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Evaluation of Volatile Composition, Antitumor and Antioxidant Activities of Libyan *Sonchus oleraceus*

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Abstract: *Gas chromatography mass spectrometry (GC-MS) data showed the identification of eighteen volatile compounds in the chloroform extract of the sonchus oleraceus, among them two were found to be the major components 1,1-Diethoxypropan-2-ol 45.20% and Phytol 25.27%. The extract have a good antitumor activity against all tested cell lines under investigation. Also antioxidant activity measured by 1,1'-Diphenyl Picrylhydrazyl (DPPH) radical showed a relatively strong antioxidant scavenging affinity as compared to vitamin C.*

Keywords: *Sonchus oleraceus; GC-MS; antitumor; antioxidant activity.*

1. INTRODUCTION

Most developing countries depend on starch-based foods as the main staple food for the supply of both energy and protein. This accounts in part for protein deficiency which prevails among the populace as recognized by the Food and Agriculture Organization of the United Nations.[1-2] *Sonchusoleraceus* L., a member of the Compositae, is a traditional medicinal plant, which has been used to treat tumor, in-flammatory diseases, infection and so on in Chinese folk culture[3]. Recent studies have indicated that extracts of *S. oleraceus* are bioactive, with antioxidant[4], anti-ageing[5] and anti-tumor[6]. Many workers [7-11] have reported the compositional evaluation and functional properties of various types of edible and wild plants which are in use in the developing countries. Many local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials. The proximate analysis showed that the plants contained an appreciable percentage of moisture content, ash content, crude protein, lipid and carbohydrate. The plants are also rich in minerals, flavonoids, flavonols, proanthocyanidins, total phenols and low levels of saponins and alkaloids. Phytochemical screening *of Sonchuswightianus* of Nepalese origin was carried out. The results showed the presence of fatty acids, triterpenes, sterols, polyphenols, emodins, quinones, glycosides, polyose and anthracenosides. Compounds *β*-sitosterol, *β*-sitosterol glycoside, 1-Hexacosanol and Hexadecanoic methyl ester were isolated. 1-Hexacosanol and Hexadecanoic methyl ester are isolated for the first time from *Sonchus wightianus.*[12] In continuation of our studies,[13-15] we aim in the present study the to evaluate the volatile constituents, antitumor and the antioxidant activity of the chloroform extract of *Sonchus oleraceus*.

2. EXPERIMENTAL SECTION

Collection of Plant Material

The plant was collected from Benghazi city in Libya in 2014 and was identified by the botany department, Faculty of Science, Zagazig University.

Preparation of Extracts

About 0.5 kg of *Sonchus oleraceus* was extracted exhaustively with chloroform using a Soxhlet apparatus for 24 hours. The extract was collected after filtering through filter paper and concentrated on a Rota -vapor to give a solid gum which was subjected to GC-MS [16].

GC-MS (Gas Chromatography/Mass Spectrometry) Analysis

The analytical GC-MS analyses were performed in two different equipment's: (a) Hewlett Packard 5973–6890 system, operating on EI mode and equipped with a HP 5 MS 30 m × 0.25 mm × 0.25 µ film thickness capillary columns. The carrier gas was Helium (flow rate = 1 mL/min). Temperature program: initial column temperature 60°C (for 5 min.), was raised to 280°C within 3°C/min, and held there for 15 min. The injector and detector temperatures were 220 and 280°C, respectively, (b) Finnegan trace GC ultra-system operating on EI mode and equipped with AT™ Aqua wax 30 m × 0.32 mm × 0.25 µm film thickness capillary column. The carrier gas was Helium (flow rate = 1.5 ml/min, constant flow) and Split ratio, 1:10. Temperature program: initial column temperature 60°C (for 5 min.), then was raised to 235°C within 3°C/min, and held there for 30 min (injector temperature 290°C, detector temperature 300°C). MS details (for both organs): ionization energy = 70 eV; emission = 200 µÅ; mass range = 35–650 Da; scan time = 1.25 s; scan rate (amu/s) = 500.0, scans/s = 0.7974.

All compounds were identified by comparison of their retention times (Rt) and mass spectra with those of authentic samples and/or mainlib, Wiley [12], replib, NISTD-EMO libraries spectra and through international literature.[19]

Material and Methods for Biological Activities

Cell Culture

Human hepatocarcinoma cell lines (Hep-G2), Colon carcinoma cells (HCT-116), breast adenocarcinoma cells (MCF-7) was purchased from ATCC, USA, were used to evaluate the cytotoxic effect of the tested extract. Cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium), except colon cells that were cultured in McCoy's media. Media were supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulphate, and 250 ng/ml amphotericin B. Cells were maintained at sub-confluency at 37ºC in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested sample was dissolved in dimethyl sulphoxide (DMSO), and then diluted thousand times in the assay. All cell culture material was obtained from Cambrex Bio-Science (Copenhagen, Denmark). All chemicals were from Sigma/Aldrich, USA, except mentioned. All experiments were repeated three times, unless mentioned.

Anti-Tumor Activity

Cytotoxicity of tested samples was measured against different tumor cells using the MTT Cell Viability Assay. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which is largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm.[18]

Reagents Preparation

MTT solution: 5mg/ml of MTT in 0.9%NaCl. Acidified isopropanol: 0.04 N HCl in absolute isopropanol.

Procedure

Cells (0.5X105 cells/well), in serum-free media, were plated in a flat bottom 96-well micro plate, and treated with 20 µl of different concentrations of each tested sample for 48 hours at 37ºC, 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 hours MTT crystals were solubilized by adding 180 µl of acidified isopropanol/well and plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using a micro plate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability.

Calculation

Percentage of relative viability was calculated using the following equation:

[Absorbance $_{\text{treated cells}}$/Absorbance $_{\text{control cells}}$)] X 100

Then the half maximal inhibitory concentration (IC_{50}) was calculated from the equation of the dose response curve

Antioxidant Activity

1,1-diphenyl-2-picrylhydrazyl is a stable deep violet radical due to its unpaired electron. In the presence of an antioxidant radical scavenger, which can donate an electron to DPPH, the deep violet color decolorize to the pale yellow non-radical form Ratty *et al.*[19] The change in color and the subsequent fall in absorbance are monitored spectrophotometrically at 520 nm.

Reagents Preparation

Ethanolic DPPH: 0.1mM DPPH/absolute ethanol

Standard ascorbic acid solution: Serial dilutions of ascorbic acid in concentrations ranging from 0-25 µg/ml in distilled water. A standard Calibration curve was plotted using serial dilutions of ascorbic acid in concentrations ranging from 0-25 µg/ml in distilled water.

Procedure

In a flat bottom 96 well-microplate, a total test volume of 200 µl was used. In each well, 20 µl of different concentrations (0-25 µg/ml final concentration) of tested sample were mixed with 180 µl of ethanolic DPPH were mixed and incubated for 30 min at 37ºC. Triplicate wells were prepared for each concentration and the average was calculated. Then photometric determination of absorbance at 515 nm was performed by microplate ELISA reader.

Calculation

The half maximal scavenging capacity (SC_{50}) values for each tested sample and ascorbic acid was estimated via dose curve.

SC_{50} of each sample was calculated using the curve equation.

3. Results and Discussion

Volatile Composition of *Sonchus oleraceus*

The chemical volatile of *sonchus oleraceus* constituents were characterized and identified by using GC-MS and the data listed in Figure 1 and Table 1.

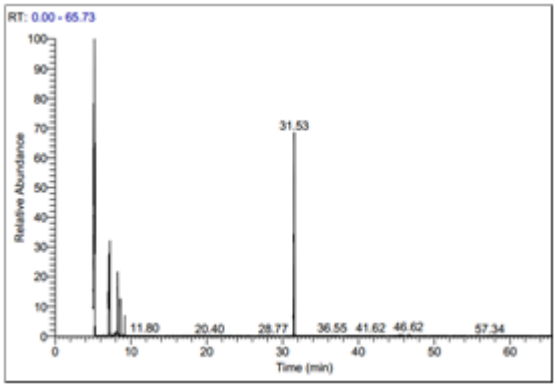

Figure1. *Gas chromatography-mass spectroscopy (GC-MS) of the chloroform extract of sonchus oleraceus.*

Table1. *The chemical compounds identified from the chloroform extract of sonchus oleraceus by using GC-MS*

Volatile compound	Rt	%
1,1-Diethoxypropan-2-ol	5.17	45.20
Cis-7,7-dimethyl-1,3,5-Cycloheptatriene	7.11	9.75
Trans-7,7-dimethyl-1,3,5-Cycloheptatriene	7.21	10.02
5-Methylocta-1,6-dien-3-yne	7.72	0.23

2-ethyl-4-methyl-1-Pentanol	7.94	0.52
1-Hexanol, 2-ethyl-	8.11	0.56
2-Propyl-1-pentanol	8.23	5.06
1,7-Octadiyne	8.43	0.09
Benzene,2-ethyl-1,4-dimethyl-	8.60	2.18
P-Cymene	9.14	0.43
Phytol	31.53	25.27
(Z)-9-Octadecenoic acid	46.69	0.65
Hexadecanoic acid,2,3-dihydroxypropyl ester	48.51	0.62
Hexadecadienoic acid, methyl ester	49.88	1.01
Hexanedioic acid, mono (2-ethylhexyl) ester	50.60	3.51
Dotriacontane	52.47	0.73
Diisooctyl phthalate	53.55	5.47
Quercetin-7,3',4'-trimethoxy	56.15	0.43

As was seen from the above data in Figure 1 and table 1, eighteen compounds were characterized and identified by using GC-MS for chloroform extract of *sonchusoleraceus*, among them, two compounds were found to be represents the major components of this extract. These compounds were 1,1-Diethoxypropan-2-ol 45.20% and Phytol 25.27%.

Results and Discussion of Anti-Tumor Activity

The chloroform extract was tested against three human cell lines including HepG-2, HCT- 116, and MCF-7 cells.

The results indicated that chloroform extract *sonchusoleraceus* have a good antitumor activity against all tested cell lines. The highest inhibition was for colon HCT-116 as concluded from their IC_{50} value 46 µg/ml (Figure 2 and Table 2), while for HepG2 cells was with low inhibition as concluded from their IC_{50} 74 µg / ml (Figure 2 and Table 2).This anticancer activity due to presence of major chemical constituents in the extract such as phytol and volatile compounds which reported for their anticancer activity [20-21].

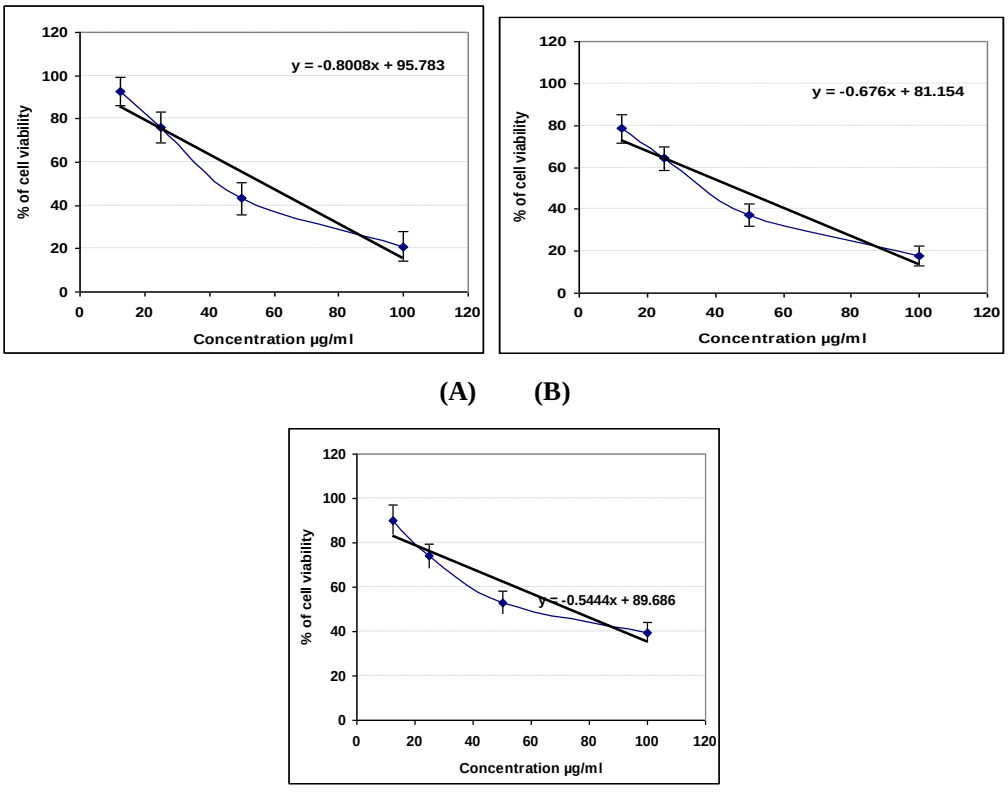

Figure2. *Cell viability of liver HepG2 (A) colon HCT-116 (B) and MCF-7 9C) cells after the treatment with different concentrations for 48 hours, as measured by MTT assay. The data are presented as (Mean ± SE) of µg/ml*

Table2. *Half maximum inhibitory concentration of extract in cell viability of liver HepG-2, of colon HCT-116 and breast MCF-7 cells after the treatment for 48 hours, as measured by MTT assay. The data are presented as µg/ml.*

Name of cell line	IC_{50} value
MCF7	58 µg / ml
HCT-116	46 µg / ml
HEPG-2	74 µg / ml

Antioxidant Activity

The chloroform extract of *sonchus oleraceus* possessed a relatively strong antioxidant scavenging affinity against DPPH radicals as concluded from their low SC_{50} value as compared with the activity of the standard antioxidant: vitamin C (SC_{50} 1.84 µg/ml) (**Figures 3**).

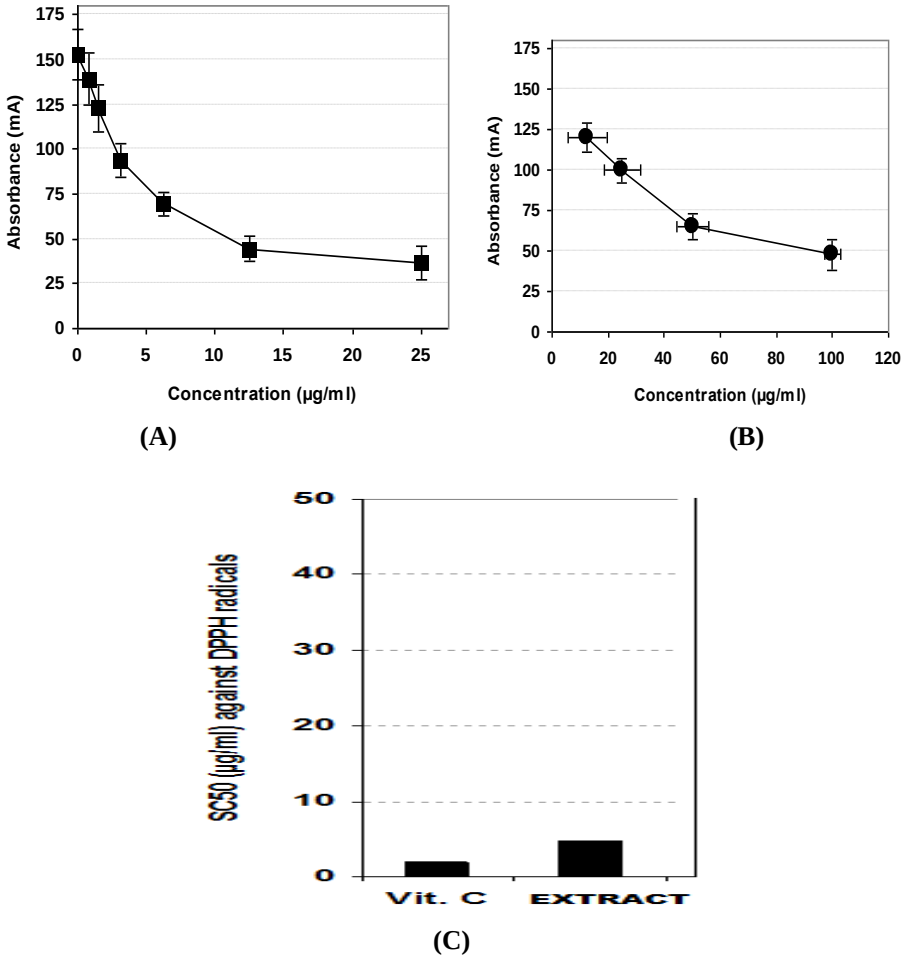

Figure3. *Antioxidant activity of chloroform extract of sonchus oleraceus, (A) Vit. C SC_{50} 1.84 µg/ml (B) and half maximum scavenging concentration of chloroform extract of sonchus oleraceus and vit. C (C) against in DPPH radicals. The data are presented as µg/ml.*

4. Conclusions

sonchusoleraceus were found to contain two major components 1,1-Diethoxypropan-2-ol 45.20% and Phytol 25.27%. The extract have a good antitumor activity against all tested cell lines under investigation. Also antioxidant activity measured by 1,1'-Diphenyl Picrylhydrazyl (DPPH) radical showed a relatively strong antioxidant scavenging affinity.

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