Journal of Medicinal Plants Research Vol. 3(10), pp. 799-808, October, 2009
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875© 2009 Academic Journals

Full Length Research Paper

Anti-cancer and anti-oxidant activity of some Egyptian medicinal plants

Amr A. Nassr-Allah[1], Ahmed M. Aboul-Enein[1], Khalid M. Aboul-Enein[2], David A. Lightfoot[3], Alan Cocchetto[4] and Hany A. El-Shemy[1*]

[1]Faculty of Agriculture Research Park (FARP) and Department of Biochemistry, Faculty of Agriculture, Cairo University, 12613 Giza, Egypt.
[2]Department of Clinical Pathology, National Cancer Institute, Cairo University, Cairo, Egypt.
[3]Department of Plant, Soil, and Agricultural Systems, Southern Illinois University at Carbondale, Carbondale, IL 62901-4415, USA.
[4]National CFIDS (NCF) Foundations, Inc., Needham, Massachusetts, USA.

Accepted 17 August 2009

Natural products from plants are rich sources of chemical diversity and most of the pharmacologically active principles currently used as drugs, including anticancer agents are plants products. Egypt, a semi- arid region has abundant plants resources, which are traditionally used for various disorders. The aim of the present study was to evaluate some Egyptian flora as anticancer agents. The materials used were leaves of *Luffa aegyptiaca* (sponge gourd), *Solenostemma arghe* (argel), *Cassia italica* (senegal senna), *Ocimum basillcum* (basil), *Colocasia antiguorum* (taro), *Beta vulgaris* (beet) and fruit of *Capsicum frutescens* (chili pepper). Antioxidant activity was assayed using the 2, 2'diphenylpicrylhydrazyl (DPPH) radical method. Anticancer activity was assayed *in vitro* against acute myeloid leukemia (AML) and acute lymphocyte leukemia (ALL); and *in vivo* against Ehrlich ascites carcinoma cells (EACC). The results showed significant antioxidant activity of most of extracts in DPPH assay. *Solenostemma arghel* hot water extract significantly reduced EACC induced tumor growth and delayed animal death (with EACC) by 29 days. Among all the extracts *S. arghel* showed high cytotoxicity (66 - 90%) on ALL and AML cells from patients. DNA fragmentation patterns showed cytotoxicity may due to the induction of apoptosis. In conclusion, some natural products from Egyptian flora have potential for use as therapeutics for diseases such as cancer.

Key words: Natural products, anticancer, antioxidants, acute myeloid leukemia, ehrlich ascites carcinoma cells.

INTRODUCTION

Natural products from plants have been valuable sources for anticancer drug discovery (Schwartsmann et al., 2002). A screening program was initiated by Leven et al. (1979) that identified many antibacterial antifungal, anti-viral, antiparasitic, and other pharmacologically active substance activities in higher plants (Jang et al., 1997).

Herbal or 'botanical', medicines, recorded in developing countries with ancient civilizations, such as Egypt and China, provide an abundant Pharmacopoeia of products that have been prescribed for many diseases over many centuries. The natural products underlying traditional medicines have received increased scientific attention recently (Han et al. 2002; Vickers 2002). Since there are national and indigenous rights over plant derived resources, basic scientific investigations based on medicinal plants and indigenous medical systems have increased in developing countries (Han et al., 2002; El Shemy et al., 2007).

Ancient herbal medicines may have some advantages over single purified chemicals (Vickers, 2002). Often the different components in a herb have synergistic activities or buffer toxic effects. Mixtures of herbs are even more complex and so might have more therapeutic or preventive activity than single products alone. In fact, several studies have demonstrated that extracts from several

*Corresponding author. E-mail: helshemy@hotmail.com. Tel: +202-37742600. Fax: +20-2-3571-7355.

herbal medicines or mixtures had an anticancer potential *in vitro* or *in vivo* (Bonham et al., 2002; Hu et al., 2002; Lee et al., 2002; El-Shemy et al., 2007). Phenolic and flavonoid contents provide antioxidant activities that may underlie the anticancer potential (Meyers et al., 2003).

Aqueous extracts from willow (*Salix* sp.) leaves prevented proliferation of three cancer cells AML, acute ALL and EACC (El-Shemy et al., 2003, 2007). Alcohol extracts of *Ganoderma lucidum* (Hu et al., 2002) induced apoptosis in MCF-7 human breast cancer cells. An aqueous extract of *Paeoniae lactiflora* induced apoptosis in HepG2 and Hep3B hepatoma cells (Lee et al., 2002). An aqueous extract of Bu-Zhong-Yi-Qi-Tang (a mixture of ten herbs) could induce apoptosis in hepatoma cells (Kao et al., 2001). Water-soluble ingredients of Sho-Saiko-To (a mixture of seven herbs) inhibited the proliferation of KIM-1 human hepatoma cells and KMC-1 cholangiocarcinoma cells (Yano et al., 1994). PE-SPES (mixture of eight herbs) had been developed as a clinical treatment of prostate cancer (Bonham et al., 2002). Chemical and pharmacological studies of various extracts or compounds purified from the herbs were found to increase myocardial blood blow, reduce radiation damages and purify blood quality (Wang et al., 2001; Xie et al., 2001; Kim et al., 2002). Luffin from the seeds of Luffa aegyptiaca is cytotoxic to the human metastatic melanoma, with approximately 10 times greater potency in Ehrlich cells (Poma et al., 1998).

Here the antioxidant and anti-tumor effects of extracts from the leaves of some Egyptian flora were examined.

MATERIALS AND METHODS

Plant materials

The plants used in this study were collected from New-Valley area in the desert region (Egypt; during Months of January to November 2006). Plants were frozen on dry ice and transported back to Cairo in the frozen state within 3 days. Seed from the plants used are maintained at Cairo University and are available on request.

Plant extraction

Crude plant extracts of *Luffa aegyptiaca* (LLA), *Solenostemma arghe* (LSA), *Cassia italica* (LCI), *Ocimum basillcum* (LOB), *Colocasia antiguorum* (LCA), Beta vulgaris (LBV) and fruit of *Capsicum frutescens* (FCA) were prepared for *in vitro* screening as follows. The extractions used 20 g of powdered plant material with 200 ml of either hot water, cold water, ethanol 80% (v/v) or methanol: methylene chloride (1:1). Extracts were made by mechanical stirring for 12 h at room temperature (25ºC) except the hot water extract (80ºC) that was made in 30 min. The extracts were completely dried in rotary evaporator at 40ºC and subsequently stored at 4ºC.

This study was partly performed on cells harvested from adult leukemia patients or their healthy relatives, aged 20 - 70 years that were admitted to the National Cancer Institute, Cairo University. International protocols governing the ethical treatment of patients were followed. In addition, the study used white albino mice that were transplanted with EACC from an immortal culture obtained from National Cancer Institute, Cairo University. International protocols governing the ethical treatment of animals were followed.

Determination of total phenolic and flavonoid contents

The total phenolic content of the plant extracts were determined by the Folin–Ciocalteu method (Meda et al., 2005). Briefly, aliquots of 0.1 g of the lyophilized powder of plant samples were dissolved in 1 ml of deionized water. This solution (0.1 ml) was mixed with 2.8 ml of deionized water, 2 ml of 2% (w/v) sodium carbonate (Na2CO3), and 0.1 ml of 50% (v/v) Folin–Ciocalteau reagent. After incubation at room temperature for 30 min, the relative absorbance of the reaction mixture at 750 nm was compared to deionized water with a spectrophotometer (Hitachi, Tokyo, Japan; Model 100 - 20). Gallic acid (GA) was chosen as a standard phenolic to construct a seven point standard curve (0–200 mg/l), the total phenolic contents in plant extracts were determined in triplicate. The data were expressed as milligram gallic acid equivalents (GAE)/g lyophilized powder. The data were then converted into GAE/g dry matter from plant samples. The adjustment was based on the moisture content of lyophilized powder.

The total flavonoid contents were determined by the aluminum chloride colorimetric method described by Chang et al. (2002). Briefly, aliquots of 0.1 g of plant extracts were dissolved in 1 ml deionized water. This solution (0.5 ml) was mixed with 1.5 ml of 95% (v/v) alcohol, 0.1 ml of 10% (w/v) aluminum chloride hexahydrate (AlCl3), 0.1 ml of 1 M potassium acetate (CH3COOK), and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the absorbance was measured at 415 nm compared todeionized with a spectrophotometer (Hitachi, Model 100 - 20). Quercetin was chosen as a standard to construct a seven point standard curve (0 - 50 mg/l). The total flavonoid contents in plant extracts were determined from triplicated assays. The data were expressed as milligram quercetin equivalents (QE)/g dry weight of lyophilized powder. The data were then converted into QE/1 g dry matter from plant samples by adjusting for the moisture content of lyophilized powder.

Antioxidant activity

The antioxidant activity of the leaf plant extracts was evaluated by using the 2, 2'diphenylpicrylhydrazyl (DPPH) assay (Cuendet et al., 1997; Burits and Bucar, 2000). Fifty microliters of the extracts were added to 5 ml of a 0.004% (w/v) of DPHH in methanol (100% v/v). After, a 30 min incubation period at room temperature the absorbance at 517 nm was compared to DPPH in methanol without an extract sample (blank). The percent inhibition of free radical formation (I%) was calculated as;

$$I\% = (A_{blank} - A_{sample} / A_{blank}) \times 100;$$

Where; A blank is the absorbance of the control reaction (containing all reagents except the extract) and A sample is the absorbance of the mixture containing the extract. The IC50 (defined as the concentration of extract required to produce 50% of the maximum inhibition) was calculated from graphing inhibition percentage against extract concentration. Determinations were carried out in triplicate.

In-vitro assay for cytotoxic activity

The cytotoxicity of each extract on AML, ALL and EACC cells was determined by the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay of mitochondrial dehydrogenases (Selvakumaran et al., 2003). AML had been diagnosed by peripheral blood and bone marrow examinations; cytochemistry; and in some cases, immunological markers. Mononuclear cells were separated from other blood cells by Ficoll Hypaque density gradient

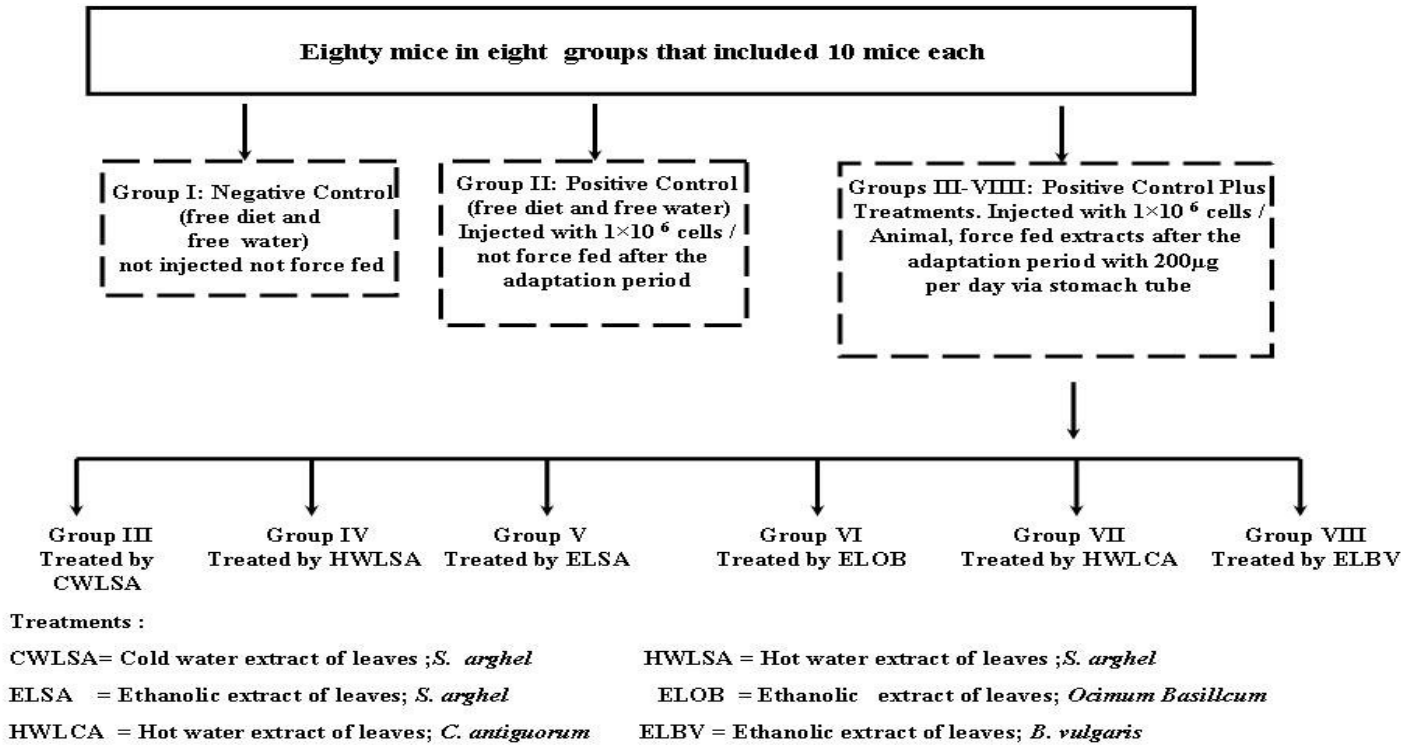

Figure 1. Flow chart of the animal experimental design.

centrifugation at 1,500 g (Pharmacia, Uppsala, Sweden). The cells were then washed with three changes of PBS. The cell counts were adjusted to (3 × 103 cell/well) and plated in 100 µl of medium/well in 96-well plates (Costar Corning, Rochester, NY). After incubation (overnight), extracts were added in various concentrations (10, 20 µg/ml) to a human normal lymphoid cell line (unpublished); 3 wells were included at each concentration. After treatment with extracts for one day, 20 µl of 5 mg/ml MTT (pH 4.7) was added per well and incubated for another 4 h, The supernatant fluid was removed and 100 µl of DMSO was added per well and shaken for 15 min. The absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without cells as blanks. The effect of extracts on the proliferation of human AML cells was expressed as the percent cytoviability, using the following formula:

% cytoviability = A570 of treated cells / A570 of control cells × 100.

Tumor transplanted animals

A total of 80 normal female Swiss albino mice weighing between 20 - 25 g each were fed on normal diet and used as follows: Group I of mice was spared a cell transplant and referred to as the control (negative). Group II was transplanted to the i.p. cavity with EACC at 2 x 106 cells (0.2 ml), the positive control. Group III was transplanted with EACC like the second group and each mouse was daily forced to ingest orally via a stomach tube about 0.2 ml of cold aqueous extract of LSA (E x 1; 10% w/v) in addition to the normal diet. Group IV was treated daily like the third group but with hot water LSA extract (Ex2). Group V was treated daily like the third group but with ethanol extract of LSA (E x 3).

Group VI was treated daily like the third group but with methylene chloride: methanol (1:1) extract of LSA (E x 4). Group VII was treated daily like the third group but with the hot water extract of LCA (E x 5). Group VIII was treated daily like the third group but with the ethanol extract of LBV (E x 6). A flow chart of the experimental design forms Figure 1. The total experimental period was 32 days after the mice were transplanted with EACC into the i.p. cavity (the adaptation period)

Biochemical analysis

At week one and week two samples of blood and EACC cells were taken. At the end of the experimental period, the surviving animals were sacrificed by decapitation and blood was collected. In blood samples several enzyme activities were determined as follows; the superoxide dismutase (EC 1.15.1.1) activity was estimated by the method of Nishikimi et al. (1972); glutathione peroxidase (EC 1 .11.1.9) by the method of Paglia and Valentine (1967); glutathione-S-transferase (EC 2.5.1.18) by method of (Habig and Pabst, 1974). In EACC samples, cells were counted microscopically and the lactic dehydrogenase (LDH; EC 1.1.1.27) activity was determined by method of Kaplan and Pesce (1996). LDH activity was determined from cells that were isolated from mice when the number of EACC was calculated for each animal each week.

DNA extraction and measurement of apoptosis

DNA was extracted from mature (normal) and immature (leukemic) white blood cells before and after treatment with the each extract. Cells were washed with PBS and then lysed in cold lysis solution (5 mmol/L of Tris, pH 7.4, 20 mmol/L of EDTA, 0.5% (v/v) Triton X-

100) for 20 min (Gao et al., 2002). Cell lysates were centrifuged at 27,000 g for 15 min, and DNA was extracted from the aqueous phase with phenol: chloroform: isoamyl alcohol (25:24:1 (v:v:v) containing 0.1% (w/v) hydroxyquinoline. DNA was precipitated with 0.3 mol/L of sodium acetate and 2 volumes of cold 100% (v/v) ethanol. Agarose gel electrophoresis (3% w/v) at 30 mA for 2 h followed by ethidium bromide staining and UV fluorescence was used to determine the degree of apoptotic DNA fragmentation (Gao et al., 2002).

Statistical analysis

The statistical analysis was computed using analysis of variance procedure described in SAS/STAT software (SAS Institute, Cary, NC, USA). The significant differences between treatment means were separated by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents

The amount of total phenolics varied slightly within each plant's extracts but ranged significantly from 6.25 ± 0.19 to 33.1 ± 1.03 mg of gallic acid/g dry sample among extraction methods and species. Among extraction methods within species the widest range was about 21 mg/g (ethanol compared to cold water in *O. basillcum*; Table 1). The highest amount of phenolics was found in the *O. basillcum* ethanolic extract and the lowest in the L. aegyptiaca cold water extract (Table 1). Similar amounts of, and ranges among, phenolics extracted from herbs and medicinal plants collected in Finland have been reported recently (Jin-Yuarn and Ching-Yin, 2007).

The amount of total flavonoids are ranged from 0.21 ± 0.01 to 2.0 ± 0.06 mg/g dry weight (quercetin equivalents). The highest amount was found in *O. basillcum* ethanolic extract and the lowest in *C. frutescens* cold water extract (Table 1). Flavonoid contents were at least an order of magnitude lower than the phenolic contents in all extracts.

DPPH radical scavenging activity

The plant extracts each showed a concentration dependent scavenging activity by quenching DPPH radicals (Table 2). As judged by this assay the *O. basillcum* ethanolic extract had the highest antioxidant activity at 92.70 ± 2.87% inhibition of radical formation (Table 2) and the lowest EC50 value of 0.19 ± 0.011 mg/ml. This high antioxidant capacity may be due to the high concentration of phenolics and flavonoids in *O. basillcum* extracts. Phenolic and flavonoid compounds are common in medicinal plants, spices, vegetables, fruits, grains, pulses and other seeds. These compounds are an important group of natural antioxidants with possible beneficial effects on human health (Meyers et al., 2002). They can participate in protection against the harmful action of reactive oxygen species, mainly oxygen free radicals.

Phytochemicals, especially the phenolics found in medicinal plants, fruits and vegetables, have been proposed as the major bioactive compounds providing the health benefits associated with diets rich in plant-foods (Halliwell and Gutteridge, 1999). In this context, redox and antioxidant systems are among the most promising targets for functional food science. For this reason, many functional foods aim to increase human intake of antioxidants to reduce the risk of chronic diseases linked to oxidative stress. Among the most common dietary sources of natural antioxidants are grapes and berries that are rich in phenolic compounds and particularly flavonoids (Robards et al., 1999; Moure et al., 2001). The results of this study suggest extracts of the leaves of herbs like basil can substitute for grapes and berries.

Cell viability and cytotoxicity analysis

After 24 h incubation of the mononuclear AML and ALL cells with plant extracts, the cytotoxicity on the tumor cell lines was evaluated using the MTT assay. The cold water, hot water and ethanolic extracts obtained from *S. arghel* showed highest activities in these assays. The maximal inhibition of cell growth (92, 82 and 79% respectively) was obtained with 200 µg/ml of the extracts (Tables 3 and 4). The cold water extract obtained from *C. antiguorum* also possessed high cytotoxic activity and inhibited the cell growth (by 81%) at the 200 µg/ml concentration. The other extracts tested had only weak to moderate (10 - 60%) cytotoxic activity against both AML and ALL cells (Tables 3 and 4). The extracts did not cause cell death in health white blood cells (Table 5). The antileukemic activity of the leaf extracts of *S. arghel* was from compounds that were soluble in water and/or ethanol. Hot water extracts from *C. antiguorum* leaves have almost the same effect. Since the active compounds were easily dissolved in hot water, they could be used as a natural antitumor medicine for home therapies.

Some extract might not be suitable for intravenous delivery because lyophilized extract resuspended in saline of *C. italica* showed very low destructive effect on tumor cells (viability 81 - 92%). Only the ethanolic extract of *O. basillcum* retained cytotoxicity through lyophilization.

Undesirable side effects might limit the use of some extracts. For example, *C. frutescens* ethanolic and methylene chloride: methanol extracts were effective in killing tumor cells but also showed relatively high cytotoxic effects (20 - 25%) on the normal cells (Table 5). However, there were several extracts with major destructive effects solely on AML and ALL cells, especially from the polar organic solvents (water and ethanol (Table 3; Table 4). It is known that the majority of phenolic compounds, most glycosides, and many types of tannins dissolve in both water and ethanol solutions (Bravo, 1988). Therefore, these groups of compounds may contain the major active components for the destruction of leukemia and carcinoma cells (El-Shemy et al., 2007). In support of third hypothesis, previous reports showed the

Table 1. Phenolic and flavonoid contents in the plant extracts (mg/g).

Plant samples	Cold water		Hot water		Ethanolic Extract		Methylene chloride:ethanol	
	* Total phenolics	** Total Flavonoids	* Total phenolics	** Total Flavonoids	* Total phenolics	** Total Flavonoids	* Total phenolics	** Total Flavonoids
Luffa aegyptiaca	***6.25 ± ***0.19 f	0.35 ± 0.01 e	15.00 ± 0.47 b	0.95 ± 0.03 c	13.16 ± 0.41 f	1.20 ± 0.04 d	9.80 ± 0.30 g	0.86 ± 0.03 e
Solenostemma arghel	17.90 ± 0.55 c	1.30 ± 0.04 c	8.76 ± 0.27 e	0.89 ± 0.03 d	21.00 ± 0.65 b	1.80 ± 0.06 c	18.40 ± 0.57 c	1.65 ± 0.05 b
Cassia acutifolia	22.30 ± 0.69 a	0.90 ± 0.03 d	8.28 ± 0.26 e	0.38 ± 0.01 f	14.30 ± 0.44 e	0.23 ± 0.01 f	24.82 ± 0.77 a	1.21 ± 0.04 c
Capsicum frutescens	14.74 ± 0.46 d	0.21 ± 0.01 f	25.80 ± 0.80 a	1.60 ± 0.05 a	16.38 ± 0.51 d	0.61 ± 0.02 e	13.32 ± 0.41 e	0.53 ± 0.02 f
Ocimum basillcum	17.90 ± 0.55 c	1.44 ± 0.04 b	10.22 ± 0.32 d	0.85 ± 0.03 d	33.10 ± 1.03 a	2.00 ± 0.06 b	21.08 ± 0.65 b	1.20 ± 0.04 c
Colocasia antiquorum	10.30 ± 0.32 e	0.87 ± 0.03 d	10.30 ± 0.32 d	0.64 ± 0.02 e	17.70 ± 0.55 c	2.67 ± 0.08 a	16.60 ± 0.51 d	1.80 ± 0.06 a
Beta vulgaris	19.42 ± 0.60 b	1.80 ± 0.06 a	12.24 ± 0.38 c	1.30 ± 0.04 b	14.00 ± 0.43 ef	1.12 ± 0.03 d	11.40 ± 0.35 f	0.97 ± 0.03 d
LSD (0.05)	0.952	0.063	0.776	0.056	1.066	0.085	0.931	0.071

* values were determined as mg gallic acid / g sample on a dry basis.
** values were determined as mg quercetin / g sample on a dry basis.
*** Each value represents the mean ± S.D (Standard Division) and mean of three replicates.
**** Values in the same column with the same letter are not significantly different at ($p \leq 0.05$).

Table 2. Antioxidant activity of plant extracts measured using DPPH and showing the derived IC_{50} values.

Plant samples	The antioxidants activity of extract (0.5 mg)							
	Cold water		Hot water		Ethanol extract		Methylene chloride: ethanol	
	Antioxidant activity	IC_{50}	Antioxidant activity	IC_{50}	Antioxidant activity	IC_{50}	Antioxidant activity	IC_{50}
Luffa aegyptiaca	17.37* ± 0.54 f**	1.19 ± 0.04 a	17.90 ± 0.55 f	1.15 ± 0.04 a	29.00 ± 0.90 e	0.75 ± 0.02 a	59.30 ± 1.84 c	0.45 ± 0.01 c
Solenostemma arghel	35.50 ± 1.10 d	0.61 ± 0.02 c	33.30 ± 1.03 d	0.67 ± 0.02 d	59.60 ± 1.85 c	0.43 ± 0.01 d	55.00 ± 1.71 d	0.47 ± 0.01 bc
Cassia acutifolia	43.00 ± 1.33 c	0.58 ± 0.02 c	39.00 ± 1.21 c	0.61 ± 0.02 e	45.20 ± 1.40 d	0.59 ± 0.02 b	54.70 ± 1.70 d	0.48 ± 0.01 b
Capsicum frutescens	25.80 ± 0.80 e	0.69 ± 0.02 b	22.20 ± 0.69 e	0.72 ± 0.02 c	31.80 ± 0.99 e	0.57 ± 0.02 b	25.60 ± 0.79 e	0.69 ± 0.02 a
Ocimum Basillcum	85.30 ± 2.64 a	0.21 ± 0.01 e	53.00 ± 1.64 a	0.48 ± 0.01 g	92.70 ± 2.87 a	0.19 ± 0.01 f	87.50 ± 2.71 a	0.20 ± 0.01 e
Colocasia antiquorum	50.20 ± 1.56 b	0.49 ± 0.02 d	49.70 ± 1.54 b	0.52 ± 0.02 f	74.50 ± 2.31 b	0.27 ± 0.01 e	67.20 ± 2.08 b	0.42 ± 0.01 d
Beta vulgaris	50.00 ± 1.55 b	0.50 ± 0.02 d	22.00 ± 0.68 e	0.78 ± 0.02 b	56.80 ± 1.76 c	0.48 ± 0.01 c	54.60 ± 1.69 d	0.49 ± 0.02 b
L.S.D. (0.05)	2.624	0.037	1.967	0.036	3.232	0.027	3.265	0.024

* Each value represents the mean ± S.D (Standard Division) and mean of three replicates.
** Values in the same column with the same letter are not significantly at ($p \leq 0.05$).

allamandin derivatives that are extracted by water and/or ethanol from *Allamanda catharica* (Apocynaceae; dogbane family) had significant activity *in vivo* against the p-388 leukemia in the mouse (Kupchan, 1976). Also cancer cells could be killed by water and ethanolic extracts of willow leaves containing salicin and saligenin (El-Shemy et al., 2007).

Signaling between cells is commonly regarded

Table 3. The effect of the plant samples on the percentage of viable AML cells after 24 h of incubation.

Plant samples	Extract concentration (µg/ml)							
	Cold water		Hot water		Ethanol		Methylene chloride: Methanol	
	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg
	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %
Luffa aegyptiaca	2*	11	14	26	57	75	39	53
Solenostemma arghel	73	82	87	92	47	79	58	72
Cassia acutifolia	7	38	2	9	49	69	52	71
Capsicum frutescens	29	45	24	67	38	90	33	77
Ocimum basillcum	38	51	30	34	69	75	53	63
Colocasia antiquorum	20	67	50	81	44	67	13	24
Beta vulgaris	7	13	4	9	20	81	38	83
L.S.D. $_{(0.05)}$	2.7	3.6	3.2	3.6	3.4	5.0	3.1	11.4

*Each value represents the mean of three replicates.

Table 4. The effect of the plant extracts on ALL cells percentage viability after 24 h of incubation.

Plant samples	Extract concentration (µg)							
	Cold water		Hot water		Ethanol		Methylene chloride: Methanol	
	10 µg	20 µg	10 µg	20 µg	10 µg	20 µg	10 µg	20 µg
	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %
Luffa aegyptiaca	26*	39	55	71	70	82	51	72
Solenostemma arghel	69	76	74	80	63	85	67	81
Cassia acutifolia	32	43	20	27	50	79	52	71
Capsicum frutescens	35	41	56	64	74	89	40	84
Ocimum Basillcum	36	40	35	42	45	76	60	77
Colocasia antiquorum	63	85	11	66	44	60	12	80
Beta vulgaris	10	35	18	29	63	85	45	60
L.S.D. $_{(0.05)}$	3.0	3.6	3.2	3.9	10.7	5.2	3.5	5.0

*Each value represents the mean ± S.D (Standard Division) and mean of three replicates.

Table 5. The effect of the extracts on non-Hodgkin's lymphomas (normal bone marrow cells) as determined by percent cytotoxicity after 24 h of incubation.

Plant samples	Extract concentration							
	Cold water		Hot water		Ethanolic extract		Methylene chloride : EtOH	
	10 µg	20 µg	10 µg	20 µg	10 µg	20 µg	10 µg	20 µg
	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %	Dead %
Luffa aegyptiaca	2	5	3	4	5	9	11	15
Solenostemma arghel	5	7	1	6	7	11	3	8
Cassia acutifolia	3	6	2	6	7	8	2	7
Capsicum frutescens	11	22	7	15	9	16	13	25
Ocimum basillcum	6	9	4	10	8	11	5	13
Colocasia antiquorum	3	8	6	10	4	7	5	8
Beta vulgaris	2	7	2	5	6	6	9	14
L.S.D. $_{(0.05)}$	0.4	0.7	0.3	0.6	0.5	0.7	0.5	0.9

*Each value represents the mean ± S.D (Standard Division) and mean of three replicates.

as the most important mechanism by which cell-type differences arise in development and by which patterns of tissue organization are established (Freeman et al., 2002). At almost every stage in development, cells emit and receive signals from other nearby cells and these signals are necessary for normal differentiation and function (Freeman et al., 2002). Epidemiological studies have suggested that an inverse association exists between consumption of vegetables and fruits and the risk of human cancers at many sites (Riboli and Norat, 2003). Phenolic compounds, including flavonoids, are especially promising candidates for cancer prevention (Bravo, 1998). Much information is available on the reported inhibitory effects of specific plant phenolic compounds and extracts on mutagenesis and carcinogenesis (Meyers et al., 2003).

The potential ability of polyphenol combinations to prevent cancer progression has not been adequately studied. Scientists have suggested that it appears extremely unlikely that any one substance is responsible for all of the associations seen between plant foods and cancer prevention because of the great variety of dietary phenolics, including flavonoids and the many types of potential mechanisms reported (Birt and Wang, 2001; El-Shemy et al., 2007). It has been suggested that the combination of phytochemicals in fruits and vegetables is crucial for their potential anticancer activities (Sun et al., 2002).

Mortality in experimental animals

Analyses of transplanted animals after tumor transplantation showed that extracts of *S. arghel* delayed the death and inhibited the tumor growth of transplanted animals compared with untreated transplanted animals (Figure 2). The active principles in both water and ethanol extracts reduced EACC growth after transplantation (Figure 2). There was a significant ($p \leq 0.05$) increase in the extent of life span of mice by *S. arghel* extract (GIV) when compared to control (GII) animals.

On the other hand, the other extracts did not induce significant differences in mortality compared with control (data not shown).

Biochemical analyses of EACC cells in experimental animals

Biochemical parameters including, super oxide dismutase, glutathione peroxidase and glutathione-S-transferase activity in the serum of each experimental group was examined (Table 6). Significant ($p \leq 0.05$) increases in the amount of each enzyme activity was found in animal groups IV and VII when compared with the control (GII) animals and a significant ($p \leq 0.05$) increase in the amount of lactic dehydrogenase activity in groups IV and VII (Table 6). The effect of plant extracts on viability EACC tumor cells number was evaluated (Table 7).

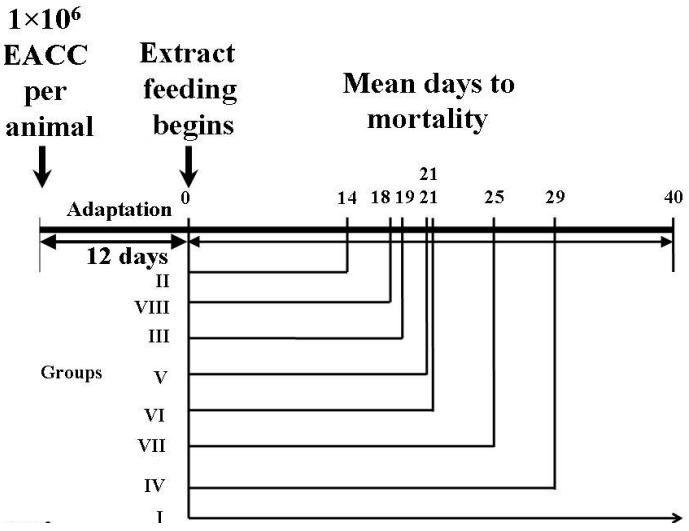

Figure 2. Life span of animals transplanted with EACC.
Group I of mice was spared a cell transplant and referred to as the control (negative). Group II was transplanted to the intraperitoneal (i.p) cavity with EACC at 2x106 cells (0.2 ml; positive control). Group III was transplanted with EACC like the second group and each mouse was daily forced to ingest orally via stomach tube about 0.2 ml of cold aqueous extract of LSA (E x 1) (10% w/v) in addition to the normal diet. Group IV was treated daily like the third group but with hot water LSA extract (E x 2). Group V was treated daily like the third group but with ethanol extract of LSA extract (E x 3). Group VI was treated daily like the third group but with methylene chloride : methanol (1:1) of LSA extract (E x 4). Group VII was treated daily like the third group but with hot water extract of LCA. Group VIII was treated daily like the third group but with ethanol extract of LBV (E x 6).

Administration each of the plant extracts for one week after EAAC transplantation decreased the tumor cell number over their untreated count expert. When plant extracts GIV, GVII and GII were administrated to mice the numbers of EACCs in tumor-bearing mice were significantly reduced (Table 7). These extracts appear to activate EACC tumor cell death possibly by immunosuppression.

Suman et al. (2001) evaluated the EACC number in mice after different doses of curcumin and found that the doses between 25 and 50 mg/kg body weight of curcumin restored the depressed cell number to normal level. Although, at doses above 50 mg/kg body weight EAC killing was much more pronounced.

DNA fragmentation analyses

DNA-agarose gel electrophoresis was performed to detect plant extract-induced DNA laddering. DNA fragmentation was observed in each plant extract treated AML and ALL cells (Figure 3). The patterns were consistent with nuclear fragmentation and condensation that occurs during apoptotic cell death (Figure 3). The exposure of AML cells to 100 and 200 µg/ml plant extracts for 24 h may be effective in inducing apoptosis (Figure 3).

Table 6. Antioxidant enzyme activities *in vivo* at 2 weeks after treatment.

Group (n)	SOD activity (U/ml)	GPx activity (mU/ml)	GST activity U/L	LDH activity (U/L)
Negative control (GI)	82.80 ± 2.57 [a]	95.10 ± 2.95 [a]	53.11 ± 1.65 [a]	22.90* ± 0.71** [c]
Positive control (GII)	28.12 ± 1.32 [g]	56.10 ± 2.64 [f]	20.90 ± 0.98 [ef]	12.14 ± 0.57 [f]
Group1 (GIII)	61.00 ± 1.59 [c]	67.01 ± 1.74 [de]	37.50 ± 0.98 [c]	17.53 ± 0.46 [e]
Group2 (GIV)	78.12 ± 2.81 [b]	84.30 ± 3.03 [b]	43.50 ± 1.57 [b]	39.12 ± 1.41 [a]
Group3 (GV)	36.00 ± 1.87 [f]	62.70 ± 3.26 [e]	21.60 ± 1.12 [e]	22.90 ± 1.19 [c]
Group4 (GVI)	54.70 ± 1.59 [d]	73.50 ± 2.13 [c]	28.43 ± 0.82 [d]	20.20 ± 0.59 [d]
Group5 (GVII)	48.06 ± 1.44 [e]	88.60 ± 2.66 [b]	19.37 ± 0.58 [f]	28.33 ± 0.85 [b]
Group6 (GVIII)	14.06 ± 0.59 [h]	71.30 ± 2.99 [cd]	16.19 ± 0.68 [g]	16.19 ± 0.68 [e]
LSD $_{(0.05)}$	3.193	4.703	1.918	1.496

***** Each value represents the mean ± S.D (Standard Division) and mean of three replicates.
**Values in the same column with the same letter are not significantly at ($p \leq 0.05$).

Table 7. Effect of the extracts on the number of viable cells in transplanted mice.

Group (n)	1st week (- ×10^6)	2nd week (- ×10^6)
Positive control (GII)	83* ± 3.90** [b]	479 ± 22.51 [a]
Group1 (GIII)	82 ± 2.13 [b]	116 ± 3.02 [d]
Group2 (GIV)	81 ± 2.92 [bc]	47 ± 1.69 [e]
Group3 (GV)	76 ± 3.95 [cd]	131 ± 6.81 [d]
Group4 (GVI)	89 ± 2.58 [a]	255 ± 7.40 [c]
Group5 (GVII)	75 ± 2.25 [d]	112 ± 3.36 [d]
Group6 (GVIII)	85 ± 3.57 [ab]	320 ± 13.44 [b]
LSD $_{(0.05)}$	5.472	18.857

***** Each value represents the mean ± S.D (Standard Division) and mean of three replicates**.**
**Values in the same column with the same letter are not

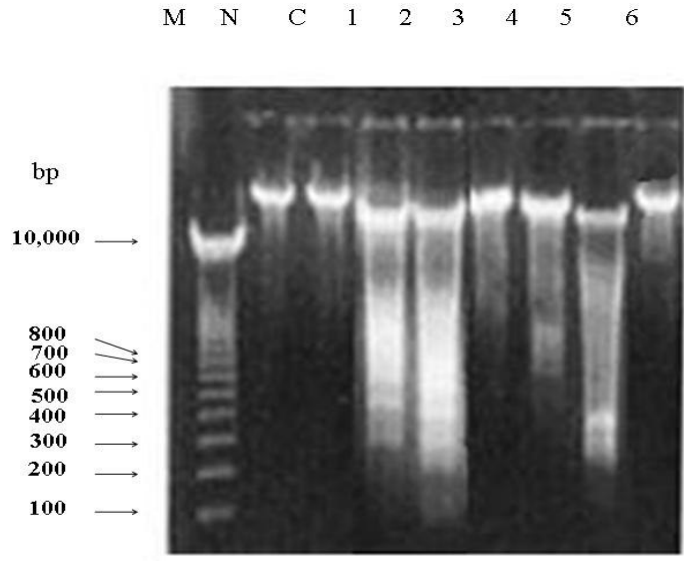

Figure 3. Agarose gel electrophoresis of DNA extracted from AML cells treated with plant extracts. Lane M:DNA ladder (distinct marker bands were arrowed with their size in bp); lane N, AML negative control cells; lane C, AML control cells; lane 1, AML cells treated with E x 1; lane 2, AML cells treated with E x 2; lane 3, AML cells treated with E x 3; lane 4, AML cells treated with E x 4; lane 5, AML cells treated with E x 5; lane 6, AML cells treated with E x 6. Each well was loaded with 5 µg of DNA.

Gao et al. (2002) showed that resveratrol induced DNA fragmentation in 32Dp210 leukemic cells. Resveratrol induced of internucleosomal DNAfragmentation and cleavage of procaspase-3 in treated cells (Gao et al., 2002). These results supported the previously reported apoptosis-inducing activity of resveratrol against tumor cell lines (Clement et al., 1998; Hsieh and Wu, 1999). Willow (Salix sp.) extracts also caused DNA fragmentation (El-Shemy et al., 2007). In the other hand, ethanol extract from leaves of *L. aegyptiaca* were showed highest activities against AML and ALL (Tables 3 and 4). Luffin from seeds of *L. aegyptiaca* was found to induce an increase in cytosolic oligonucleosome-bound DNA in both melanoma and Ehrlich ascites tumour cells, the level of DNA fragmentation in the former cell line being higher than in the latter (Poma et al., 1998). Experiments with melanoma cells indicate that an increase in cytosolic nucleosomes could be supportive of apoptosis as the type of cell death induced by luffin (Poma et al., 1998).

The results reported here suggested that hot water and ethanolic extracts of *S. arghel* and hot water extracts of *C. antiquorum* may have an immuno-modulatory potential via stimulating antiproliferation of tumor cells. However, hot water and ethanolic extracts of *S. arghel* and hot water extracts of *C. antiquorum* significantly inhibited the growth of AML, ALL and EACC cells *in vitro* and *in-vivo*. This appears to involve apoptosis-induced cell loss; a lowering in the proliferation rate of AML cells. The immuno-modulatory components were associated with the content of phenolics, including flavonoids. Further, The total phenolic contents correlated significantly ($p < 0.05$) and positively with the activity of antioxidation enzymes *in vivo* and the percent inhibition of oxidation *in vitro*.

In conclusion, it has become clear that in Egypt some plants and herbs might provide effective anti cancer therapeutics. Such extracts should be more widely used in developing countries for prevention and treatment of dangerous diseases like cancer. The extracts should be considered as good sources for drug discovery.

ACKNOWLEDGMENTS

The authors thank colleagues, technicians for their helpfulness and assistance during the experiments. This study was partly supported by grants from the National CFIDS Foundations Inc, Needham, MA 02492-3931, USA.

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