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Protective Effect of Ginger and Cactus Saguaro Extract Against Cancer Formation Cells

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ABSTRACT

This study aimed to determine protective effect ginger extract and cactus saguaro extract against cancer cells. The current study has been on some analysis for both Ginger extract and Cactus Saguaro were carried out. Results showed Moisture content ranged between 91:92% and Protein were 34.1 and 35.5 respectively for ginger and cactus. Total phenol, Total flavonoids, DPPH (radical scavenging activity) were also determined. Total phenol were 43.36 and 133.98 respectively. Results of Total flavonoids was ranged from 33.64 and 27.15 for ginger and cactus while ginger exhibited high amount DPPH was 36.55% and 45.21% ginger and cactus . Cancer cells have been treated with different concentrations (6.25, 12.5, 25, 50 and 100 µg/ ml) from plants extracts and IC50 (The inhibition concentration) was calculated. The anticancer activity of ginger and cacti saguaro against human liver HEPG-2, breast MCF-7 and colon HCT116 cancer cells . So we recommend that using the extracts of both of ginger and cactus them as natural components used in the treatment of cancer.

Keywords: Ginger, Cactus Saguaro, cancer cells, Cell culture.

INTRODUCTION

Cancer is a disease which occurs when changes in a group of normal cells within the body lead to uncontrolled growth causing a lump called a tumor; this is true of all cancers. Breast cancer is characterized by the uncontrolled growth of abnormal cells in the milk producing glands of the breast or in the passages (ducts) that deliver milk to the nipples (ACS, 2014) .

Hepatocellular carcinoma (HCC) or liver cancer is much more common in males than in females. Much of this is probably because of behaviors affecting some of the risk factors (Ferlay *et al.*, 2010).

Colorectal cancer is cancer that starts in the colon or rectum. The colon and the rectum are parts of the large intestine, which is the lower part of the body's digestive system. Colorectal cancer is the third most common type of cancer in men and women in the United States (NCI, 2014).

Recently, researchers become more interested in the development of new drugs from natural resources, such as fruits, vegetables, oil seeds and herbs to overcome the complications accompanied by the synthetic chemicals. Natural products provided the only source of pharmaceuticals for thousands of years, and have made enormous contributions to human health. The potential of using natural products as anticancer agents was recognized in the 1950s by the U.S. National Cancer Institute (NCI) (*Bauml et al., 2015*).

Zingiber officinal is Roscoe, commonly known as ginger belongs to family Zingiberaceae is cultivated commercially in India, China, South East Asia, West Indies, Mexico and other parts of the world. It is consumed worldwide as a spice and flavoring agent and is attributed to have many medicinal properties.

The British Herbal Compendium reported its action as carminative, anti- emetic, spasmolytic, peripheral circulatory stimulant and anti-inflammatory (*Bradley.,1992*).

In addition to its culinary use, ginger also possess medicinal properties, and has been used since antiquity to treat some diseases like common cold, headaches, nausea, stomach upset, urinary infections, digestive, gastrointestinal disturbances, diarrhea, nausea, asthma and parasitic infections, rheumatic arthritis,and muscular discomfort in the various alternative and folk systems of medicine in the world (*Baliga et al.,2011 and Haniadka et al., 2013*).

Cactus fruit contains substantial amounts of ascorbic acid, vitamin E, carotenoids, fibers, amino acids and antioxidant compounds (phenols, flavonoids, betaxanthin and betacyanin) which have been put forward to account for its health benefits such as hypoglycemic and hypolipidemic action, and antioxidant properties (*Osorio et al., 2011*) and (*Schaffer et al.,2005*). Several reports have documented the abundance of vitamins and minerals in cactus (*Stintzing et al., 2003*).

This study was carried out extraction and identification of some bioactive phytochemicals (flavonoids and phenols) from ginger extract and cactus saguaro to assess their anticarcinogenic effect on human cancer cell line of breast, colon and liver cancer, which may develop new chemotherapy treatments for cancer diseases in human.

MATERIALS AND METHODS

Preparation of plant extract.

Ginger extracts were prepared were collected and dried for 12 hours at 40º C and ground finely by the blender. The powder (100g) was extracted with 300 ml aqueous 80% ethanol in a soxhelet apparatus for (72ºh) the solvent was filtered and then evaporated by Rtavapor apparatus after the extraction. The extract yield was 9% the produced alcoholic extract was kept at 20º C until usage. The extract was prepared according to (Eidi *et al*., 2007). While Cactus Saguaro was prepared by cutting it for small parts then gel substance was taken and used**.**

Chemical composition:

Moisture, Ash, fat, protein and fiber were determined according to the methods of (A.O.A.C., 2005). While carbohydrates was calculated by difference as follows: Carbohydrates=100- (% protein+ % fat + % ash).

Fractionation of phenolic compounds:

Phenolic compounds were determined by HPLC according to the method of *(Goupy et al., 1999)* as follow: 5g of sample was extracted by methanol and centrifuged at 10000 rpm for 10 min and the supernatant was filtered through a 0.2-µm Millipore membrane filter then 1-3 ml was collected in avail for injection into HPLC Hewllet Packared (series1050).

Equipped with auto sampling injector, solvent degasser, ultraviolet (UV) detector set at 280 nm and quaternary HP pump (series1100). The column temperature was maintained at 35ºC. Gradient separation was carried out with methanol and acetonitrile as a mobile phase at flow rate of 1ml/min. phenolic acid standard from sigma Co. were dissolved in a mobile phase and injected into HPLC. Retention time and peak area were used to calculation of phenolic compounds concentration by the data analysis of Hewllet Packared software.

Determination of total phenolic compounds:

The total phenolic content of sample was determined using Folin- Ciocalteau reagent (*Velioglu et al., 1998*).

Determination of total flavonoids

Two milliliters of the samples (10 g/L) was transferred to a 10mL volumetric flask containing 2 mL of AlCl3 (20 g/L ethanol) and 6 mL of sodium acetate (CH3COONa) (50 g/L ethanol). by HPLC according to the method of (*Zhisen., 1999*).

Determination of 1, 1- Diphenyl - 2 - picrylhydrazyl (DPPH):

By HPLC according to the method of (Burda and Oleszek., 2001). AA DPPH (%) = (A DPPH-A sample)/ A DPPH × 100 A DPPH: The absorbance of the methanolic DPPH solution, A sample: The absorbance in the presence of the juice.

Cell lines and culture maintenance.

Human breast cancer cell line (MCF-7), human hepatocellular carcinoma cell line (HePG-2), and colon carcinoma cell line (HCT116) were obtained from VACSERA - Cell Culture Unit, Cairo, Egypt. This cell lines originally obtained from the American Type Culture Collection (ATCC). The cell count was done and the cell viability was tested by trypan blue using haemocytometer. Cells were cultured for carrying out various assays according to (Doyle and Bryan, 1998).

Measurements of cytotoxicity by sulphodiamine-B assay (SRB) (*Skehan et al., 1990*)

The cytotoxic assay was performed at VACSERA - Cell Culture Unit, using the sulforhodamine B assay. The relationship between the surviving fraction and the drug concentration was plotted to determine inhibitory concentrations (IC50) for each tumor cell line. The IC50 values will be calculated using sigmoidal concentration response curve fitting models (Sigmaplot software).

RESULTS AND DISCUSSION

Ginger was recognized with higher protein and fiber content when compared with cacti saguaro, while cacti saguaro scored a higher content of carbohydrate and ash as presented in in the Table (1) Fig (1).This priding were in agreement with (Prakash, 2010).

Table 1. Chemical composition of raw materials ginger extract and cacti saguaro extract (mg/100g) in dry weight:-

Plant extract	Chemical composition				
	protein%	Fiber%	Fat%	Ash %	Carbohydrate%
Ginger	34.1	38.5	11.9	11.00	4.5
Saguaro	33.5	31.25	12.5	12.5	10.25

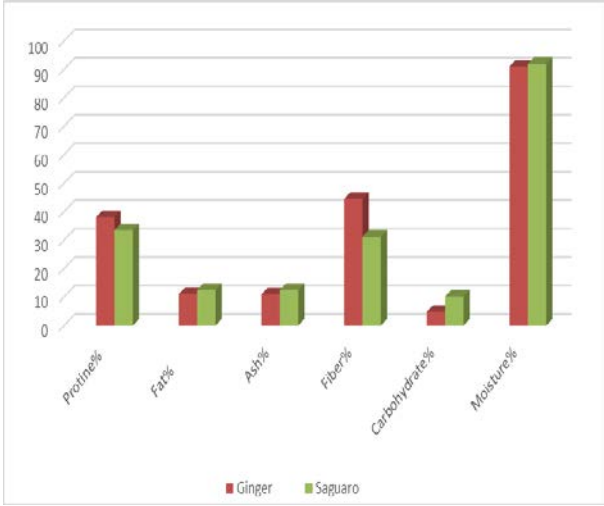

Figure 1. Chemical composition of ginger and cacti saguaro in dry weight

As we illustrated in the Table (2) and Fig (2)that the Cacti Saguaro and Ginger have many important phenolic compounds that have ability to inhibition cancer cells and this consistent with the study of *(Sohi et al., 2003)*.

Table 2. Phenolic compounds of Ginger and Cacti Saguaro extract:-

Identified Constituents	Phenolic compounds (mg / 100 g)	
	Ginger	Cacti Saguaro
Gallic	0. 06090	0. 45201
Pyrogallol	1.95554	4.83037
3-OH-Tyrosol	1.47799	7.49081
4-Amino-benzoic	. 17896	0. 42815
Protocatchuic	2.22687	1.88705
Chlorogenic	1.23529	17.42067
Catechol	2.83914	20.36976
Catechein	1.13943	8.98746
Caffeine	0. 26209	9.51395
P-OH-benzoic	0. 78287	8.34487
Caffeic	0. 31367	3.78775
Vanilic	0. 40767	2.89264
Ferulic	0. 86837	1.63502
Iso-Ferulic	0. 24330	---------
e-vanillic	17.35667	19.00648
Reversetrol	---------	0. 69966
Ellagic	5.80286	16.84320
Alpha-coumaric	2.32437	----------
Benzoic	1.41798	6.19150
3,4,5-methoxy-cinnamic	1.99124	0. 57639
Salycilic	---------	2.48955
Coumarin	0. 23530	----------
P-coumaric	0. 24545	0. 14055

who reported that phenolic compounds as a free radical scavenger and as an inducer of apoptosis in leukemia, lung cancer, and colon adenocarcinoma cell lines, as well as in normal lymphocyte cells which due to natural phenolic compounds with various structural features and possessing widely differing antioxidant activity.

The radical scavenging activity relationships of a large number of representative phenolic compounds (e.g., flavanols, flavonols, chalcones, flavones, flavanones, isoflavones, tannins, stilbenes, curcuminoids, phenolic acids, coumarins, lignans, and quinones) have been reported by *(Gao et al., 2001)* to possess potent antioxidant activity and by to who proved that phenolic compound possess have anticancer or anticarcinogenic/ antimutagenic *(Tapiero et al., 2002)*

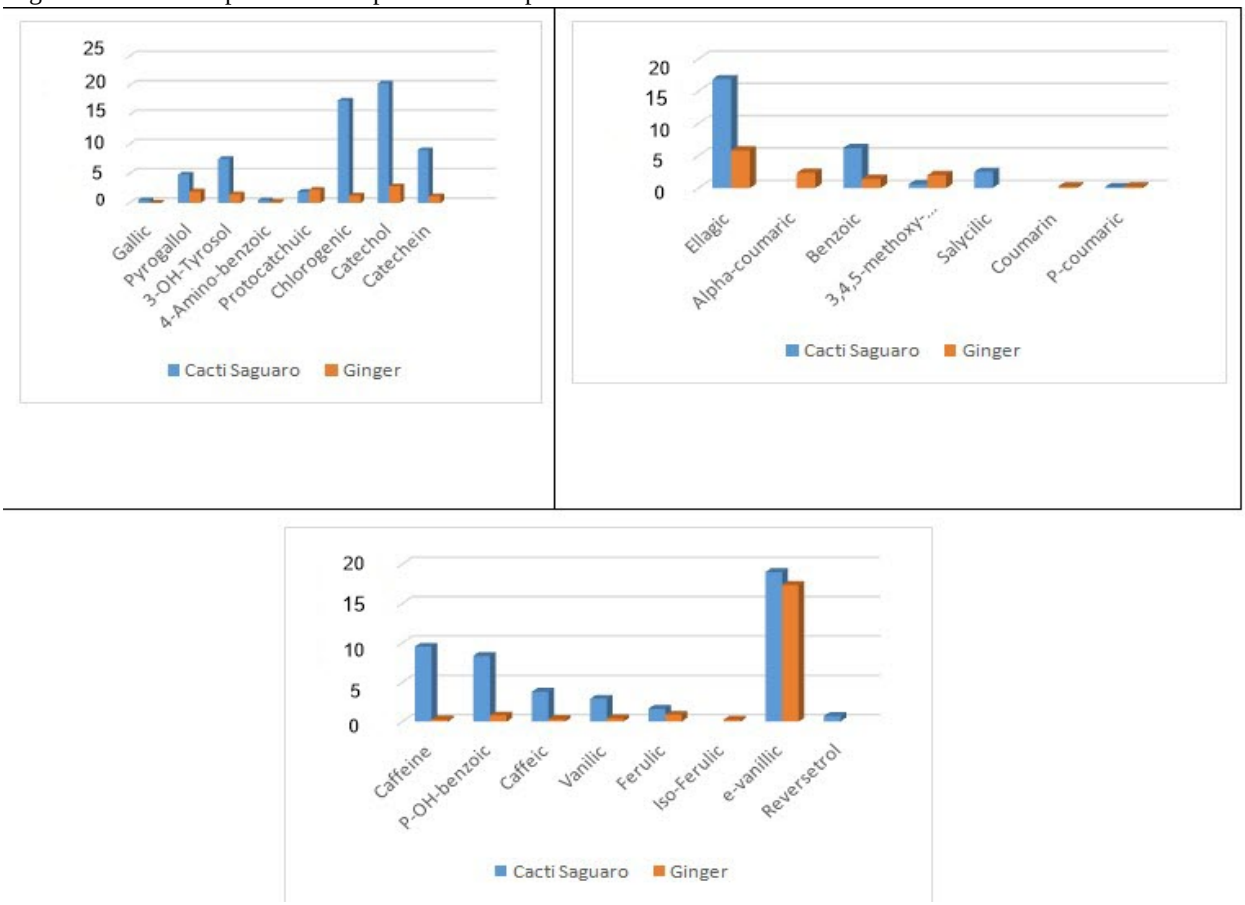

Figure 2: Phenolic compounds of Ginger and Cacti Saguaro.

Table 3. Total Phenol and total Flavonoids of raw materials in ginger and cacti saguaro (mg/100g):-

Plant extract	Total Phenol	Total Flavonids
Ginger	43.36	33.64
Saguaro	133.98	27.15

The consumption of food products containing high amounts of flavonoids have been reported to lower the risk of various cancers. The mechanisms underlying the cancer-protective effects of these naturally occurring polyphenolic compounds, is not known *(Brusselmans et al., 2005)*. Intake of beverages or food products containing flavonoids has been frequently associated with a reduced risk for developing various cancers *(Knekt et al., 2002)*.

Table 4. DPPH Radical scavenge activity in ginger extract and cacti saguaro extract:-

Plant	D PPH%
Ginger	36.55
Saguaro	45.21

Stoilova *et al.* (2007) proved that the antioxidant activity of cacti saguaro extracts containing polyphenol components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals.

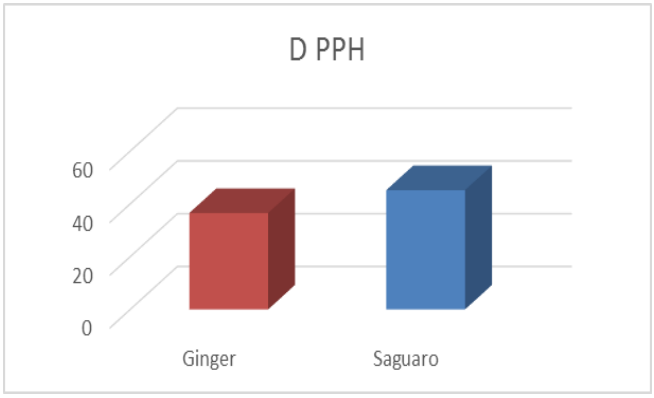

Figure 3. DPPH in ginger extract and cacti saguaro mg/g

Results in table (5) showed the effect of ginger on cancer cell by the measure ments of cytotxicity by using sulphadiamine

Ginger aqueous extract contains good amount of polyphenols and flavones–flavonoids, as nature polyphones and flavonoids–flavonols show anticancer activity. In vitro studies, was demonstrated that aqueous extract of ginger inhibit cancer cell growth. Results of cytotoxicity of ginger extract were an in accordance with the study of *(*Lee *et al*, 2008) who reported that the extracts of ginger has anti cancer properties.

Table 5. Effect of Ginger on Cancer cells by using Measurements of cytotoxicity by sulphodiamine-Bassay (SRB)

Concentrations µg/ml	Surviving fraction% ±SE		
	MCF-7	HCT116	HEPG-2
0.00 (control)	100	100	100
6.25	77.03 ± 0.06	83.98 ± 0.09	83.81 ±0.06
12.5	73.83 ± 0.03	74.23 ± 0.01	77.20 ±0.03
25	73.77 ± 0.03	73.50 ± 0.04	76.42 ±0.01
50	71.05 ± 0.01	57.08 ± 0.01	73.68 ±0.04
100	43.60 ± 0.01	51.03 ± 0.03	67.57 ±0.02
IC_{50}	53.97 ± 0.04	89.25 ± 0.05	195.91± 0.01

Effect of ginger extract likely that antitumor effect on colon cancer cells functions by Inhibiting the growth of cancer cells this is consistent with *(*Abdullah *et al*., 2010), who reported that ginger extract possess anti-tumor effect on colon cancer cell.

Jeena (2013) reported that 6-shogaol has induce apoptotic cell death of liver cells via an oxidative stress-mediated capsize-dependent mechanism. Ginger and its constituents show a vital effect in the control of tumor development through up regulation of tumor suppressor gene, induction of apoptosis and inactivation of VEGF pathways.

Table 6. Effect of Cacti Saguaro on Cancer cells

Concentrations µg/ml	Surviving fraction%±SE		
	MCF-7	HCT116	HEPG-2
0.00 (control)	100	100	100
6.25	72.03 ±0.02	86.96 ± 0.07	81.43 ± 0.04
12.5	66.99 ±0.01	77.20 ± 0.02	80.64 ± 0.08
25	65.02 ±0.03	72.00 ± 0.01	79.36 ± 0.09
50	64.31 ±0.06	67.23 ± 0.03	73.96 ± 0.04
100	41.70 ±0.05	61.44 ± 0.04	71.79 ± 0.05
IC_{50}	88.36± 0.07	99.47 ± 0.06	7242.6±0.07

Comprising between ginger extract with cacti saguaro results showed higher inhibition effect on cancer cells viability at all tested concentrations which could be due to its higher content of phenolic compounds that possess an anticancer effect on cancer cells as shown in table (6).

This results showed cacti saguaro concentration's have a positive effect on cancer that consists of bioactive ingredients for cancer therapy. The National Cancer Institute explains that antioxidants protect healthy cells from damage caused by free radicals, which are byproducts of oxidation. Free radical damage can lead to illnesses such as cardiovascular disease and cancer *(Valko et al., 2007).*

Colon, liver, breast and prostate cancer, the cactus pear's photochemical compounds could inhibited the growth of cells in all four cancers without affecting the healthy cells of the body due to its compound this is consistent with *(Joy., 2014).*

Antigenic factor such as VEGF play a significant role in the development and progression of tumors. Therefore, Inhibition of VEGF is an important step in the prevention of tumor development/management. *(Bode et al., 2001).*

Results showed cacti saguaro concentration's effect on cancer that consists of bioactive ingredients for cancer therapy .

Tumor development and progressions are multi step process including genetic and metabolic changes *(Rahmani et al., 2012)*. Earlier study summarized the role of medicinal plant in the diseases management via modulation of various biological activities including cancer *(Rahmani et al., 2014) .*

The Cacti Saguaro and Ginger have many important phenolic compounds that have ability to inhibition cancer cells and this consistent with the study of *(Sohi et al., 2003)* who reported that phenolic compounds as a free radical scavenger and as an inducer of apoptosis in leukemia, lung cancer, and colon adenocarcinoma cell lines, as well as in normal lymphocyte cells which due to natural phenolic compounds with various structural features and possessing widely differing antioxidant activity.

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التأثير الوقائي لمستخلص الزنجبيل ومستخلص الصبار وضد تكوين الخلايا السرطانية معمليا

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أجريت هذه الدراسه بهدف دراسه التاثير الوقائى مستخلص الزنجبيل وصبار ساجوار ضد الخلايا السرطانية. و أجريت بعض التحاليل المعمليه لكلا من مستخلص الزنجبيل ومستخلص صبار السجوار. و كانت اهم نتائج التحليل الكيماوى كالتالى تراوحت نسبه الرطوبه ٩٢:٩١ % والبروتين ٣٤.١ : ٣٥.٥ % على التوالي لمستخلص الزنجبيل والصبار . وايضا تم تقدير المحتوى الكلى للفينولات والفلافونويدات ومضادات الاكسده النشطه . وبلغ اجمالى المركبات الفينوليه ٤٣.٣٦: ١٣٣.٩٨ على التوالي . وتراوحت مجموعه الفلافونيدات ٣٣.٦٤: ٢٧.١٥ على التوالي وكانت نسبه مضادات الاكسده النشطه ٣٦.٥٥ : ٤٥.٢١ % على التوالي لكل من الزنجبيل وصبار السجوار. وتمت معالجه الخلايا السرطانيه مع تركيزات (٦.٢٥ - ١٢.٥ - ٢٥ - ٥٠ - ١٠٠ ميكرو جرام / جم) من المستخلصات النباتيه وتم تقدير التركيز المثبط IC50% واظهرت النتائج نشاط مضاد للسرطان لمستخلص الزنجبيل والصبار ضد الخلايا السرطانيه (سرطان الكبد وسرطان الثدى وسرطان القولون) .لذلك ننصح باستخدام مستخلص الزنجبيل والصبار كمواد طبيعيه لعلاج الخلايا السرطانيه.