Ecballium elaterium (L.) A. Rich. seed oil: Chemical composition and antiproliferative effect on human colonic adenocarcinoma and fibrosarcoma cancer cell lines

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ORIGINAL ARTICLE

Ecballium elaterium (L.) A. Rich. seed oil: Chemical composition and antiproliferative effect on human colonic adenocarcinoma and fibrosarcoma cancer cell lines

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KEYWORDS

Ecballium elaterium;
Seed oil;
Chemical composition;

Abstract In this work the physicochemical characteristics including fatty acids, tocopherols and sterols composition of *Ecballium elaterium* (L.) A. Rich seed oil was determined. Results showed that linoleic acid (48.64%) and punicic acid (22.38%) were the major polyunsaturated fatty acids. Among the phytosterols, β-sitosterol was the most abundant (396.25 mg/100 g), while the major

Abbreviations: *E. elaterium*, *Ecballium elaterium* (L.) A. Rich.; GC–FID, Gas chromatography equipped with flame ionization detector; HPLC, high-performance liquid chromatography; HT29, human colon adenocarcinoma cell lines; HT1080, human colon fibrosarcoma cell lines

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Antiproliferative activity;
Colon cancer

tocopherol form was γ-tocopherol (44.23 mg/100 g). In addition, we evaluated for the first time the effect of *E. elaterium* seed oil on the growth of human colonic adenocarcinoma (HT29) and fibrosarcoma (HT1080) cell lines. The original finding was its potent antiproliferative effect on both tumour cell lines. This effect was dose-dependent, with half-maximal inhibition values of IC_{50} = 4.86 μg/ml and 4.16 μg/ml respectively. This pilot study opens the way for further investigation about the potential use of *E. elaterium* as an anticancer agent.

1. Introduction

Wild plant species have always played an important medicinal role in the history of man. Through academic research, pharmaceutical sciences may take profit from the available bioresources, and ethnobotanical as well as ethnopharmacological approaches that would be of interest.

Ecballium elaterium (L). A. Rich. a wild perennial herb with sub-succulent, hairy leaves and stems, is widely used in Tunisian folk medicine and possesses therapeutic activities against a wide range of ailments (Le Floc'h, 1983; Boukef, 1986). The mature seeds are the oil-bearing part of the fruit (Alapetite, 1979) and the watery juice inside the fruit yields a powerful drug called "elaterium". It also contains proteins, lipids, cucurbitacins (B, D, E, I and L,) and cucurbitacin derivatives such as glycosylcucurbitacins and triterpenoids glycosides (Lavie and Szinai, 1958; Erclyes et al., 1989; Heitz et al., 1989; Greige-Gerges et al., 2007). *E. elaterium* is of interest because its fruits extracts are still used in the Mediterranean region in different medicinal systems (Rust et al., 2003; Uslu et al., 2006). Several biological activities of *E. elaterium* and its components, including anticancer activity, have also been reported (Lavie and Szinai, 1958; Chan et al., 2010), but there are no reports on the anticancer activities of the fixed oil of this endemic Mediterranean plant.

Cancer remains one of the most dreaded diseases causing an astonishingly high death rate, second only to cardiac arrest. A high number of new drugs derived from plant secondary metabolites have been applied the treatment and prevention of cancer (Newman et al., 2003; Balunas and Kinghorn, 2005).

To our knowledge the cited literature about seeds oil from *E. Elaterium* only described fatty acid composition (Erclyes et al., 1989). In this work we analyse the physical and chemical properties of the seed oil. Moreover, cell proliferation was fundamental in several biological processes, especially in cancer (Li and Galileo, 2010), we evaluated the effect of *E. elaterium* seed oil on the proliferation of human colon adenocarcinoma (HT29) and fibrosarcoma (HT1080) cell lines.

2. Materials and methods

2.1. Materials

Dulbecco's modified Eagle's medium (DMEM) was purchased from GIBCO (Cergy-Pontoise, France) and foetal calf serum (FCS) from BioWhittaker (Fontenay-sous-Bois, France). Penicillin, streptomycin, silylant reagent *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide, sterols standards, fatty acid methyl esters standards, β-carotene, silica gel F254 plate and aluminium oxide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Potassium hydroxide, *n*-hexane, diethyl ether, ethanol, acetonitrile, acetone, chloroform were purchased from Fluka Chemical Co. (Buchs, Switzerland).

2.2. Extraction of the seed oil

Fruits of *E. Elaterium* were collected in January 2011 from a region of SidiThabet, area of Ariana (Northern Tunisia, latitude 36°54′45.25″N, longitude 10°06′02.10″E, altitude 30 m). The identification was made by Professor S. BEN SAAD (Department of Botany, Faculty of Sciences of Tunis) and voucher specimens (BPn 02-January 2011) were deposited at the above-mentioned laboratory of Natural Substances to serve as a future reference.

Seeds were removed from the mature fruits, washed with water, dried, and then ground by a grinder to pass 500 μm screens. The ground dried *E. elaterium* seeds (50 g) were extracted with 250 mL *n*-hexane using 24 cycles Soxhlet extraction at 80 °C. The solvent was removed via a rotary vacuum distillation at 40 °C. The residue was weighed and stored at −20 °C until analysis. The result was expressed as lipid percentage in the seed powder dry matter.

2.3. Physico-chemical analysis

The refractive index of the seed oil was determined using a Sopelem Series 3296 refractometer (Sopelem, France). The content of chlorophyll pigment was determined according to AOCS Official Method Cc 13i-96 (AOCS, 1998) by measuring absorbance at 630, 670 and 710 nm. The carotenoid content was determined according to official method (958.05) (AOAC, 1999), and expressed as μg of β-carotene per g of oil. The absorbance was recorded at 440 nm (JASCO V- 530, WITEG Labortechnik., Gmbh, Germany) using hexane as blank.

For chemical analysis, acid, iodine and saponification values were determined respectively according to the Norm ISO 660, ISO 3961 (ISO, 1996) and ISO 3657 (ISO, 2002).

2.4. Fatty acid composition

The fatty acid methyl esters (FAME) composition was determined by the conversion of oil to fatty acid methyl esters prepared by adding 1 ml of *n*-hexane to 40 mg of oil followed by 200 μL of sodium methoxide 2 mol/L. The mixture was heated at 50 °C for few seconds followed by the addition of 200 μL HCl 2 mol/L. The top layer (1 μL) was injected onto a Gas chromatography (GC) (Agilent 6890 N, California, USA)

equipped with a flame ionization detector (FID) and a polar capillary column (HP-Innowax polyethylene glycol, 0.25 mm internal diameter, 30 min length and 0.25 μm film in thickness) to obtain individual peaks of fatty acid methyl esters. The detector temperature was 275 °C and the column temperature was 150 °C held for 1 min and increased at the rate of 15 °C/min to 200 °C and the rate of 2 °C/min to 250 and held for four min. The FAME peaks were identified by comparing their retention times with individual standards. The relative percentage of the fatty acid was calculated on the basis of the peak area of a fatty acid species to the total peaks areas of all the fatty acids in the oil sample.

2.5. Tocopherol composition

Prior to the HPLC analysis, 0.5 g of seed oil was diluted with 5 mL *n*-hexane and 5 μL samples were injected. The tocopherol composition of *E. elaterium* seed oil was determined using HPLC (Agilent 1100, CA, USA) consisting of a G1354 quaternary pump, a G1313A standard auto sampler, a G1321A fluorescence detector set at λ excitation of 295 nm, and λ emission of 330 nm according to norm ISO 9936 (ISO, 2006). A normal phase column (Pinnacle II silica); 150 mm × 3.2 mm × 3 μm was used with hexane/isopropanol (99.5/0.5, v/v) as a mobile phase. The system was operated isocratically at a flow rate of 0.5 mL/min at 30 °C. The quantification was based on an external standard method. The mixed tocopherol standards in a hexane solution (2 mg/mL) were prepared from the standard compounds: α-, β-, γ-, and δ- tocopherols (Sigma Chemical Co., St. Louis, MO, USA).

2.6. Phytosterol composition

Separation of phytosterols was performed according to the method ISO 12228 (ISO, 1999). Lipids (250 mg) were refluxed for 15 min with 5 mL ethanolic KOH solution (3%, w/v) after addition of cholesterol (1 mg) as an internal standard. The mixture was immediately diluted with 5 mL of ethanol. The unsaponifiable part was eluted over a glass column packed with slurry of aluminium oxide in ethanol and evaporated in a rotary evaporator at 40 °C under reduced pressure. For the characterization of sterols, a silica gel F254 plate was developed in *n*-hexane/diethyl ether (1:1, v/v) solvent system for the detection of sterols, the thin-layer plate was sprayed with methanol. The sterol bands were scraped from the plate and recovered by extraction with diethyl ether. The sterol trimethylsilyl ether (TMS) derivatives were prepared by adding 100 μL of a silylant reagent *N*-methyl-*N*-(trimethylsilyl) trifluoroacetamide/ pyridine (1/10, v/v) in a capped glass vial and heated at 105 °C for 15 min. A mixture of standard solutions of sterols was prepared by derivatization. The TMS derivatives were analysed using the GC system (Agilent 6890N, California, USA) equipped with a FID (flame ionization detector) and the GC chemstation software. A HP-5 column was used (0.32 mm i.d × 30 m in length; 0.25 μm film in thickness; Agilent, CA, USA). The carrier gas (helium) flow was 1.99 mL/min (split–splitless injection with a split ratio of 1:200). Sterols peak identification was carried out according to the ISO 12228 reference method and confirmed by GC–MS (NIST 2002 database) operating in the same conditions as used for the GC–FID. All analytical determinations were performed at least in triplicate.

2.7. Anticancer activities

2.7.1. Cell culture

The human colonic adenocarcinoma (HT29) and fibrosarcoma (HT1080) cancer cell lines were purchased from American type culture Collection Company. Cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% foetal calf serum and 2 mM L-glutamine as a complete growth medium. Cells were maintained in culture and were incubated at 37 °C in a humid atmosphere of 5% CO_2. When confluence was about 80%, cells were sub-cultured by splitting with fresh medium at needed density for cytotoxicity and proliferation assay.

2.7.2. Cytotoxicity assay

The cytotoxicity was assessed first by measuring the release of lactate dehydrogenase (LDH) activity into the culture medium upon damage of plasma membrane. Suspended HT29 and HT1080 cells were incubated with *E. elaterium* seed oil in 96-well plates (100 μl/well). Total release of LDH (100% toxicity) was obtained by adding 0.1% Triton-X100 in culture medium. The supernatants were collected, clarified by centrifugation 5 min at 600 g and 80 μL was submitted to LDH-based cytotoxicity kit (Sigma) in accordance with the manufacturer's instructions.

In the second time cell viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. Cells were cultured in standard conditions until confluence in microtiter plates. MTT solution (500 μg/mL final concentration) was added to the culture medium 4 h before the end of treatment. Subsequently, the MTT solution was removed and replaced by 100 μL of DMSO in order to dissolve the precipitated formazan crystals. Finally, the absorbance was measured at 550 nm.

2.7.3. Proliferation assay

For the Proliferation assay cells were obtained in single cell suspension by treatment of subconfluent cell monolayers with trypsin. After centrifugation, cells were washed twice and resuspended with culture medium (5000 cells/100 μL). After 2 h, seed oil was added and cells allowed to proliferate for 72 h at 37 °C in a cell culture incubator. Cells were washed and then fixed by 1% glutaraldehyde. After staining by 0.1% crystal violet, cells were lysed with 1% SDS and the optical density was measured at 600 nm by a microplate reader (Sarray et al., 2007).

2.8. Statistical analysis

All data were expressed as means ± standard deviation of three experiments performed in triplicate. Evaluation of the statistical significance for observed differences between amounts of chemical compounds was performed by one-way analysis of variance (ANOVA) followed by Tukey's HSD post-hoc test (RStudio, Version 0.97, Boston, USA). Differences between means are considered statistically significant at $P < 0.05$.

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3. Results and discussion

3.1. Physical properties of seed oil

The yield of oil extraction (weight of extracted oil × 100/dry weight of seed) from *E. elaterium* seeds was 22%. This yield was very close to that of C*ucurbita maxima* belonging to the cucurbitaceae family with a yield of 21.83% (Stevenson et al., 2007). At room temperature, *E. elaterium* seed oil was a yellow liquid having a refractive index of 1.48. This value was similar to that of hexane-extracted almond oils, and exceeded those of most common vegetable oils (1.400–1.478) (Padley et al., 1994). The strong absorbance at 470 nm indicates the presence of a high level of carotenoids (4.69 ± 0.03 mg/kg of oil), accounting for the intense yellow colour of *E. elaterium* seed oil. These carotenoids are beneficial, since they protect against the development of cancer (Verhoeven et al., 1997). The chlorophyll content measured at 630–710 nm, was not significant (0.026 ± 0.04 mg/kg of oil).

3.2. Chemical composition of seed oil

E. elaterium seed oil showed high iodine value (160.12 ± 0.14 g I_2/100 g of oil) due to its high content in unsaturated fatty acids. This value gave the oil a good quality for the production of drying oil used for paint purposes (Eromosele et al., 1997). The iodine value of this plant seed oil was higher than that of *C. maxima* (153.66 g I_2/100 g of oil) (Rezig et al., 2012). The high saponification value (191.57 ± 0.034 mg KOH/g of oil) of *E. elaterium* seed oil indicates high amounts of low molecular weight fatty acids and suggests that this oil can be used for shampoo, shoe-polish and skin cream manufacture (Ajiwe et al, 1998; Cerchiara et al., 2010). This saponification value was higher than that reported for *Cucurbita pepo* (185.3 ± 0.034 mg KOH/g of oil) (Nyam et al., 2009). *E. elaterium* seed oil showed a low acid value (2.06 ± 0.02 mg KOH/g of oil), a value lower than that reported in the literature for kernel seed oil (2.82 ± 0.02 mg KOH/g of oil) (El-Adawy and Taha, 2004). The lower acidity showed that this oil is edible and could have a long shelf life (Nyam et al., 2009).

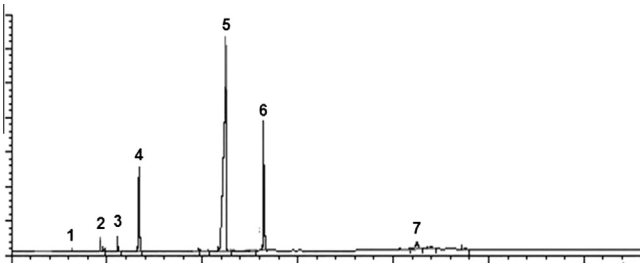

Figure 1 GC-FID chromatogram of fatty acids from *E. elaterium* seed oil. 1: Myristic acid; 2: Palmitic acid; 3: Stearic acid; 4: Oleic acid; 5: Linoleic acid; 6: Punicic acid; 7: Arachidic acid.

3.2.1. Fatty acid composition

Fatty acid composition of the oil extracted from *E. elaterium* seed was shown in (Fig. 1). *E. elaterium* seed oil is characterized by high amounts of unsaturated fatty acids: linoleic acid (48.64%), punicic acid (22.38%) and oleic acid (15.58%) representing about 86% of the total fatty acids (Table 1). These results are in agreement with those found for *Citrullus colocynthis* (L.) containing high levels of linoleic acid (Nehdi et al, 2013). However, in the Cucurbitaceae family, only *E. elaterium* growing wild in Mediterranean region was characterized by the presence of punicic acid (Erclyes et al., 1989).

3.2.2. Tocopherols

Due to its antioxidant properties, the dietary intake of the fat-soluble vitamin E has been suggested to reduce cancer risk (Jiang et al., 2004). Thus, the determination of tocopherol content is extremely important, especially in *E. elaterium* seed oil because there are no data about its chemical composition and biological activities. Table 2 shows the tocopherols content of *E. elaterium* seed oil. As shown in Fig. 2 the major tocopherols were γ- and δ-tocopherols (44.2 mg/100 g and 12.44 mg/100 g, respectively), while α- and β-tocopherols were less abundant (3.62 and 1.82 mg/100 g, respectively).

Table 1 Fatty acid composition of *E. elaterium* seed oil.

Peak number	Retention time (min)	Fatty acid	Carbon length	Composition (%)[a]
1	3.268	Myristic	C14:0	0.09 ± 0.03
2	4.962	Palmitic	C16:0	4.09 ± 0.03
3	5.968	Stearic	C18:0	4.93 ± 0.04
4	7.012	Oleic	C18:1	15.58 ± 0.01
5	11.221	Linoleic	C18:2	48.64 ± 0.03
6	13.248	Punicic	C18:3 (*n*-5)	22.38 ± 0.03
7	21.658	Arachidic	C20:0	0.84 ± 0.02
		SAFA[b]	–	9.95
		MUFA[c]	–	15.58
		PUFA[d]	–	71.02
		P/S[e]	–	7.13

[a] The relative percentage of the fatty acid was calculated on the basis of the peak area to the total peak areas of all the fatty acids in the oil sample. The values are mean ± SD of three experiments.
[b] SAFA, saturated fatty acids.
[c] MUFA, monounsaturated fatty acids.
[d] PUFA, polyunsaturated fatty acids.
[e] P/S: ratio of polyunsaturated/saturated fatty acids.

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Table 2 Tocopherols content of *E. elaterium* seed oil.

Peak number	Retention time (min)	Compounds	Oil[a] (mg/100 g)
1	1.896	α-Tocopherols	3.62 ± 0.03
2	4.112	β-Tocopherols	1.82 ± 0.01
3	5.234	γ-Tocopherols	44.23 ± 0.02
4	7.846	δ-Tocopherols	12.44 ± 0.04
		Total	62.11

[a] The tocopherol composition of *E. elaterium* seed oil was determined using HPLC according to norm ISO 9936. The quantification was based on an external standard method. The mixed tocopherol standards in a hexane solution (2 mg/ml) were prepared from the standard compounds: α-, β-, γ-, and δ-tocopherols. The values are mean ± SD of three experiments.

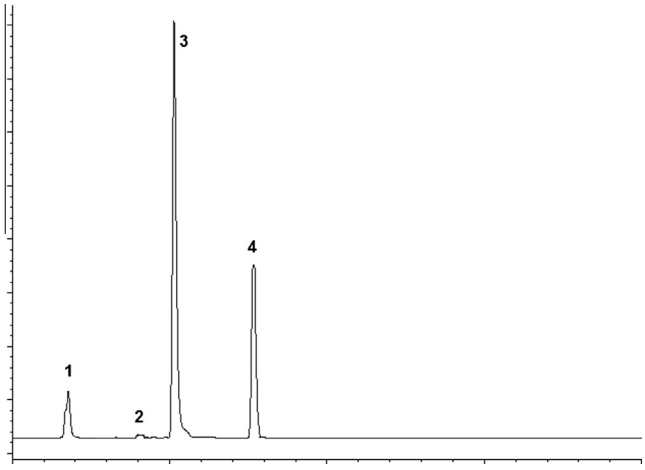

Figure 2 HPLC-FLD chromatogram of tocopherols from *E. elaterium* seed oil. 1: α-tocophérol; 2: β-tocophérol; 3: γ-tocophérol; 4: δ-tocophérol.

γ-Tocopherol represents 71% of total tocopherols. The level of tocopherols in *E. elaterium* (62.11 mg/100 g oil) was very close to that of *C. pepo* (68 mg/100 g oil), a cultivated species of the cucurbitaceae family (Francois et al., 2006) containing high amounts of γ-tocopherol (96% of the total tocopherols). The oil extracted from *C. colocynthis* also contained high amount of γ-tocopherol (116 mg/100 g oil) confirming that γ-tocopherols the major tocopherol isomer in seed oil of the cucurbitaceae family (Nehdi et al., 2013).

3.2.3. Sterols

Sterols were one of the unsaponifiable fractions in many oils. They were of interest due to their impact on health. Recently, sterols have been added to vegetable oils to produce functional food (Ntanios, 2001). Phytosterols played major roles in pharmaceutical, nutrition and cosmetic areas (Fernandes and Cabral, 2007). The phytosterol content in vegetable oils was used as a mean of oil characterization, oil derivatives identification, and for quality determination (Ramadan et al., 2006). Table 3 summarizes the phytosterols content in *E. elaterium* seed oil. As shown in Fig. 3 the most abundant was β-sitosterol (396.25 mg/100 g), which constituted about 50.24% of the total sterol content, followed by campesterol, delta-7-avenasterol, delta-7-stigmastenol, and sitostanol.

Table 3 Phytosterols content of *E. elaterium* seed oil.

Peak number	Retention time (min)	Compound	Oil[a] (mg/100 g)
1	26.168	Desmosterol	8.89 ± 0.01
2	28.985	Campesterol	139.63 ± 0.02
3	32.896	Stigmasterol	6.29 ± 0.01
4	34.942	Delta-7–campesterol	19.18 ± 0.06
5	39.762	β-Sitosterol	396.25 ± 0.08
6	40.248	Sitostanol	21.29 ± 0.09
7	42.734	Delta-5-avenasterol	16.44 ± 0.03
8	44.081	Delta-7–stigmastenol	86.68 ± 0.01
9	46.294	Delta-7-avenasterol	93.81 ± 0.02
		Total	788.57

[a] Separation of sterols was performed according to the method ISO 12228. The unsaponifiable part was eluted over a glass column. For the characterization of sterols, a silica gel F254 plate was developed. The sterols trimethylsilyl ether derivatives were analysed using the GC–FID system after derivatization with silylant reagent. Values are mean ± SD of three experiments.

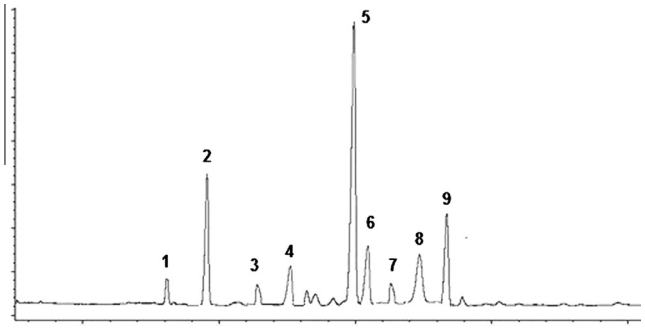

Figure 3 GC-FID chromatogram of sterols from *E. elaterium* seed oil. 1: Desmostérol, 2: Campestérol, 3: Stigmastérol, 4: Delta-7–Campesterol, 5: β-sitostérol, 6: Sitostanol, 7: Delta-5-Avenastérol, 8: Delta-7-Stigmasténol, 9: Delta-7-Avenastérol.

Among the different plant sterols, sitosterol has been one of the most intensively investigated. A high β-sitosterol content was found in the majority of vegetable oils, such as those extracted from olive, grape seed and sunflower seed oil (Feinberg et al., 1987). The present study revealed that β-sitosterol content in seed oil of *E. elaterium* was lower than that of its related family *Citrullus lanatus* (64%), but higher than that of *C. colocynthis* (16.95%) (Badifu, 1991; Talabani and Tofiq, 2012).The presence of phytosterols in food systems may bring nutritional and functional benefits (Ntanios, 2001). It thus seems interesting to test the possibility of incorporating *E. elaterium* seed oil into food and pharmaceutical products.

3.3. Anticancer activities

3.3.1. Cytotoxicity

Cytotoxicity was first assessed by the release of lactate dehydrogenase (LDH), as a marker for cell membrane integrity after exposure to seed oil. Using the LDH assay, we found that a concentration up to 5 μg/mL of seed oil did not significantly induce detectable cytotoxicity on human colonic adenocarcinoma HT29 (Fig. 4a) and fibrosarcoma HT1080 (Fig. 4b) cell lines. Thus, *E. Elaterium* seed oil did not induce membrane damage after a short-term incubation mimicking the pre-

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incubation step. In the second time, long-term cell viability was evaluated by MTT assay. As displayed in Fig. 4c, the viability of HT29 and HT1080 cells incubated for 72 h was not affected by 5 μg/mL oil and only slightly affected by concentrations up to 25 μg/mL.

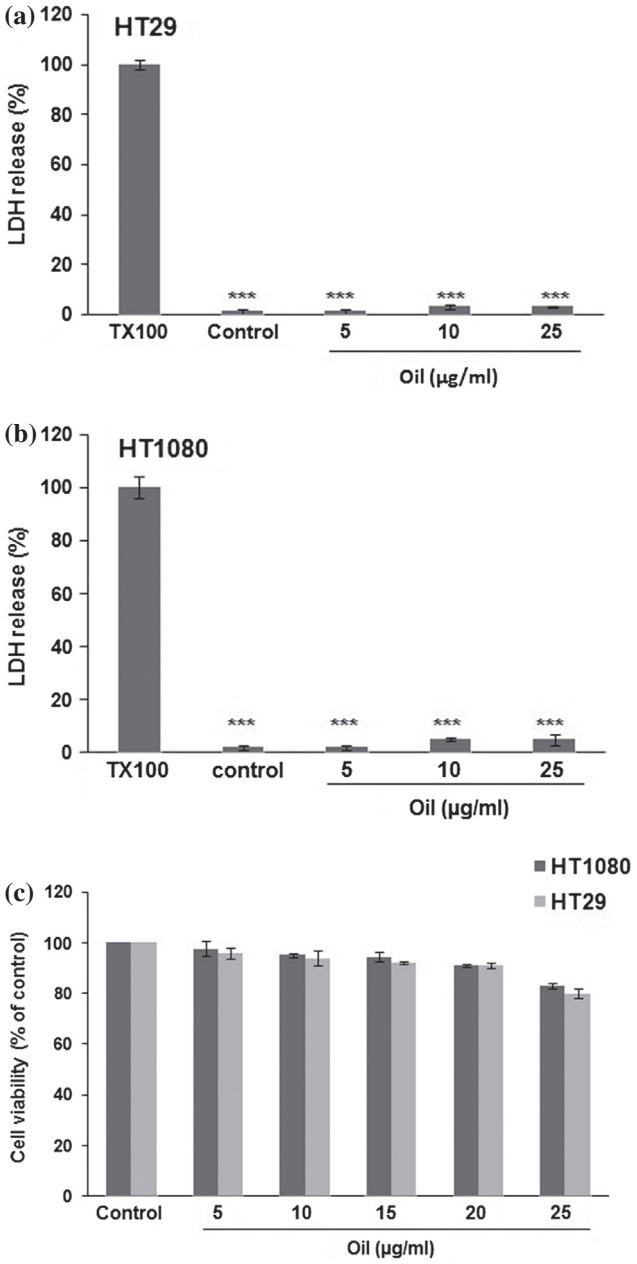

Figure 4 *E. elaterium* seed oil cytotoxicity. LDH assay: Suspended HT29 (a) and HT1080 (b) cells were incubated at room temperature with various concentrations of *E. elaterium* seed oil in 96-well plates. Supernatant were submitted to LDH activity using a colorimetric assay. Total release of LDH (100% toxicity) was obtained by adding 0.1% Triton-X100 during the incubation period (TX100). Data shown are means (± SD) from 3 experiments performed in triplicate. Values of $^{***}p < 0.01$ were considered statistically highly significant. (c) MTT assay: HT29 and HT1080 cells were cultured with different concentrations of *E. Elaterium* seeds oil. MTT solution was then added for 4 h. The MTT solution was removed and replaced with 100 μL of DMSO into each well in order to dissolve the precipitated formazan crystals. Finally, the absorbance was measured at 550 nm.

3.3.2. Seed oil of E. elaterium inhibits the proliferation of cancer cell lines

In order to know whether seed oil interferes with proliferation, we followed the growth of HT29 and HT1080 cells in the absence or in the presence of the oil. For both cell lines, the number of cells in the wells was reduced by the presence of the seed oil in culture medium. The proliferation of both human cell lines was dramatically decreased in the presence of 5 μg/mL of seed oil and completely abolished in the presence of 25 μg/mL, without being cytotoxic as we demonstrated using LDH and MTT assay. The inhibitory effect of tumour cell proliferation was dose-dependent with a half-maximal inhibition (IC_{50}) of 4.86 μg/mL and 4.16 μg/mL for HT29 and HT1080 cell lines respectively (Fig. 5).Thus, oil clearly appears to be a potent inhibitor of tumour cell proliferation.

Our results show that seeds oil from *E. Elaterium* was found to be a rich source of polyunsaturated fatty acid, especially linoleic and punicic acids. A high ratio of polyunsaturated/saturated fatty acids (7.13) can have beneficial effects on cancer prevention and treatment (Grossmann et al., 2010; Rosa et al., 2012). Linoleic acid has been reported to have significant activity in inhibiting mammary carcinogenesis (Ip et al., 1997). It also suppresses the proliferation of human breast adenocarcinoma cells (MDA-MB468) in SCID mice (Visonneau et al., 1997). Punicic acid isolated from *Punica granatum* has been found to have various health benefits, including antiproliferative activities against breast cancer cell line MDA-MB-231. Furthermore, it induces apoptosis in breast cancer cell lines (Grossmann et al., 2010). Many investigations have demonstrated that omega-6 (n-6) fatty acid inhibits growth of cancerous cells *in vitro*. *E. elaterium* was the only species in the Cucurbitaceae family characterized by the presence of punicic acid in seed oil. Thus *E. elaterium* can be considered as a promising source of plant-derived anticancer agents. *E. elaterium* seed oil was also characterized by the occurrence of monounsaturated fatty acids, such as oleic acid (18:1n-9), that play an active role in the prevention of human cancer. Oleic acid, the main monounsaturated fatty acid of olive oil, enhances the growth inhibitory effects of trastuzumab in breast cancer cell lines SK-Br3 and BT-474 (Menendez et al., 2005).

β-Sitosterol, the major phytosterol in *E. elaterium* seed oil, displays antiproliferative activity against colonic cancer cell lines COLO 320 DM (Baskar et al., 2010). Besides, γ-tocopherol shows a great ability to inhibit proliferation of prostate and lung cancer cells by interrupting the sphingolipid pathway synthesis (Jiang et al., 2004). This suggests that these natural compounds found as the major tocopherol forms in *E. elaterium* seed oil may have a high potential in the prevention of human cancer.

Previous studies have shown that vegetable oil is an ideal candidate for use in cancer prevention and treatment (Sotiroudis et al., 2003). There is also evidence that lin seed has anticancer effects, favourably affects breast cancer risk and decreases cell proliferation (Thompson et al., 2005). Experiments have demonstrated that virgin olive oil is capable to inhibit various key stages in the colon HT115 and HT29 carcinogenesis pathway (Gill et al., 2005). In the same way,

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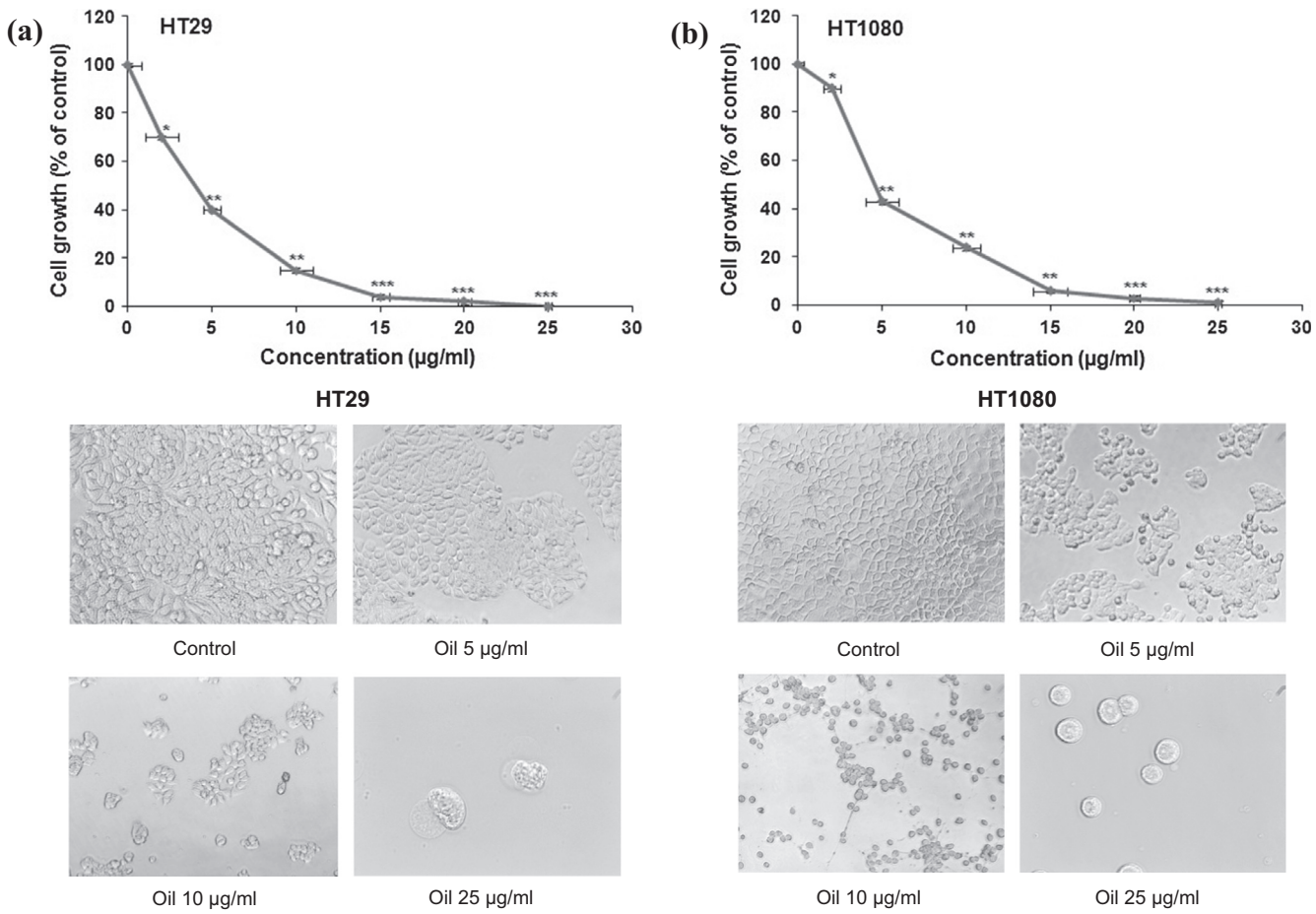

Figure 5 *E. elaterium* seed oil inhibits colon cancer proliferation of adenocarcinoma HT29 (a) and fibrosarcoma HT1080 (b) human cell lines. Cells (5,000 per well) were seeded in culture medium for 2 h at 37 °C. Different concentrations of seed oil were then added and cells were allowed to proliferate for 72 h. Cells were quantified by staining with 0.1% crystal violet, solubilization with 1% SDS and measure of absorbance at 600 nm. Metamorph imaging software was used to capture representative photographs after treatment of cells without (control) or with 5, 10 or 25 µg/ml of oil. Data shown are means (± SD) from 3 experiments performed in triplicate. $P < 0.05$ was considered statistically significant and indicated with asterisks over the value $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

polyphenols and sterols from argan oil exhibit strong antiproliferative activity against three human prostatic cell lines PC3, DU145, and LNCaP (Bennani et al., 2007).

Our data demonstrate that *E. elaterium* seed oil inhibits tumour cell proliferation of HT29 and HT1080 cell lines with a half-maximal inhibition (IC_{50}) of 4.86 µg/mL and 4.16 µg/mL, respectively. These concentrations were close to IC50 values observed with *E. elaterium* freeze-dried extract (Bohlooli et al, 2012) against growth of human gastric carcinoma cell lines (2.5 µg/mL), but much lower than that reported in the case of human oesophageal squamous cell carcinoma cell lines (500 µg/mL). This antiproliferative activities have been attributed to cucurbitacins (Bernard and Olayinka, 2010). Thus, Attard and Cuschieri (2004) have previously reported that cucurbitacin E extracted from *E. Elaterium* exhibits a marked effect on proliferation of prostate adenocarcinoma, melanoma and breast carcinoma cell lines.

4. Conclusions

This study offers an overview of the chemical composition of the seed oil extracted from *E. elaterium* growing wild in Tunisia. It revealed that the oil is a rich source of many important nutrients. The major found fatty acid is linoleic acid. The phytosterol marker is β-sitosterol and γ-tocopherol is the major vitamin form. This composition may explain the potent anticancer activity, especially inhibition of colonic adenocarcinoma and fibrosarcoma human cancer cell lines proliferation. Thus *E. elaterium* seed oil could be used in developing new strategies for the prevention and treatment of human cancer.

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