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Cytotoxic activity and phytochemical analysis of *Arum palaestinum* Boiss.

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ABSTRACT

Objective: To evaluate the *in vitro* cytotoxic activity of the fractionated extract as well as isolated compounds of *Arum palaestinum* Boiss. (*A. palaestinum*) (black calla lily), and to identify the volatile components which may be responsible for the potential antitumor activity.

Methods: *A. palaestinum* was collected from its natural habitats and subjected to phytochemical analysis for separation of pure compounds. *In vitro* cytotoxic activity was investigated against four human carcinoma cell lines Hep2, HeLa, HepG2 and MCF7 for the fractionated extract and isolated compounds. While, the diethyl ether fraction was subjected to GC–MS analysis as it exhibited the most potent cytotoxic effect to evaluate the active constituents responsible for the cytotoxic activities.

Results: Four flavonoid compounds were isolated (luteolin, chrysoeriol, isoorientin, isovitexin) from the diethyl ether and ethyl acetate. The extracts and the pure isolated compounds showed a significant high antiproliferative activity against all investigated cell lines. The GC–MS analysis revealed the separation and identification of 15 compounds representing 95.01% of the extract and belonging to different groups of chemical compounds.

Conclusions: The present study is considered to be the first report on the cytotoxic activities carried out on different selected fractions and pure compounds of *A. palaestinum* to provide evidences for its strong antitumor activities. In addition, chrysoeriol and isovitexin compounds were isolated for the first time from the studied taxa.

1. Introduction

Arum L. is a genus of flowering plants belonging to family Araceae, represented by 26 species and distributed in Northern Africa, Mediterranean Region, Western Asia, and Europe [1]. *Arum palaestinum* Boiss. (*A. palaestinum*), known as black calla lily, it is edible and considered as ornamental plant, and used in folk medicine to cure several chronic diseases such as stomach acidity, atherosclerosis, cancer and diabetes [2,3]. The preservation and conservation of *A. palaestinum* were carried out by Shibli *et al.* and Farid *et al.* [4,5].

A few publications are about the phytochemical investigation and biological activity of *A. palaestinum*. It is characterized by presence of alkaloids, proanthocyanidins, flavones and their C-glycosides and flavonols [6,7]. A comprehensive metabolite profiling of *A. palaestinum* leaves by using liquid chromatography tandem mass spectrometry was studied by Abu-Reidah *et al.* [8]. It is worth to mention that in all published reports of its phytochemical studies, leaves and flowers of *A. palaestinum* were used and evidences for strong antitumor activities of its extracts and isolated compounds were provided [1,9].

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The present study aims to evaluate the *in vitro* cytotoxic activity of the fractionated extract as well as isolated compounds of *A. palaestinum* against four human carcinoma cell lines Hep2, HeLa, HepG2 and MCF7, and to identify the volatile components which may be responsible for the potential antitumor activity by using gas chromatography–mass spectrometry (GC–MS) through preliminary analysis.

2. Materials and methods

2.1. Plant collection

A. palaestinum Boiss. was collected from their growing habitats in Bergesh protected area, Irbid, Jordan (latitude: 32°25′43.1682″ N and longitude: 35°46′47.0094″ E) in February 2012 by Dr. Ahmed El-Oqlah, Department of Biological Sciences, Yarmouk University, Jordan.

2.2. Extraction and isolation

The aerial parts of *A. palaestinum* were air dried and ground in the laboratory, and the obtained powder (1 450 g) was extracted three times at room temperature with 70% methanol for an interval of six days. The aqueous-methanol extract was evaporated under reduced pressure and temperature to obtain a residue of 302 g. The residue was dissolved in distilled water (500 mL) and fractionated by using diethyl ether (44 g), dichloromethane (26 g), ethyl acetate (40 g), butanol (75 g), methanol (56 g) and water (58 g) according to their polarity, then the fractions were subjected to biological analysis. Diethyl ether and ethyl acetate fractions were subjected to 3 mm paper chromatography by using bulk acoustic wave as eluent to yield compound 1 (7.0 mg), compound 2 (3.8 mg), and compound 3 (3.5 mg) from the diethyl ether fraction; and compound 4 (4.3 mg) was isolated from the ethyl acetate fraction. Each individual compound was subjected to a Sephadex LH-20 column for the final purification.

2.3. Structure elucidation of the isolated compounds

The identification of compounds was determined by R_f values, color reactions, ultraviolet spectrophotometry, 1D-NMR spectroscopy, and electron impact ion source/mass spectrometery. Trace compounds were identified by co-chromatography with reference samples which were obtained from Department of Phytochemistry and Plant Systematics, National Research Center, Egypt.

2.4. Cell culture and in vitro cytotoxic activity

Four human tumor cell lines, epidermal carcinoma of larynx (Hep2), cervix (HeLa), liver (HepG2) and breast (MCF7) were used in the study and obtained from the American Type Culture Collection, Minisota, USA. The cell lines were maintained at the National Cancer Institute, Cairo, Egypt, by serial subculturing. The samples were prepared by dissolving 1:1 stock solution in dimethylsulfoxide at 100 mmol/L and stored at −20 °C.

The cytotoxic activity of the fractionated extract and pure compounds were carried out by using sulforhodamine-B (SRB) assay following the method reported by Vichai and Kirtikara [10]. SRB is a bright pink aminoxanthrene dye with two sulphonic groups. It is a protein stain that binds to the amino groups of intracellular proteins under mildly acidic conditions to provide a sensitive index of cellular protein content. Cells were seeded in 96-well microtiter plates at initial concentration of 3×10^3 cell/well in a 150 µL fresh medium for 24 h before treatment with the extract to allow attachment of cells to the wall of the plate. The extract as well as pure compounds at different concentrations (0.0, 5.0, 12.5, 25.0 and 50.0 µg/mL) were added to the cell monolayer in triplicate. Monolayer cells were incubated with the compounds for 48 h at 37 °C in an atmosphere of 5% CO_2. After 48 h, cells were fixed, washed and stained with SRB. Excess stain was washed with acetic acid and attached stain was recovered with ethylenediaminetetraacetic acid–Tris buffer. Color intensity was measured at 570 nm with an ELISA reader. The relation between surviving fraction and drug concentration was plotted to obtain the survival curve of each tumor cell line as compared with doxorubicin, the control anticancer drug.

2.5. GC–MS

GC–MS analysis was performed on a thermo scientific TRACE GC ultra gas chromatograph directly coupled to ISQ single quadrupole mass spectrometer, by using non-polar 5% phenyl methylpolysiloxane capillary column (30.00 m × 0.25 mm × 0.25 µm) type TG-5MS under the following conditions: oven temperature program from 60 °C (3 min) to 280 °C at 5 °C/min, then isothermal at 280 °C for 5 min. The carrier gas was helium at flow rate of 1 mL/min. The volume of injected sample was 1 µL of sample in diethyl ether using splitless injection technique, ionization energy 70 eV, in the electronic ionization mode. The identification of the compounds were carried out by matching their fragmentation patterns in mass spectra with those in Wiley Registry 9th and NIST11 GC–MS libraries.

3. Results

3.1. Structure elucidation of the isolated compounds

The present work dealed with the isolation and characterization of four compounds: luteolin (Compound 1), chrysoeriol (Compound 2), isoorientin (Compound 3), isovitexin (Compound 4). Compounds 2 and 4 were isolated for the first time from the studied taxa.

Table 1

IC_{50} of the antitumor activity of fractionated extract and pure compounds of *A. palaestinum* against the four carcinoma cell lines. µg/mL.

Extract	IC_{50}			
	Hep2	HeLa	HepG2	MCF7
Crude extract	23.60	23.90	14.20	17.80
Diethyl ether fraction	3.83	4.13	3.68	3.53
Dichloromethane fraction	10.30	11.30	8.63	6.23
Ethyl acetate fraction	33.80	31.90	12.50	27.20
Methanol fraction	38.90	29.50	15.20	28.40
Water fraction	22.70	18.80	19.60	26.90
Luteolin	9.98	16.00	17.80	21.80
Chrysoeriol	12.20	16.40	23.00	18.20
Isoorientin	18.80	32.30	20.00	19.90
Isovitexin	22.70	20.50	21.70	18.50
Doxorubicin standard	3.73	4.19	4.28	4.43