle, cells were treated with the predetermined K_d of test compounds for 24 h and collected by trypsinization, washed with ice-cold PBS, and re-suspended in 0.5 ml of PBS. Ten ml of 70% ice-cold ethanol was added gently while vortexing, and cells were kept at 4°C for 1 hr and stored at −20°C until analysis. Upon analysis, fixed cells were washed and re-suspended in 1 ml of PBS containing 50 µg/ml RNase A and 10 µg/ml propidium iodide (PI). After 20 min incubation at 37°C, cells were analyzed for DNA contents by FACSVantageTM (Becton Dickinson Immunocytometry Systems, San Jose, CA). For each sample, 10,000 events were acquired. Cell cycle distribution was calculated using CELLQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA). Cells treated with 5-FU was used as positive control sample.

Statistical evaluation. Data are presented as mean ± SD. Analysis of variance (ANOVA) with Tukey's post hoc test was used for testing the significance using SPSS® for windows, version 17.0.0. $p < 0.05$ was taken as a cut off value for significance.

Extraction and isolation. The air-dried powdered leaves of *E. cinerea* F. Muell. ex Benth. (500 g) was extracted by Soxhlet apparatus using Chloroform: Methanol (80 : 20) as a solvent mixture to give the dried sideroxylonal-rich extract (SRE)[13,14–17]. The SRE (20 g) was chromatographed on a VLC column (7 cm × 12.5 cm) of Silica

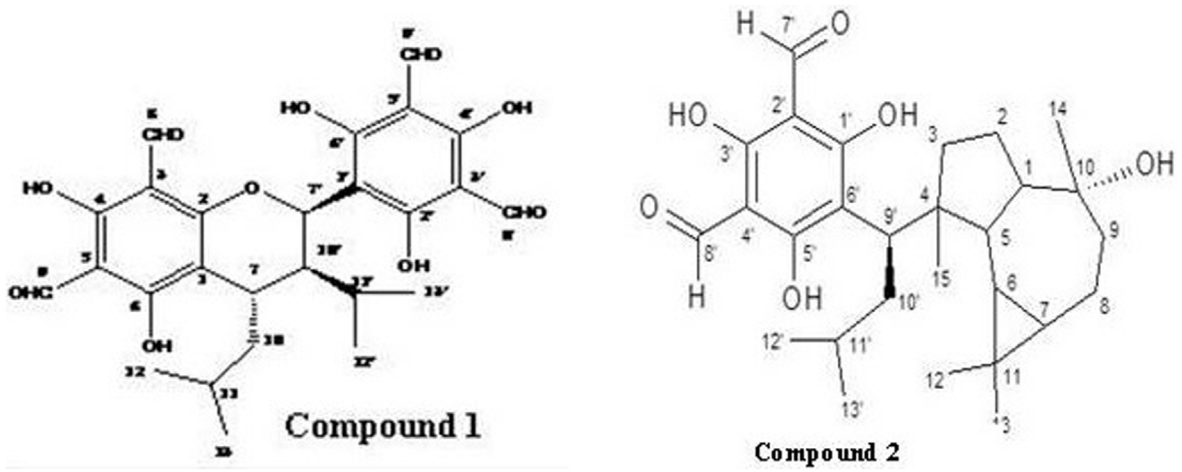

Figure 1 | **The isolated Compounds from the leaves of *Eucalyptus cinerea*.**

gel H (200 g). Gradient elution was carried out starting with *n*-hexane (100%) followed by 10% increments of EtOAc, up to 100% EtOAc, then by MeOH/$CHCl_3$ (5% increments), and finally 20% MeOH. Fractions, each of 200 ml, were collected and monitored by TLC. Similar fractions were pooled together to yield 3 collective fractions (Fr. I-Fr. III). Further isolation and purification of these fractions led to isolation of two acyl phloroglucinol compounds as follows:

Fr. II (4 g): was rechromatographed on a silica gel column (27 × 3 cm). Isocratic elution was carried out using *n*-hexane: ethyl acetate (50 : 50). Fractions, 10 ml each, were collected and monitored by TLC. Subfractions (50–60), were washed with acetone to yield 150 mg of white needle crystals (compound 1).

Fr. III (5 g): was rechromatographed on a silica gel column (27 × 3 cm). Isocratic elution was done with chloroform: methanol (95 : 5) and yielded 500 mg of subfraction (30–40). This subfraction was purified on silica gel pre-packed column; size B applying Medium Pressure Liquid Chromatography (MPLC). Elution was done using chloroform:methanol (95 : 5) as eluent to yield two subfractions named III-A (40–45) and III-B (53–60). Subfraction III-A (40–45), 160 mg, was chromatographed on Sephadex LH-20 column (28 × 1.5 cm) using methanol for elution. Subfractions (10–25) were collected and evaporated (97 mg) and finally chromatographed on silica gel column (40–63 μm, particle size) and (25 × 1 cm) in dimensions, using Chloroform: methanol (95 : 5) for elution to give 37 mg of yellow prisms (compound 2).

Results

Two acyl phloroglucinol compounds (1) and (2) (Figs. 1&2) were isolated from the SRE of the juvenile leaves of *E. cinerea*. Compounds were identified based on their M.P, EI-MS and NMR analyses.

Compound 1. White needle crystals (MeOH, 150 mg), M.P: 215–217°C, UV (MeOH): λ_{max} 280, 380, EI-MS: m/z (rel. int. %): 500 (M^+, 8.24%), 305 (1.25%), 249 (80.11%), 235 (16.8%), 222 (69%), 207 (49%), 195 (100%) ^{1}H-NMR δ ppm (300 MHz, $CDCl_3$): δ_H 0.66 (3H, d, *J* = 6.9, H-12′ or 13′), 0.87 (3H, d, *J* = 6.9, H-12′ or 13′), 0.91 (3H, d, *J* = 6.3, H-12 or 13), 1.01 (3H, d, *J* = 6.0, H-12 or 13), 1.45 (1H, m, H-10a), 1.52 (1H, m, H-10b), 1.62 (1H, m, H-11), 1.90 (1H, m, H-11′), 1.95 (1H, m, H-10′), 2.88 (1H, dd, *J* = 1.5, 10.5, H-7), 5.84 (1H, d, *J* = 2.7, H-7′), 8.53 (1H, s, 2′ or 6′-OH), 9.96 (1H, s, H-8), 10.1 (1H, s, H-8′), 10.14 (1H, s, H-9′), 10.17 (1H, s, H-9). ^{13}C-NMR δ ppm (75 MHz, $CDCl_3$): δ_C 19.62 (C-12′ or 13′), 20.96 (C-12 or 13), 23.74 (C-12′ or 13′), 24.18 (C-12 or 13), 25.54 (C-11), 26.38 (C-11′), 26.62 (C-7), 41.92(C-10′), 44.63 (C-10), 77.55 (C-7′), 100.45 (C-1′), 103.48 (C-3), 103.97 (C-3′, 5′), 105.16 (C-5),108.21 (C-1), 160.67 (C-2), 165.58 (C-2′ or 6′),167.16 (C-2′ or 6′), 167.43 (C-4), 168.78 (C-4′), 169.43 (C-6), 190.07 (C-8), 191.95 (C-8′), 192.16 (C-9′), 192.26 (C-9).

Compound 2. Yellow prisms (Acetone, 37 mg), M.P.: 193–195°C, UV (MeOH): λ_{max} 276, 390, ESI-MS (–ve mode): 471[M-H]$^-$. ^{1}H-NMR δ ppm (600 MHz, $CDCl_3$): δ_H 0.518 (1H, m,H-7), 0.54 (1H,m, H-6), 0.68 (3H, s, H-15), 0.76 (3H, d, *J* = 6.24, H-12′), 0.78 (3H, d, *J* = 6.18, H-13′), 0.92 (2H, m, H-8), 1.07 (3H, s, H-14), 1.089 (3H, s, H-12), 1.089 (3H, s, H-13), 1.12 (1H, m, H-11′), 1.3 (1H, t, H-5),1.4 (2H, qd, H-3), 1.5 (2H, t, *J* = 13.02, H-9a),1.6 (2H, t, *J* = 6.18,12.5, H-9b), 1.64 (2H, m, H-2), 1.8 (1H, m, H-10′a), 1.9 (1H, m, H-1), 2.27 (1H, td, H-10′b), 3.4 (1H, dd, *J* = 4.8,13.08, H-9′), 10.06 (1H, s, H-7′), 10.06 (1H, s, H-8′). ^{13}C-NMR δ ppm (150 MHz, $CDCl_3$): δ_C 16.79 (C-12), 18.8 (C-14), 19.34 (C-11), 20.1 (C-8), 20.4 (C-13′), 21.4 (C-15), 23.6 (C-12′), 24.15 (C-2), 25.8 (C-11′), 27.09 (C-7), 27.5 (C-13), 28.08 (C-6), 34.2 (C3), 35.12 (C-10′), 35.4 (C-9′), 43.7 (C-5), 43.9 (C-9), 48.19 (C-4), 54.06 (C-1), 75.26 (C-10), 105.1 (C-2′), 105.1 (C-4′), 109.17 (C-6′), 168.8 (C-3′), 169.22 (C-5′), 169.95 (C-1′), 191.5 (C-8′), 191.6 (C-7′).

Accordingly, the SRE and the two phloroglucinol derivatives were screened for their anti cancer activity against the three cell lines (MCF7, HEP2 and CaCo cell lines) and 10 FS (normal cell lines). They showed moderate to potent cytotoxic activity against the 3 tested human cancer cell lines and less cytotoxicity against normal cell lines (Table 1). These three human cancer cell lines are the common cancer types in Egypt.

Furthermore, the antiproliferative effect of Sideroxylonal-B and Macrocarpal-A against MCF-7 cell line in addition to the potential inherent resistance of MCF-7 cells was assessed using E_{max} model. The K_d values for Sideroxylonal-B and Macrocarpal-A in MCF-7 cell line was 69.1 ± 4.3 μM and 37.5 ± 5.3 μM, respectively. MCF-7 cell line showed relatively high resistance fraction to treatment with

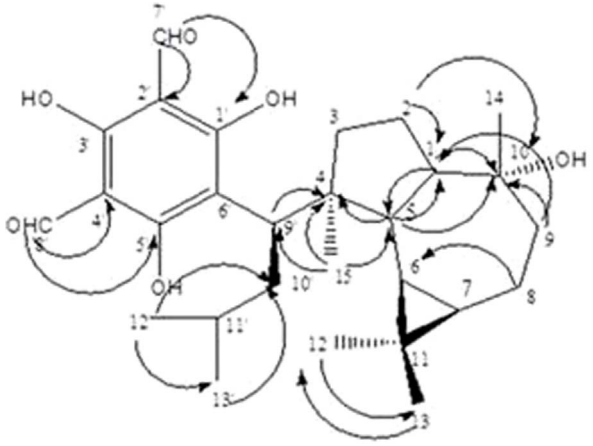

Figure 2 | **HMBC key for Macrocarpal A.**

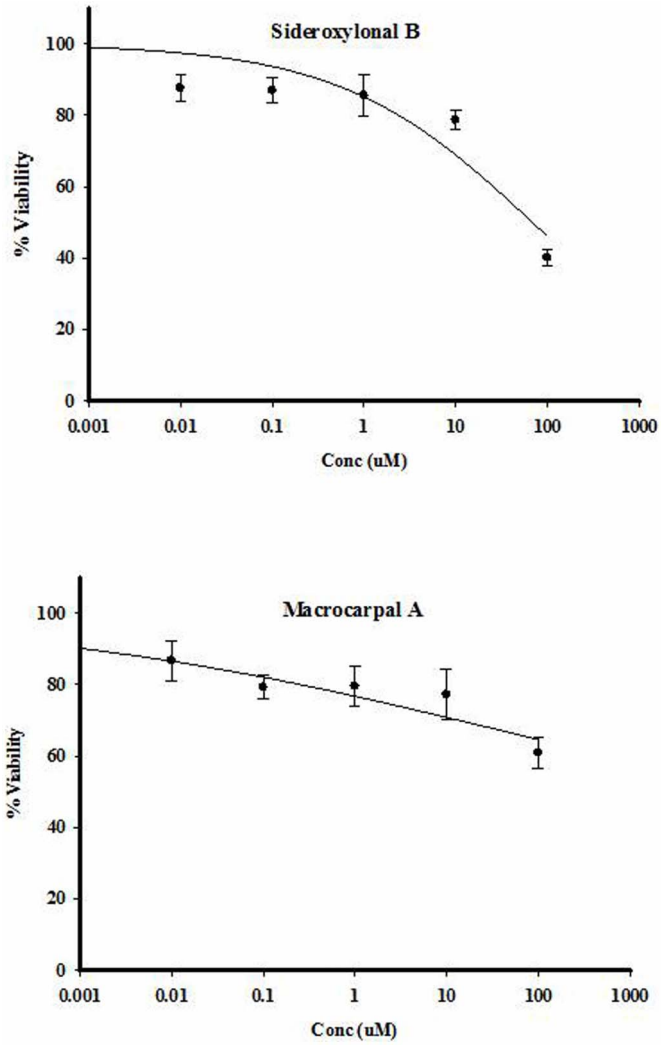

Figure 3 | Dose response curve of Sideroxylonal B (A) and Macrocarpal A (B) against MCF-7 cell line fitted to E_{max} model.

Macrocarpal-A (R-fraction of 35.5 ± 8.7%) while there were significantly lower R-values for Sideroxylonal-B (3.9 ± 0.5%) (Fig. 3).

DNA flow-cytometry was used to evaluate the detailed effect of Sideroxylonal-B and Macrocarpal-A on the cell cycle distribution of MCF-7 cell line compared to 5-FU (Fig. 4). Sideroxylonal-B moderately decreased cell population in S-phase (33.2 ± 0.5%) (Fig. 4-A) to 26.3 ± 0.2% (Fig. 4-B). Reciprocally, Sideroxylonal-B induced compensatory increase in the non-proliferating cell fraction (G_0/G_1-phase) from 61.1 ± 0.6% (Fig. 4-A) to 63.8 ± 0.2% (Fig. 4-B). In addition, Sideroxylonal-B induced significant increase in G_2/M-phase. In contrast, Macrocarpal-A only induced marginal increase in the non-proliferating cell fraction (Fig. 4-C&D) with significant increase in the late apoptotic cell fraction (Pre-G phase).

Discussion

The UV data of compounds (1&2) were similar to those reported for euglobals[19,20]. The EI-MS spectrum of compound 1, showed a molecular ion peak at (m/z) 500 calculated for $C_{26}H_{28}O_{10}$. A characteristic fragment ion at (m/z) 249 resulted from retro-Diel's Alder cleavage of the molecular ion, which further fragmented to give another characteristic ion at (m/z) 195 $[C_9H_7O_5]^+$. The ^{1}H-NMR spectrum of compound 1, showed four methyl groups (δ_H 0.66, 0.87, 0.91 and 1.01, each 3H, d, J = 6.9, 6.9, 6.3 and 6 Hz, respectively) suggesting two isopropyl groups and five methine protons at: δ_H 2.88 (1H, dd, J = 1.5, 10.5, H-7), δ_H 1.95 (1H, m, H-10′), δ_H 1.9 (1H, m, H-11′), δ_H 1.62 (1H, m, H-11), another doublet at δ_H 5.84 (1H, d, J = 2.7 Hz) was typical of sideroxylonals[13] and could be ascribed to oxymethine proton at H-7′. The ^{13}C-NMR spectrum of compound 1, exhibited twelve aromatic carbons, six of which were in the oxy-aromatic region.

^{1}H-NMR spectrum [δ_H 9.96 (1H, s), 10.1 (1H, s), 10.14 (1H, s) and 10.17 (1H, s).] and ^{13}C-NMR spectrum [δ_C 190.07, 191.95, 192.16 and 192.26] confirmed the presence of four formyl groups. Relative stereochemistry of compound 1 was determined by the magnitude of spin coupling constant (J = 2.7 Hz) between H-7′ and H-10′ suggesting a *cis*-relationship between these protons. From the above data and comparing with data of previously isolated sideroxylonals, compound 1 was identified as sideroxylonal B.

The ESI-MS of compound 2, in the negative ion mode, showed a molecular ion peak at 471 $[M-H]^-$. This indicates that the molecular weight of compound 2 is 472, calculated for $C_{28}H_{40}O_6$. The ^{1}H-NMR spectrum of compound 2, showed: two formyl groups attached to a benzene ring at δ_H10.06 (2H, s) corresponding to H-7′ & H-8′. A methine proton adjacent to phloroglucinol moiety at δ_H3.4 (1H, dd, J = 4.8, 13.08) assigned to H-9′ and an isobutyl side chain.

The ^{13}C-NMR spectrum of compound 2, exhibited twenty eight carbon signals confirming the above molecular formula. The ^{13}C-NMR spectrum showed: two formyl groups at (δ_C191.5&191.6) which were consistent with ^{1}H-NMR data, a quaternary carbon attached to a hydroxyl group at δ_C75.26 assigned for C-10. Three aromatic carbons at δ_C105.1, 105.1 and 109.17 which were assigned to C-2′, 4′ and 6′, respectively. Three oxy-aromatic carbons at δ_C 169.95, 168.8 and 169.22 accounting for C-1′, 3′ and 5′, respectively. From the above data and with the aid of DEPT, HMQC and HMBC (Figure 2) spectra, compound 2 was identified as macrocarpal A. The physico-chemical properties, ^{1}H-NMR and ^{13}C-NMR data were also in accordance with those reported for macrocarpal A[21].

This is the first report for isolation of sideroxylonal B and macrocarpal A from *E. cinerea* juvenile leaves. However, sideroxylonal B was previously isolated from *E. sideroxylon* (leaves)[14], *E. loxophleba* (leaves)[13], *E. robusta* (leaves)[22], and macrocarpal A was isolated from the leaves of *E. macrocarpa*[21].

Cancer is a fatal disease and a leading cause of death worldwide with projected 12 million deaths in 2030[23]. Several classes of anticancer drugs have been developed and many of them are of natural origin. Natural products have been the mainstay of cancer chemotherapy for the past 30 years[24]. However, most of the currently used anticancer drugs cause undesirable side effects due to lack of tumor specificity and multidrug resistance. Therefore the search for potent, safe and selective anticancer compounds is crucial for new drug development in cancer research. Natural products, due to their structural diversity, provide excellent templates for the construction of novel compounds[24].

In the course of our continuing search for novel cancer chemotherapeutic agents from natural sources, the Sideroxylonal Rich Extract (SRE) as well as the two phloroglucinol derivatives were screened for their anti cancer activity against the three cell lines (MCF7, HEP2 and CaCo cell lines) along side with human normal cell line.

According to the National Cancer Institute guidelines[25] the extracts with $IC_{50} < 20$ values μg mL^{-1} were considered active. They showed moderate to potent cytotoxic activity against the three tested human cancer cell lines (Table 1) which are considered the most common cancer types in Egypt, where the strongest effect was detected against MCF7 cell line indicating the specific and selective cytotoxicity against this type of cancer cells. On the other hand, Sideroxylonal B (1) was more potent as cytotoxic especially against MCF7 (IC_{50} = 4.4 ± 0.25 μg mL^{-1}) and CaCo cell line (IC_{50} = 4 ± 0.36 μg mL^{-1}) relative to SRE and Macrocarpal A (2).This is the first report for the cytotoxic activity of these naturally occurring compounds. Results confirm that the extract as well as the compounds

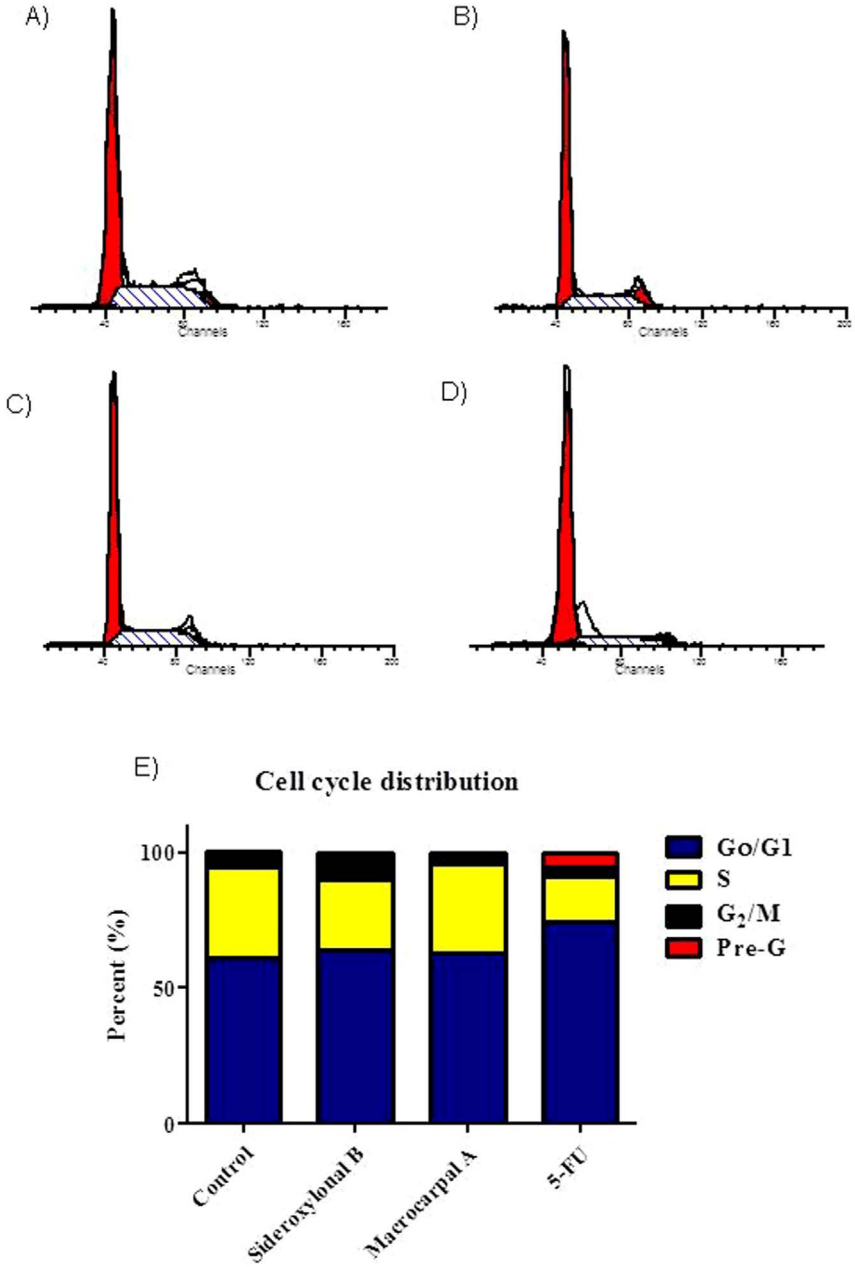

Figure 4 | Cell cycle distribution of MCF-7 cells (A) after treatment with Sideroxylonal B. (B) and Macrocarpal A (C) for 24 h and compared to cell treated with 5-FU (D). Percent of cell phases after treatment are compared in bar chart (E).

could kill cancer cells but do little damage to normal cells and hence are selectively active[25]. The influence of Sideroxylonal-B on cell cycle progression of MCF-7 cells was similar to the antiproliferative profile of 5-FU which decreased the S-phase cell population with compensatory increase in the non-proliferating cell fraction (G_0/G_1-phase) (Fig. 4-D). On the other hand, 5-FU and Macrocarpal-A showed higher cytotoxic potential manifested as increased pre-G apoptotic fraction of MCF-7 cells (Fig. 4-C&D) compared to control untreated group. The antiproliferative effect of 5-FU is attributed to its thymidinylate synthase inhibition and S-phase cell cycle arrest. Herein, the cell cycle profile of cells treated with Sideroxylonal-B and Macrocarpal-A indicates possible S-phase specific effects.

The mode of action of the compounds could be similar to those of euglobals isolated from other species of Eucalyptus[26].

Conclusion. Two cytotoxic acyl phloroglucinol compounds; Sideroxylonal B and macrocarpal A were isolated from the sideroxylonal-rich extract (SRE) of the juvenile leaves of *E. cinerea* F. Muell. ex Benth. cultivated in Egypt. The SRE and the two compounds showed activity against the three tested human cancer cell lines; HEP2, CaCo and MCF7 and low cytotoxicity against normal cell lines indicating their selectivity. The present work is the first report concerning the isolation of these compounds from natural origin and their antiproliferative effect that might help as chemotherapy of three different types of cancer which are common in Egypt.

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Author contributions

A.M.A.; carried out antiproliferative activity, M.M.S.; wrote manuscript and prepared the article in the journal's format, F.R.S. and A.M.H. isolated and identified of the compounds. All authors reviewed the manuscript.

Additional information

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