International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

CHEMICAL AND BIOLOGICAL EVALUATION OF THE DIETHYL ETHER EXTRACT OF WILD *SONCHUS ASPER* AND *SONCHUS OLERACEUS* GROWING IN LIBYA

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ABSTRACT

Twelve compounds were identified from *Sonchus asper* by using GC-Mass spectroscopy, the main constituents were found to be Phytol 33.89 % and trans-anethole 20.22 %. While, eighteen compounds were identified from *Sonchus oleraceus*, among them Ethyl linoleate 43.05 %, (E)-9-Octadecenoic acid ethyl ester 24.02 % were found to represent the major constituents. Anti-tumor activity of the two *Sonchus* species showed that *Sonchus oleraceus* has a moderate inhibition with Hep-G2 and HCT-116 cells, while *Sonchus asper* has low inhibition against all cells (Hep-G2, HCT-116 and MCF-7). The antioxidant activity of *Sonchus oleraceus* showed a relatively strong antioxidant scavenging affinity against DPPH radicals.

Keywords: *Sonchus asper*, *Sonchus oleraceus,* Gas Chromatography/Mass spectroscopy, anti-tumor activity and antioxidant activity.

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] *Sonchus* is a perennial herb. The genus *Sonchus* comprises 50 species distributed in Euroasia, around the Mediterrianean and tropical Africa. The word "*Sonchus*" comes from the Greek word meaning "hollow" which refers to hollow stems. Some of the important species of the genus are *S. alpinus*, *S. arvensis*, *S. asper*, *S. macrocarpus*, *S. brachyotus*, *S. kirkii*, *S. wightinaus*, *S. hymanii*, *S. oleraceus*, *S. crataegifolium*, *S. tuberifer sventet*c.[3] Though several works reporting compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries abound in literature, much still need to be done. Many workers[4-8] have reported the compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries. Many local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials. For this reason, the nutritional, phytochemical, antioxidant and antibacterial activities of the acetone, methanol and water extracts of the leaves of *Sonchus asper* and *Sonchus oleraceus* were investigated. The proximate analysis showed that the plants contained an appreciable percentage of moisture content, ash content, crude protein, crude lipid and carbohydrate. The plants are also rich in minerals, flavonoids, flavonols, proanthocyanidins, total phenols and low levels of saponins and alkaloids. The extracts of the 2 plants also showed strong antioxidant antibacterial properties.[9] Phytochemical screening *of Sonchus wightianus* of Nepalese origin was carried out. Phytochemical screening 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.*[10] *In vivo* study was designed to evaluate the anticancer effects of these three types of the extracts on the AM-3 (Murine mammary adenocarcinoma). Hot and cold aqueous extracts showed inflammatory mononuclear cell infiltration in the kidney and lung, section with necrosis in most of the cancer cells in the lung tissue; with few necrotic changes in the normal tissue were noticed after one month of treatment by hot and cold aqueous extracts. [11] In continuation of our studies,[12-14] we report here to study the chemical constituents and the biological investigation of the diethyl ether extract of two species of *Sonchus*.

MATERIALS AND METHODS:

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 400 grams of the plant was extracted under reflux over a boiling water bath with 4 liters of a methanol/bidistilled water (3: 1) mixture for 3 hours. The extract was filtered; the process was repeated 3 times. The solvent was removed by using rotatory evaporator. The process yielded finally 25 grams of aqueous methanolic extract which was transferred into conical flask and refluxed with 500 ml diethyl ether for 2 hours, after then the solvent was removed using a rotatory evaporator to give finally 3 grams of diethyl ether extract.[15]

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 ($\mathbf{R_t}$) and mass spectra with those of authentic samples and/or mainlib, Wiley 9, replib, NISTD-EMO libraries spectra and through international literature.[16]

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[17].

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% CO2 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 of treated cells/Absorbance of 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 decolourize to the pale yellow non-radical form Ratty *et al*.[18] The change in colourization 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 37°C. 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.

RESULTS AND DISCUSSION:
Chemical constituents of the diethyl ether extract of *Sonchus asper:* The chemical constituents of this extract were characterized and identified by using GC-MS as shown in figure 1 and table 1.

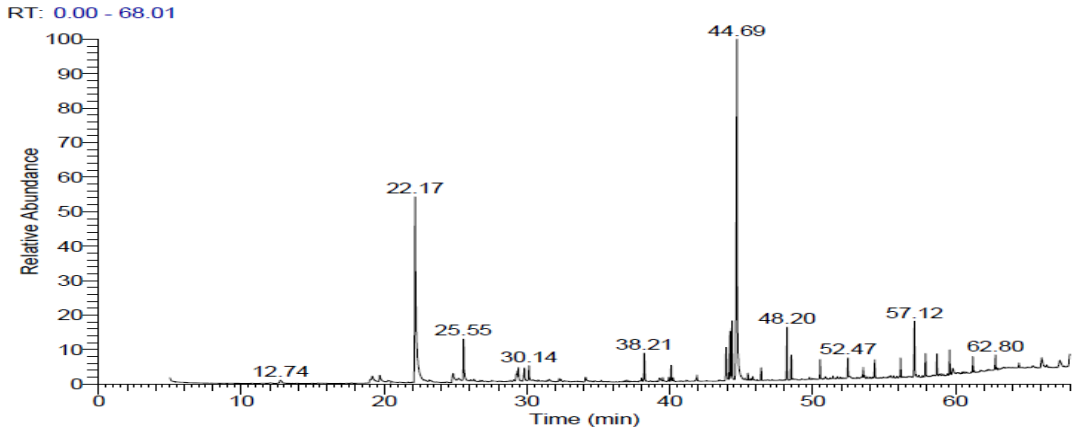


Figure (1) Gas chromatography-mass spectroscopy (GC-MS) of the diethyl ether extract of *Sonchus asper*:

Table- 1: The chemical compounds identified from the diethyl ether extract of *Sonchus asper* by using GC-MS.

Compound name	Retention time in min.	Peak area %
Trans-anethole	22.17	20.22
2-Diethoxymethyl-3-methylbutanol	25.56	3.84
2-Pentadecanone,6,10,14-trimethyl	38.21	2.54
1-Dodecanol,3,7,11-trimethyl	40.09	1.26
Heneicosane	44.21	4.01
2-Hydroxy-1,1,10-tri methyl-6,9-epidioxyde	44.35	5.09
Phytol	44.69	33.89
Phytol isomer	48.20	4.05
Hexatriacontane	48.50	1.85
Heptacosane	52.47	1.74
1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	57.12	4.58
Dotriacontane	62.80	1.26

As was seen from the above data in figure 1 and table 1, twelve compounds were characterized and identified by using GC-MS, and the main constituents of this extract were found to be Phytol 33.89 % and Trans-anethole 20.22 %.

Chemical constituents of the diethyl ether extract of *Sonchus oleraceus*:

Also the same, this extract characterized and identified by using GC-MS and the data listed in figure 2 and table 2.

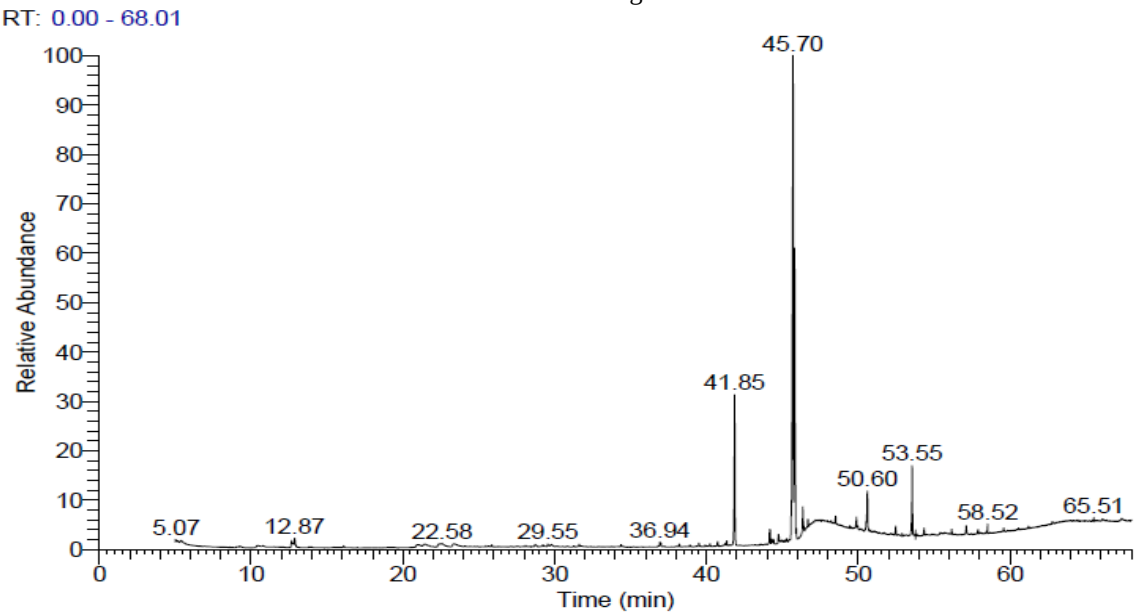


Figure (2) Gas chromatography-mass spectroscopy (GC-MS) of the diethyl ether extract of *Sonchus oleraceus*:

Table- 2: The chemical compounds identified from the diethyl ether extract of *Sonchus oleraceus* by using GC-MS.

Compound name	Retention time in min.	Peak area %
O-Cymene	12.67	0.81
1,8-Cineole	12.87	0.95
1-Heptatriacotanol	40.74	0.45
Ethyl-9-hexadecenoate	41.34	0.35
Hexadecanoic acid,ethyl ester	41.85	11.77
11,14-Eicosadienoic acid, methyl ester	44.18	1.45

Phytol	44.75	0.92
Ethyl linoleate	45.70	43.05
(E)-9-Octadecenoic acid ethyl ester	45.81	24.02
Ethyl Oleate	45.92	0.22
Octadecanoic acid,ethyl ester	46.36	2.28
(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 2 and table 2, eighteen compounds were characterized and identified by using GC-MS, among them, three compounds were found to be represents the major components of this extract. These compounds were Ethyl linoleate 43.05 %, (E)-9-Octadecenoic acid ethyl ester 24.02 % and Hexadecanoic acid, ethyl ester 11.77 %.

Results of biological investigation of date palm pollen:

Results of Anti-tumor activity:

The samples were tested against different human cancer cell lines including Hep-G2, HCT-116, and MCF-7 cells. In Hep-G2 cells the results indicated that ***Sonchus*** *oleraceus* was with moderate inhibition, (Figure 3) as concluded from their relatively low IC_{50} (Figure 4), while sample ***Sonchus*** *asper* was with low inhibition (Figure 3) of the cell viability as concluded from their high IC_{50} (Figure 6).

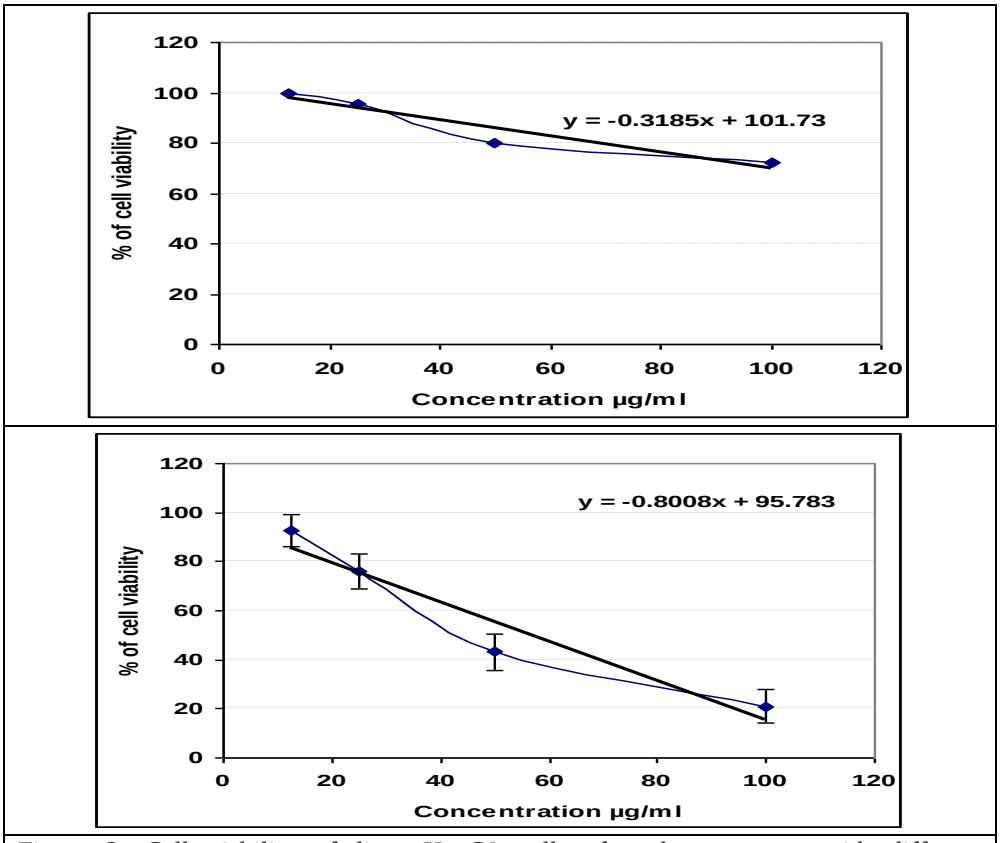
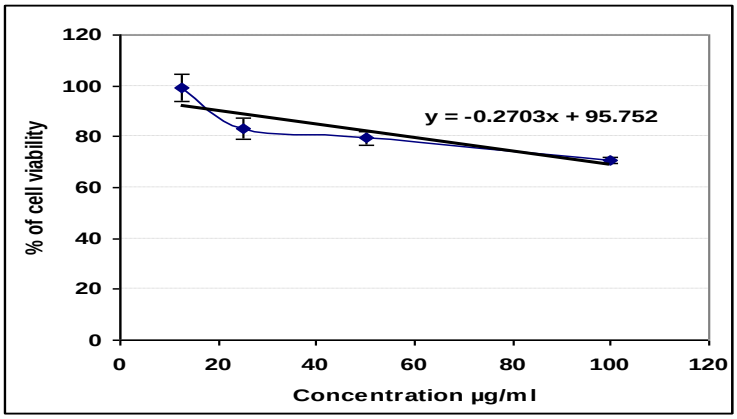


Figure 3: Cell viability of liver HepG2 cells after the treatment with different concentrations of *Sonchus asper* and *Sonchus oleraceus* for 48 hours, as measured by MTT assay. The data are presented as (Mean ± SE) of µg/ml.

Also the same results were obtained for colon HCT-116 cells; the results indicated that *Sonchus oleraceus* was with moderate inhibition, (Figure 4) as concluded from their relatively low IC_{50} (Figure 6) but have much lower value as compared with Hep-G2 cells, while sample *Sonchus asper* was with low inhibition (Figure 4) of the cell viability as concluded from their high IC_{50} (Figure 6).



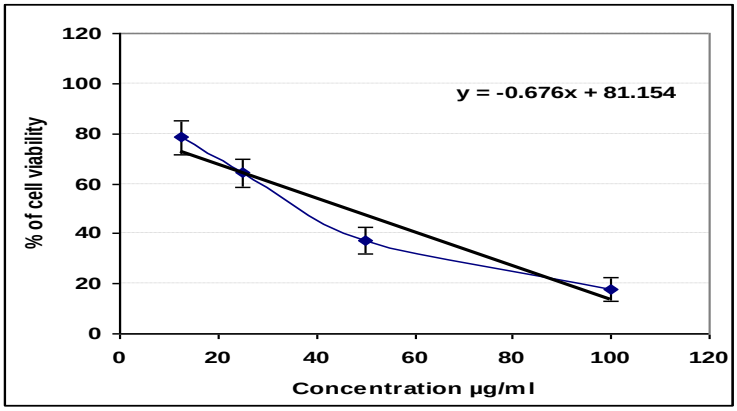
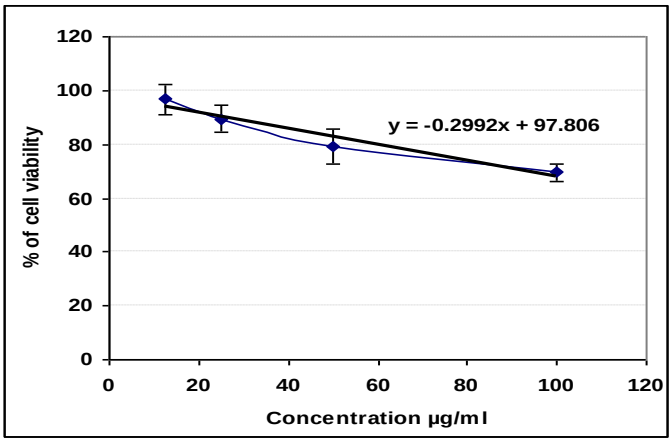


Figure 4: Cell viability of colon HCT-116 cells after the treatment with different concentrations of *Sonchus asper* and *Sonchus oleraceus* for 48 hours, as measured by MTT assay. The data are presented as (Mean ± SE) of µg/ml.

Finally in breast MCF-7 cells the results indicated that all of the tested samples were inactive as anticancer agents (Figures 5 and 6).



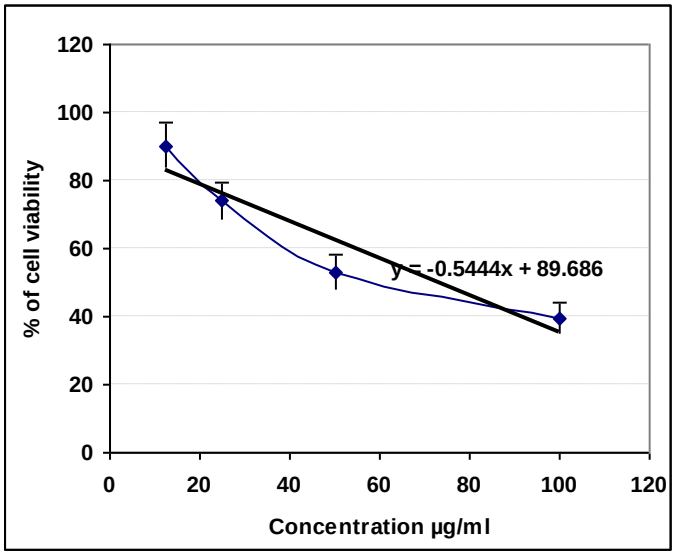


Figure 5: Cell viability of breast MCF-7 cells after the treatment with different concentrations of *Sonchus asper* and *Sonchus oleraceus* for 48 hours, as measured by MTT assay. The data are presented as (Mean ± SE) of µg/ml.

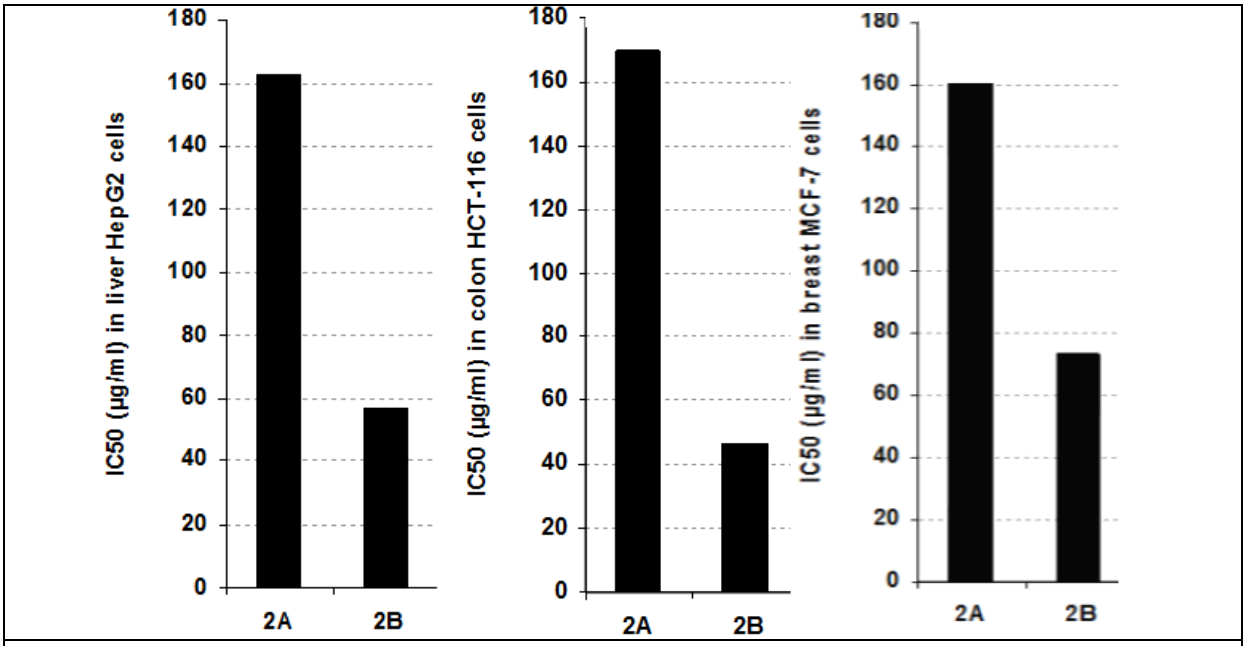


Figure 6: Half maximum inhibitory concentration of all samples in cell viability of liver HepG2, of colon HCT-116 and breast MCF-7 cells after the treatment for 48 hours, as measured by MTT assay. The data are presented as µg/ml.

Where; *Sonchus asper* will be represented by 2A and *Sonchus oleraceus* represented by 2B.

Antioxidant activity:

The tested sample *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), but for *Sonchus asper* possessed low antioxidant scavenging affinity against DPPH radicals as concluded from their high SC_{50} value (Figures 7, 8 and 9).

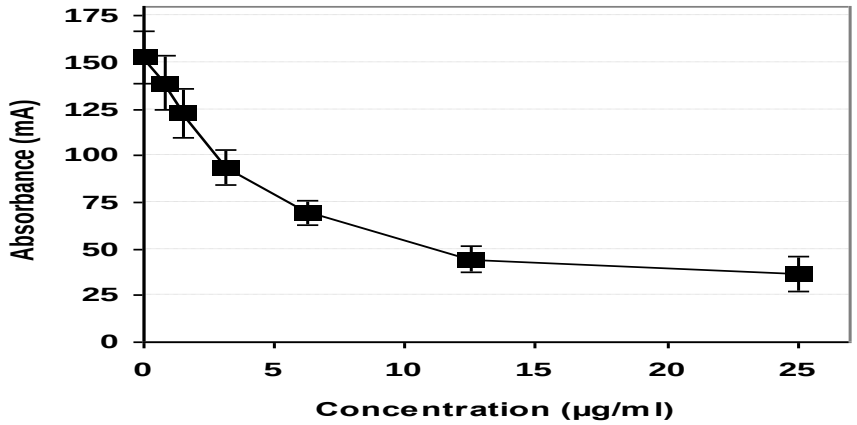
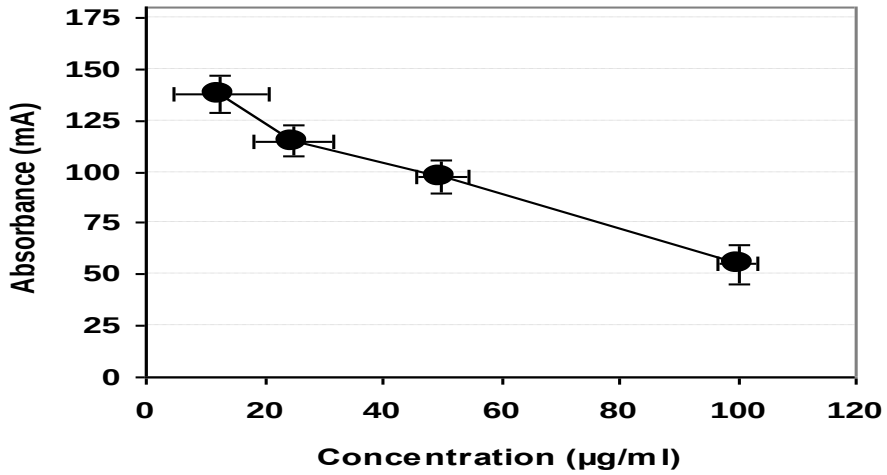


Figure7: Antioxidant activity of ascorbic acid (vitamin C) against DPPH radicals: SC_{50} 1.84 µg/ml



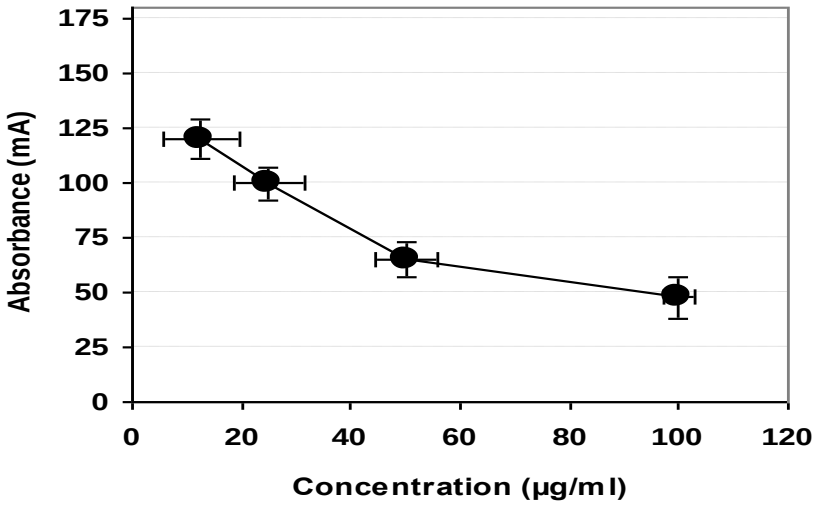


Figure 8: Antioxidant activity of *Sonchus asper* and *Sonchus oleraceus* against DPPH

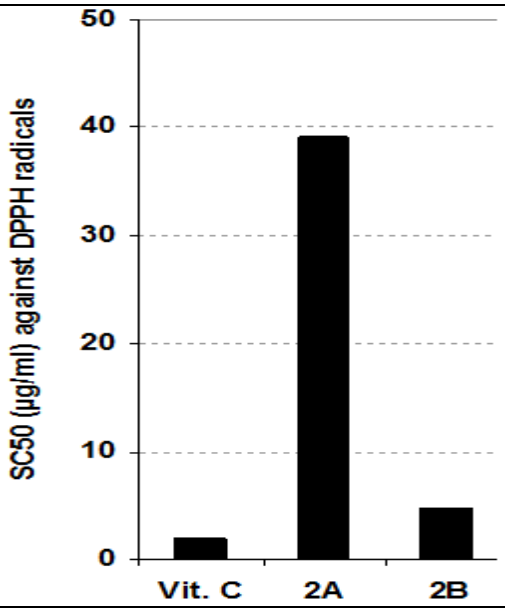


Figure 9: Half maximum scavenging concentration of all samples in DPPH radicals. The data are presented as µg/ml.

Also here in antioxidant activity; *Sonchus asper* will be represented by 2A and *Sonchus oleraceus* represented by 2B.

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