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Biochemical Study of Some Active Ingredients in *Helianthus tuberosus* L.

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ABSTRACT

Jerusalem artichoke (*Helianthus tuberosus* L.) yields a high level of carbohydrates and low calorie bulking agent. Total sesquiterpenes were extracted from leaves and one major compound, heliangine, was isolated and identified by different spectral tools. Inulin was prepared from the tubers. Cytotoxic investigations of different substances were performed on four cell lines: Total sesquiterpenes were potent followed by heliangine; inulin did not exhibit this effect.

Keywords: heliangine, identification, isolation, inulin, sesquiterpene lactone

INTRODUCTION

Jerusalem artichoke (JA) originates from America, but exact region of its origin still remains controversial. It is an alternative plant, useful in many ways. It is interesting because of its high sugar content, primarily inulin, productivity and possibility of cultivation on marginal land. JA is a good source of fructose, useful in the food industry and for pharmaceuticals (Danilčenko *et al*. 2008).

In folk medicine, JA tubers are used as a lactagogue, stimulant and tonic, having mild laxative effect for infantile and elderly patients. Leaves and flowers, as a decoction, are used as a diuretic, sedative and antipyretic in addition to their high content of potassium nitrate, which is known to be a blood purifier. The herb as a whole is beneficial for gout, rheumatism and in obesity reducing fats from tissues; furthermore, it is used for diabetic patients (Abou-Hussein 2000).

Another important secondary product which occurs in leaves is the sesquiterpene lactones, characteristic of the Asteraceae (Teixeira da Silva *et al*. 2005). These sesquiterpenoids have pronounced bitter sensory qualities and are therefore believed to contribute to the plants' defense against herbivores (Rees and Harborn 1985) and also exhibit a widely range of bioactivities which include toxicity for certain cancer cell lines (Zidorn *et al*. 1999; Im *et al*. 2007), anti-inflammatory (Wang *et al*. 2008; Büchele *et al*. 2010), and anti-infectious properties (Boulanger *et al*. 2007).

Pan *et al*. (2009) reported the isolation of different compounds from which 4,15-isoatriplicolide methylacrylate and 4,15-isotriplicolide angelate were most cytotoxic against MCF7 cell lines. These compounds are germacrane sesquiterpene lactones found only in leaves of JA. The objective of this investigation was to study the cytotoxic activity of total sesquiterpenes, heliangine and inulin isolated from JA.

MATERIALS AND METHODS

Plant material

Jerusalem artichoke was obtained from Department of Crop Science Faculty of Agriculture, Ain Shams University, Cairo, Egypt.

Isolation of inulin

Tubers were immersed immediately in boiling water for 4 min, then immersed in cold water followed by the addition of an antioxidant agent (acetic acid) to inhibit the oxidation of phenols. Sliced tubers (0.1 kg) were mixed with 100 ml of hot water >80°C to extract inulin, the mixture was kept at 80-100°C for 20-30 min, while the juice was collected by pressing through cloth filter. The colorless effluent was concentrated under vacuum after adjusting the pH to 6.5-7.0. The inulin obtained was dried and ground. The final JA inulin prepared was a colorless and odorless white powder (Kim 1975).

Isolation of sesquiterpene lactone from JA leaves

5 kg of powdered JA leaves were extracted till exhaustion with 10 l of ethanol (95%) by percolation. The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 50°C; a sticky dark green residue was obtained. A constant weight (60 g) of the residue was dissolved in 60 ml hot water, filtered and then the filtrate was successively extracted with light petroleum (60-80°), chloroform and finally with ethyl acetate. The ethyl acetate extract was fractionated by hexane which yields precipitate crystals when evaporated. A crystallized precipitate was checked on TLC which revealed one spot with a reddish violet color when sprayed with vanillin (Mallinckrodt. Inc. Paris, Kentucky, 2.5% in ethanol) (Abou-Hussein 2000).

Identification of isolated compound

The crystalline compound isolated from the leaves JA plant was spectrometrically analyzed by IR (KBr) EI/MS, Finnigan mat SSQ 7000 (Thermo Inst., system Inc., USA), ^{1}H-NMR spectra were recorded in ($CDCl_3$) at 270 or 500 MHz on a Varian Mercury.

Cytotoxic activity

1. Cell culture

The human carcinoma cell lines: hepatocellular carcinoma (Hep G2), colon carcinoma (HCT-116), breast carcinoma (MCF-7) and lymphoblastic leukemia cells (1301) were purchased from ATCC, VA, USA. Cells were routinely cultured in DMEM (Dulbeco's

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Research Note

Modified Eagle's Medium). Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 U/ml penicillin G sodium, 100 U/ml streptomycin sulphate, and 250 ng/ml amphotericin B (Cambrex, Bioscience Copenhagen, Denmark). Cells were maintained in humidified air containing 5% CO_2 at 37°C. Monolayer cells were harvested using trypsin /EDTA, except for RAW 264.7 cells, which were collected by scraping. Compounds were dissolved in DMSO (99.9%) and diluted 1000-fold in the assays (Pinello *et al*. 2009**)**. In all the cellular experiments, results were compared with DMSO-treated cells. All experiments were repeated four times, unless mentioned, and the data was represented as means ± S.D.

2. Cytotoxicity assay

The effect of the tested extracts on the growth of different carcinoma cell lines was estimated. Cells (5×10^4 cells/well) were suspended in serum-free media. The cells were plated separately in a sterile flat bottom 96-well microplate (Greiner, Germany), and treated with 20 µl of each tested extract with a final concentration of 100, 50, 25, and 12.5 µg/ml for 24 h at 37°C in a humidified 5% CO_2 atmosphere.

After incubation, media were removed and 40 µl MTT solution/well were added and incubated for an additional 4 h. MTT assay was prepared according to the method of Hansen *et al*. (1989). The color was measured photometricaly and the absorbance was measured at 570 nm using microplate ELISA reader (Rainbow, Tecan, Germany). Four repeats were prepared for each concentration and the average was calculated. Data were expressed as the percentage of relative viable cells compared with DMSO-treated cells. The percentage of relative viable cells was calculated using the following equation:

$$\frac{\text{Absorbance of extract-treated cells} \times 100}{\text{Absorbance of control cells}}$$

Cytotoxic concentration was expressed by half maximal inhibitory concentration (IC_{50}), which was calculated from the equation of the dose curve for each extract.

RESULTS AND DISCUSSION

Identification of isolated compound

Heliangine: A colorless prismatic crystal (150 mg), m.p. 238–242°C. IR (KBr): 3435 (OH), 1749 (Iactone), 1708 (ester), 1650 (C=C). EI/MS *m/z*: 362 $C_{20}H_{26}O_6$, 279 ($C_{15}H_{19}O_5$), 261 ($C_{15}H_{17}O_4$), 245 ($C_{15}H_{17}O_3$), 83 (100%) (C_5H_7O). H^1 NMR: see **Table 1**.

Table 1 NMR spectral data for compound isolated from JA.

Position	H^1NMR	Multiplicity	*J* coupling Hz
	δ_H	triplet	
1	2.45	dd	
2	2.4	singlet	
3	4.5		
4		doublet	0.5
5	6.4	doublet	0.5
6	5.6	multi	
7	2.7	doublet	2
8	6.6	dd	
9	2.8		
10			
11			
12		doublet	2
13	5.3, 6.6	singlet	
14	1.65	doublet	
15	1.8		
1'		Broad multi	
2'			
3'	6.8		
4'	1.65		
5'	1.5		

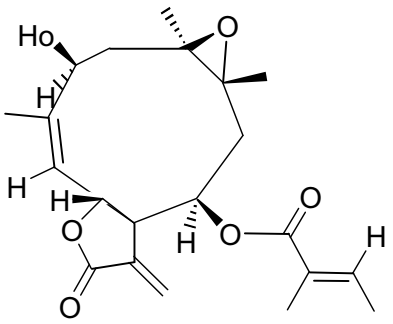

Fig. 1 Heliangine structure.

Cytotoxic activity

The results obtained are illustrated in **Figs. 1** and **2** regarding the effect of the different extracts and isolated substances in the four cell lines. Total sesquiterpene lactones (D1) was the potent cytotoxic fraction for 1301 leukemia cells; IC_{50} was 20.16 µg/ml followed by its effect on HCT 116 cells, with an IC_{50} of 34.98 µg/ml.

Heliangine (D2) ranked second regarding its effect on Hep G2 cells and breast cancer carcinoma MCF7; the IC_{50} was 46.66 for both cell carcinoma lines. The IC_{50} values for other carcinoma cells were high and hence had no potent cytotoxic activity. On the other hand inulin (D3) showed no cytotoxic activity on any of the cell lines tested, although it was reported to have cytotoxic activity *in vivo* (Taper and Roberfroid 2000a, 2000b, 2005). Total sesquiterpenes are more potent than the isolated heliangine and this may be due to the synergistic effect between sesquiterpenes. Until now, most protocols of clinical cancer treatments use a combination of several anticancer agents which act in synergism. In this regard, Düchler and Stepnik (2008) investigated the combination of caffeic acid phenethyl ester, methylglyoxal, and parthenolide showed the highest toxicity towards leukemic cells. These findings indicate that leukemia cell death could be induced efficiently and selectively by selective drug combinations.

Chen *et al*. (2007) reported that isocostunolide, a sesquiterpene lactone isolated from *Inula helenium*, effectively induced cytotoxicity in three cancer cell lines (A 2058, HT-29 and Hep G2) with an IC_{50} 3.2, 5.0 and 2.0 µg/ml, respectively.

Bruno *et al*. (2005) surveyed several natural and synthetic sesquiterpene lactones with different skeletons tested on nine cancer cell lines. Elemane, heliangolane and their hydroxy analogues, all containing an α, β-unsaturated aldehyde substituent, which were the most potent cytotoxic compounds.

Kim *et al*. (2007) isolated four known germacranolide sesquiterpene lactones from the chloroform-soluble fraction of the whole plants of *Carpesium triste* var. *manshuficum*. All the isolates showed significant cytotoxicity (ED~0 value: 4.3-16.8 ~M) against five human tumor cell lines; A549, SKor-3, SK-MEL-2, XF498 and HCT15.

CONCLUSION

In summary, the results obtained in the present study clearly demonstrate that the sesquiterpene lactones derived from JA leaves, are fairly active compounds for *in vitro* cytotoxic activity against 1031 leukemia and HCT 116 cell lines, while heliangine showed moderate activity against Hep G2 and breast cancer MCF7 cell lines. Inulin had no anticancer activity against the four cancer cell lines. Further efforts are underway to isolate and identify other compounds from this plant species. Our data may contribute to a rational basis for the use of JA extract in possible therapy of diseases related to oxidative stress. These results obtained also indicated that inclusion of JA as a dietary supplementary has beneficial effects for human health.

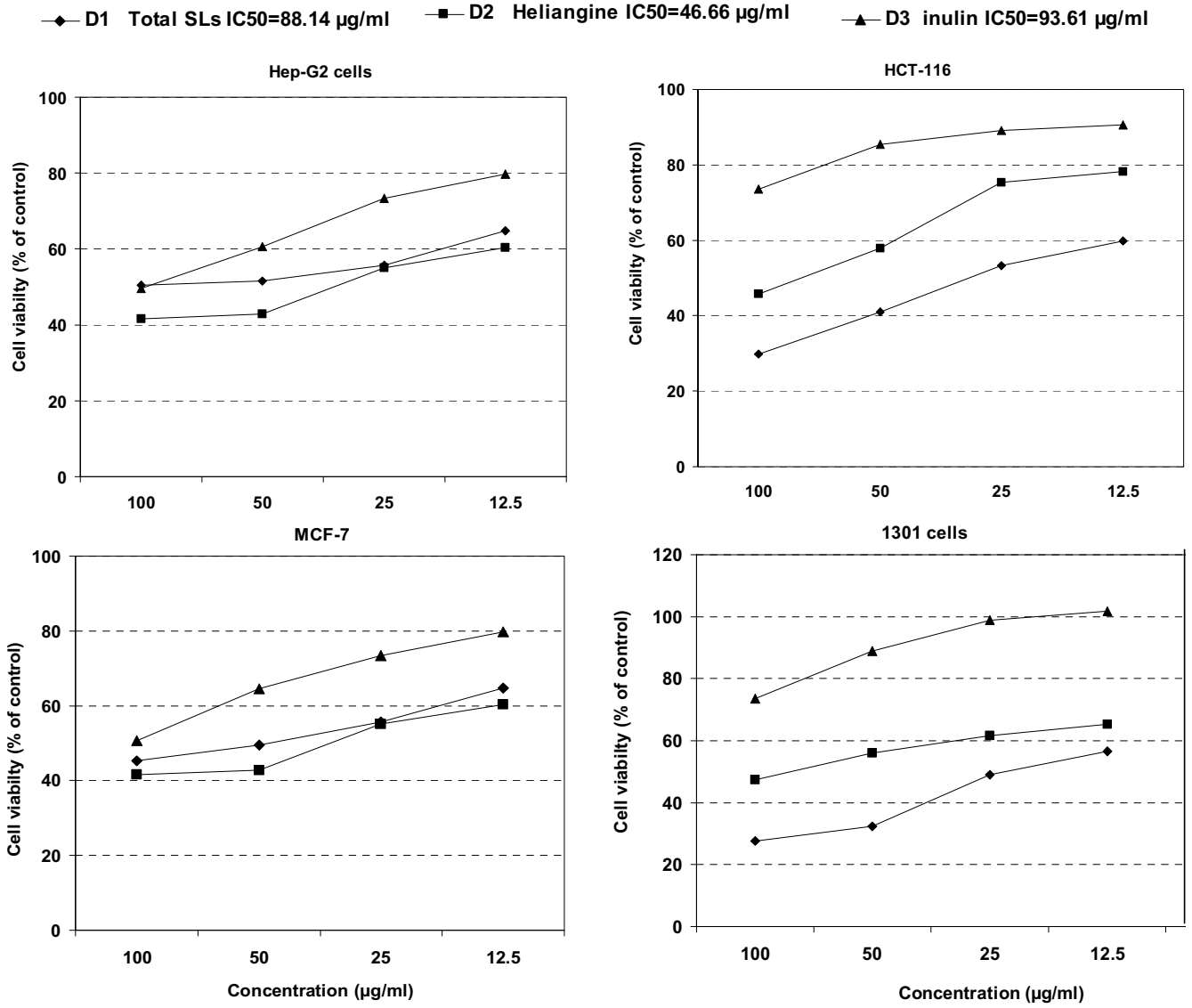

Fig. 2 Effect of total sesquiterpene (D1), heliangine (D2) and inulin (D3) on HepG2, HCT-116, MCF-7 and 1301 cells.

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