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EGYPTIAN FLORA PROVIDING POWER ACTIVITY AS ANTIOXIDANTS AND ANTICARCINOGENIC AGENTS

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ABSTRACT

In nature natural products from plants are rich sources which were used for treating a number of diseases instead of chemical therapy. Meanwhile, many of the pharmacological principles currently used as anticancer agents have been initially isolated from plants especially which grow in dry weather area like Egypt. Therefore, some Egyptian flora extracts were used for this study such as leaf of *Luffa aegyptiaca* (LLA), *Solenostemma arghel* (LSA), *Cassia acutifolia (LCAA), Ocimum Basillcum* (LOB), *Colocasia antiguorum* (LCA), *Beta vulgaris* (LBV) and fruit of *Capsicum frutescens* (FCF), as antioxidants activity using DPPH˙ radical method and for anticancer (Acute myeloid leukemia, AML, Acute Lymphocyte Leukemia ALL *In-vitro study*) and Ehrlich Ascites Carcinoma Cells (EACC, *In-vivo* study). The results showed that EACC derived tumor growth was reduced by the *Solenostemma arghel* (LSA) hot water extract and death was delayed (for 29 days). On the other hand, in *in-vitro* experiments the extracts could kill the majority (66-90%) of abnormal cells among primary cells harvested from patients with ALL and AML especially hot water extract of *Solenostemma arghel* (LSA). DNA fragmentation patterns within treated cells inferred targeted cell death by apoptosis were detected. Therefore, the mechanism on tumor cells may due to promote apoptosis, cause DNA damage, or denature proteins. On the other hand, most of extracts showed significant elevation as antioxidant derivatives. In conclusion, metabolic flora compounds could use as drug for harmful diseases such cancer without side effect.

Keywords: Natural products, Anticancer, Antioxidants, Acute myeloid leukemia, Ehrlich Ascites Carcinoma Cells.

INTRODUCTION

Natural products, including plants have been considered as valuable sources for anticancer drug discovery (Schwartsmann *et al.*, 2002). Herbal, or 'botanical', medicines, recorded in developing countries such as Egypt and China Pharmacopoeia had been prescribed in many diseases for long time, and began to match by increasing scientific attention recently (Vickers, 2002). Due to the different components in a herb may have synergistic activities or buffering toxic effects, mixtures or extracts of herbs might have more therapeutic or preventive activity than alone (Li *et al.*, 2000; Vickers, 2002). Several studies have demonstrated that extracts from several herbal medicines or mixtures had an anticancer potential *in vitro* or *in-vivo* (Bonham *et al.*, 2002; Hu *et al.*, 2002; Kao et al., 2001; Lee *et al.*, 2002; Yano *et al.*, 1994; El-Shemy, *et a.*, 2007). Recently, the use of traditional medicine based on plants has received considerable interest (Han et al 2002). There are national and indigenous rights over plant derived resources. Basic scientific investigations based on medicinal plants and indigenous medical systems

have increased. A screening program was initiated by Leven et al. (1979) that identified many antibacterial antifungal, antiviral, antiparasitic, and other pharmacologically active substance activities in higher plants (Jang et al 1997).

Aqueous extract of willow Salix leaves could induce prevention of three cancer cells AML, acute ALL and EACC (El-Shemy *et al.*, 2007). Alcohol extract of *Ganoderma lucidum* (Hu *et al.*, 2002) could induce apoptosis in MCF-7 human breast cancer cells, and water extract of *Paeoniae lactiflora* also could induce apoptosis in HepG2 and Hep3B hepatoma cells (Lee *et al.*, 2002). Water extract of Chinese Bu-Zhong-Yi-Qi-Tang (mixture of ten herbs) could induce apoptosis in hepatoma cells (Kao *et al.*, 2001). Water-soluble ingredients of Sho-Saiko-To (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 for 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; Yim *et al.*, 2000). Therefore, the aim of our study is to determine the effect of some Egyptian flora as antioxidants and antitumor in order to use for treatment of cancer instead of chemical and radiotherapy which caused sever damage in human cells.

MATERIALS AND METHODS

Plant extraction:

Crude plant leaf extracts of *Luffa aegyptiaca (LLA), Solenostemma arghel (LSA), Cassia acutifolia (LCAA), Ocimum Basillcum (LOB), Colocasia antiguorum (LCA), Beta vulgaris (LBV) and fruit of Capsicum frutescens (FCF)* were prepared for *in vitro* screening. The extraction was done using 20g of powdered plant (leaf or fruit) material with 200 ml of hot water, cold water, ethanol 80% and methanol : methylene chloride (1:1) separately for each plant with mechanical stirring, for 12 h at room temperature (25°C) except the hot water (80°C) which was used for half hour. The extracts were completely evaporated at 40°C then lyophilized and stored at 4°C.

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

Determination of total phenolic and flavonoid contents:

Total phenolic contents of plant samples were determined by the Folin–Ciocalteu method (Meda *et al.*, 2005)

The total flavonoids content was determined according as the aluminum chloride colorimetric method described by Chang *et al.* (2002).

Antioxidants activity:

The antioxidant activity of the leaf plant extracts was evaluated by using DPPH˙ assay (Cuendet et al., 1997 and Burits and Bucar, 2000) Hydrogen atom- or electron- donation ability of the corresponding oils was measured from the bleaching of the purple-colored methanol solution of DPPH. This spectrophotometric assay uses stable 2, 2'diphenylpicrylhydrazyl (DPPH) radical as a reagent. Fifty microliters of various concentrations of the oils in methanol were added to 5 ml of a 0.004% methanol solution of DPPH˙. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition of DPPH˙ free radical in percent (I %) was calculated in following way:

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

Where: A_{blank} is the absorbance of the control reaction (containing all reagents except the tested compound), and A_{sample} is the absorbance of the tested compound. Extract concentration providing 50% inhibition IC_{50} (defined as the concentration of tested compound required to produce 50% maximal inhibition) was calculated form the graph plotting inhibition percentage against extract concentration. Determinations were carried out in triplicate.

***In-vitro* assay for cytotoxic activity (MTT assay):**

The cytotoxicity of each extract on both AML, ALL and EACC cells was determined by the MTT assay (Selvakumaran *et al.*, 2003). AML had been diagnosed by peripheral blood and bone marrow examination, cytochemistry and immunological markers in some cases. Mononuclear cells were separated from other blood cells by Ficoll hypaque density gradient (Pharmacia, Uppsala, Sweden). The cells were then washed with three changes of PBS. The cell counts were adjusted to (3×10^3 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 human normal lymphoid cell line (reported in elsewhere); 3 wells were included in each concentration. After treatment with extracts for one day, 20 µl of 5 mg/ml MTT (pH 4.7) were added per well and cultivated for another 4 h, the supernatant fluid was removed, then 100 µl DMSO were 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 % cytoviability, using the following formula: %cytoviability = A_{570} of treated cells / A_{570} 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: One group of mice was spared a cell transplant and referred as the control (normal). The second group was transplanted to the intraperitoneal (i.p) cavity with EACC at 2×10^6 cells (0.2 ml) (transplanted control). The third group 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 (10% w/v) in addition to the normal diet. The fourth group was treated daily like the

third group but with hot water LSA extract. The fifth group was treated daily like the third group but with ethanol extract of LSA extract. The sixth group was treated daily like the third group but with methylene chloride : methanol (1:1) of LSA extract. The seventh group was treated daily like the third group but with hot water extract of LCA. The eighth group was treated daily like the third group but with ethanol extract of LBV. Flow chart of experimental design. Total experimental period was 32 day after mice transplanted to the intraperitoneal (i.p) cavity with EACC (adaptation period)

Biochemical analysis:

At the end of the experimental period, the animals were sacrificed by cervical decapitation and blood plasma was collected. The Superoxide dismutase (SOD) was estimated by the method of Nishikimi *et al.* (1972), glutathione peroxidase (GPx) by the me hod of Paglia and Valentine (1967) and glutathione-S-transferase (GST) by method of Habig and Pabst (1974). The Lactic dehydrogenase (LDH) was determined by method of Kaplan and Pesce, (1996) in EACC weekly which were separated from mice (number of EACC was calculated weekly in all animals). All enzymes activity determinations were done by kits from Sigma (Egypt).

DNA extraction and measurement of apoptosis:

DNA was extracted from mature (normal cells) and immature white blood cells (leukemic cells) before and after treatment with the selected extract which resulted from the screening. 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% Triton X-100) for 20 min (Gao *et al.*, 2002). Cell lysates were centrifuged at 27,000 xg 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 UV fluorescence was used to determine the degree of apoptotic DNA fragmentation (Gao X *et al.*, 2002).

Statistical analysis:

The statistical analysis was computed using analysis of variance procedure described by Sendecor and Cochran (1980). The significant mean differences between treatment means were separated by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

Total phenolic and flavonoid contents of plant samples:-

The amount of total phenolic compounds varied slightly in plant extracts and ranged from 6.25 to 33.10 mg as gallic acid/g dry sample (Table 1). The highest amount was found in *Ocimum basillcum* ethanolic extract and the lowest in one *Luffa aegyptiaca* cold water extract (Table 1). Similar amounts in plant phenolics from herbs and medicinal plants collected in Finland have been reported recently (Jin-Yuarn and Ching-Yin 2007).

Total flavonoids are ranged from 0.21 ± 0.01 to 2.67 ± 0.08 mg/g dry weight (Quercetin equivalents), the highest amount was found in clocasia *antiguorum* ethanolic extract and the lowest one in *Capsicum frutescens* cold water extract (Table 1) and the phenolic and flavonoids compounds extracted from tested plants may contain the major active principles for the destruction of cancer cells (Kupchan 1970, Aboul-Enein 1986 , Aboul-Enein *et al.*, 1991, El-Shemy *et al* and 2003 El-Shemy *et al.*, 2007)

DPPH radical scavenging activity:

The plant extracts showed a concentration-dependent scavenging activity by quenching DPPH radicals (Table 2). The hydrogen donating activity, showed that the *Ocimum basillcum* ethanolic extract as antioxidants activity was 92.70 ± 2.87 percent, with IC50 value of 0.19 ± 0.011 mg/ml. This due to the high concentration of phenolics and flavonoids in *Ocimum basillcum* extract. Phenolics and flavonoids compounds widely distributed in the 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. They can participate in protection against the harmful action of reactive oxygen species, mainly oxygen free radicals. Free radicals are produced in higher amounts in a lot of pathological conditions and are involved in the development of the most common chronic degenerative diseases, such as cardiovascular disease and cancer (Halliwell and Gutteridge, 1999).

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. Since the prevention of chronic diseases is a more effective strategy than their treatment, reducing the risk of diseases such as cardiovascular disease and cancer is a subject of great interest for medicos, scientists in general, consumers and the food industry (Liu, 2003). In this context, redox and antioxidant systems are among the most promising targets for functional food science. For this reason, many functional foods are nowadays aimed at boosting intakes of antioxidants in order to reduce the risk of chronic disease linked to oxidative stress. Among the most common dietary sources of natural antioxidants, grapes and berries are rich in phenolic compounds, particularly flavonoids, which are valued for their role in anticarcinogenic, antiatherogenic, anti-inflammatory, antimicrobial and antioxidant activities (Robards, *et al.*, 1999; Moure *et al.* 2001).

Cell viability and cytotoxicity analysis:

After 24 h incubation of the mononuclear AML and ALL cells with different plant extracts, the cytotoxicity on the tumor cell lines was evaluated using the MTT assay. The hot water, cold water and ethanolic extract obtained from *Solenostemma arghel* showed highly active in this assay. The maximal inhibition of cell growth (82, 92, 79%) was obtained at 200 µg/ml of extracts (Table 3 and 4). On the other side, cold water extract obtained from *Colocasia antiguorum* also possessed cytotoxic activity and inhibited the cell growth (81%) at 200 µg/ml concentration. The other tested extracts gave weak effects against AML and ALL (Table 3 and 4) compared with control (Table 5).

Table (1). Contents of Phenolic and Flavonoid compounds in plant samples

Plant samples	Cold water		Hot water		Ethanolic Extract		Methylene chloride:EtOH	
	* Total phenolics	** Total Flavonoids	* Total phenolics	** Total Flavonoids	* Total phenolics	** Total Flavonoids	* Total phenolics	** Total Flavonoids
Luffa aegyptiaca	6.25	0.35	15.00	0.95	13.16	1.20	9.80	0.86
Solenostemma arghel	17.90	1.30	8.76	0.89	21.00	1.80	18.40	1.65
Cassia acutifolia	22.30	0.90	8.28	0.38	14.30	0.23	24.82	1.21
Capsicum frutescens	14.74	0.21	25.80	1.60	16.38	0.61	13.32	0.53
Ocimum Basillcum	17.90	1.44	10.22	0.85	33.10	2.00	21.08	1.20
Colocasia antiquorum	10.30	0.87	10.30	0.64	17.70	2.67	16.60	1.80
Beta vulgaris	19.42	1.80	12.24	1.30	14.00	1.12	11.40	0.97
LSD $_{(0.05)}$	0.952	0.063	0.776	0.056	1.066	0.085	0.931	0.071

- 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 ($P \leq 0.05$).
- *: values were determined as mg galic acid / g sample on dry base; **: values were determined as mg qurceten / g sample on dry base

Table(2). Antioxidant activity of plant samples using DPPH and DPPH IC_{50} values

Plant samples	The antioxidants activity of extract (0.5 mg)							
	Cold water		Hot water		Ethanolic Extract		Methylene chloride:EtOH	
	Antioxidant activity	IC_{50}	Antioxidant activity	IC_{50}	Antioxidant activity	IC_{50}	Antioxidant activity	IC_{50}
Luffa aegyptiaca	17.37	1.19	17.90	1.15	29.00	0.75	59.30	0.45
Solenostemma arghel	35.50	0.61	33.30	0.67	59.60	0.43	55.00	0.47
Cassia acutifolia	43.00	0.58	39.00	0.61	45.20	0.59	54.70	0.48
Capsicum frutescens	25.80	0.69	22.20	0.72	31.80	0.57	25.60	0.69
Ocimum Basillcum	85.30	0.21	53.00	0.48	92.70	0.19	87.50	0.20
Colocasia antiquorum	50.20	0.49	49.70	0.52	74.50	0.27	67.20	0.42
Beta vulgaris	50.00	0.50	22.00	0.78	56.80	0.48	54.60	0.49
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 ($P \leq 0.05$).

From this observation, it is clear that the antileukemic activity of the leave extracts of *Solenostemma arghel* was mostly due to compounds that were soluble in water and/or ethanol. Hot water extract of *Colocasia antiguorum* leaves have the same effect. These active ingredients were easily dissolved in hot water, and in turn could be used as a natural antitumor medicine. When the fractions of each crude extract were dissolved in a saline solution after removing the solvent then incubated with the leukemia cells, the results showed that a fraction of the *Luffa aegyptiaca*, *Cassia acutifolia* and *Ocimum Basillcum* showed very low destructive effect on tumor cells (viability 81–92%), except the ethanolic extract of *Ocimum Basillcum*. Furthermore, *Capsicum frutescens* ethanolic and methylene chloride : methanol extracts were effective on tumor cells but showed high cytotoxic effect on the normal cell. However, a major destructive effect on AML and ALL were obtained by a fraction of the polar organic solvents (water and 80% ethanol (Table 3 and 4). It is known that the phenolic compounds, most glycosides, and many types of tannins dissolve in water or ethanol solutions (Bravo L 1988). Therefore, these groups of compounds may contain the major active components for the destruction of leukemia and carcinoma cells (Aboul-Enein 1986, Aboul-Enein *et al* 1991 and El-Shemy, *et al* 2007).

The allamandin derivatives that are extracted by water and/or ethanol from *Allamanda catharica* (Apocynaceae) showed significant activity *in vivo* against the p-388 leukemia in the mouse (Kupchan 1976). Based on this finding, the cancer cells could be killed through denaturation of some enzymes and proteins that are induced by salicin and saligenin (El-Shemy *et al*, 2007). Signaling between cells is commonly regarded 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 phenolics acids and 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 and Liu, 2003). Yet, 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 flavonoids and many types of potential mechanisms reported (Birt and Wang, 2001; Malak *et al.*, 2005 and 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, Chu, Wu, & Liu, 2002).

Table (3).The effect of the plant samples extracts on AML cell cytoviability % after 24 h of incubation

Plant samples	Extract concentration (µg)							
	Cold water		Hot water		Ethanolic Extract		Methylene chloride:EtOH	
	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 Basilicum	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. Each values % relative to untreated cells
- Values in the same column with the same letter are not significantly at ($P \leq 0.05$).

Table (4).The effect of the plant samplesextracts on cell cytoviability% after 24 h of incubation

Plant samples	Extract concentration (µg)							
	Cold water		Hot water		Ethanolic Extract		Methylene chloride:EtOH	
	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	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 Basilicum	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. Each values % relative to untreated cells
- Values in the same column with the same letter are not significantly at ($P \leq 0.05$).

Table (5). The effect of the plant samples extracts on normal cell HD cytotoxicity% after 24 h of incubation

Plant samples	Extract concentration (µg)							
	Cold water		Hot water		Ethanolic Extract		Methylene chloride:EtOH	
	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg	100 µg	200 µg
	Viable %	Viable %	Viable %	Viable %	Viable %	Viable %	Viable %	Viable %
Luffa aegyptiaca	98	95	97	96	95	91	89	85
Solenostemma arghel	95	93	99	94	93	89	97	92
Cassia acutifolia	97	94	98	94	93	92	98	93
Capsicum frutescens	89	78	93	85	91	84	87	75
Ocimum Basillcum	94	91	96	90	92	89	95	87
Colocasia antiquorum	97	92	94	90	96	93	95	92
Beta vulgaris	98	93	98	95	94	94	91	86
L.S.D. $_{(0.05)}$	ns 6.2	5.9	ns 6.3	5.9	ns 6.0	0.7	6.1	5.7

- Each value represents the mean ± S.D (Standard Division) and mean of three replicates. Each values % relative to untreated cells
- Values in the same column with the same letter are not significantly at ($P \leq 0.05$).

Table (6). Antioxidants and anticancer enzymes levels *in vivo*

Group(n)	Anticancer indicator	Antioxidant indicator		
	LDH activity (U/L)	GST activity U/L	GPox activity (mU/ml)	SOD activity (U/ml)
Normal control (GI)	22.90	53.11	95.10	82.80
Transplanted control (GII)	12.14	20.90	56.10	28.12
Group1 (GIII)	17.53	37.50	67.01	61.00
Group2 (GIV)	39.12	43.50	84.30	78.12
Group3 (GV)	22.90	21.60	62.70	36.00
Group4 (GVI)	20.20	28.43	73.50	54.70
Group5 (GVII)	28.33	19.37	88.60	48.06
Group6 (GVIII)	16.19	16.19	71.30	14.06
LSD $_{(0.05)}$	1.496	1.918	4.703	3.193

- 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 ($P \leq 0.05$).

Biochemical analysis of experimental animals:

Analyses of transplanted animals before and after tumor transplantation were correlated with extracts administration. The results showed that extracts of *Solenostemma arghel* delayed the death and inhibited the tumor growth of transplanted animals compared with untreated transplanted animals that died within two weeks. However, the active principles in water and ethanol extracts modified the features of EACC hampering their growth after transplantation (our data not shown). There was a significant ($P \leq 0.05$) increase in the extent of life span of mice by *Solenostemma arghel* extract (GIV) when compared to control (GII) animals.

On the other hand, in extract values did not induce significant difference compared with control (our data not shown). Biochemical parameters body weight of animals including superoxide dismutase (SOD), glutathione peroxidase (Gpx) and glutathione-S-transferase (GST) activity in plasma of various experimental groups were examined (Table 6). Significant stimulations in the extent of SOD, GPX and GST activities in group (IV and VII) when compared with the control (GII) animals and a significant stimulations in the extent of lactic dehydrogenase (LDH) in groups (IV and VII) were detected which measured in EACC (Table 6).

The effect of plant extracts on viability EACC tumor cells number was evaluated. Administration of 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 reduced when compared with the transplanted control (Table 7).This implied that these extracts activated EACC killing tumor burden itself caused immunosuppression. (Aboul-Enein *et al.*, 1986) Suman, *et al.* (2001) evaluated the EACC number in mice when administrated by 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 of curcumin body weight EAC killing was much more pronounced.

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

Group(n)	1st week (- ×10⁶)	2nd week (- ×10⁵)
Normal control (GI)	00.00	00.00
Transplanted control (GII)	83	479
Group1 (GIII)	82	116
Group2 (GIV)	81	47
Group3 (GV)	76	131
Group4 (GVI)	89	255
Group5 (GVII)	75	112
Group6 (GVIII)	85	320
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 significantly at ($P \leq 0.05$).

DNA fragmentation:

DNA-agarose gel electrophoresis was performed to detect plant extracts induced DNA laddering. Such a phenomenon was observed in plant extracts treated AML and ALL cells, which is consistent with the fact that nuclear fragmentation and condensation, occurs during apoptotic cell death (Figure 1). Overall results seem to indicate that exposure of AML cells to 100 and 200µg/ml of different plant extracts for 24 h may be effective in inducing apoptosis (Figure 1). Gao *et al.* (2002) investigated that the resveratrol induced DNA fragmentation in 32Dp210 leukemic cells. Resveratrol induced apoptosis in these cells as supported by the induction of internucleosomal DNA fragmentation and the cleavage of procaspase-3 in resveratrol 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 and Hsieh and Wu, 1999) and also with salix extract (Malak et al., 2005 and El-Shemy *et al*, 2007).

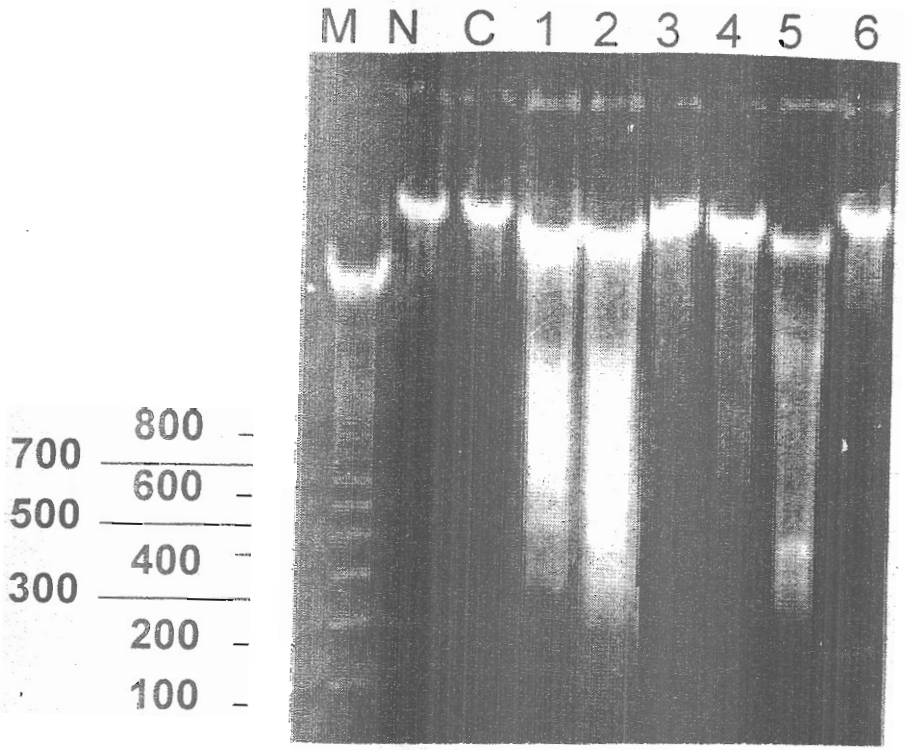

Fig. (1) Agarose gel electrophoresis of DNA extracted from AML cells treated with C-PC. M :DNA ladder(marker) ;N: AML normal control cells; C: AML control cells; Lane 1: AML cells treated with Ex1; Lane 2: AML cells treated with Ex2; Lane 3: AML cells treated with Ex3; Lane 4: AML cells treated with Ex4; Lane 5: AML cells treated with Ex5; Lane 6: AML cells treated with Ex6. all of wells were loaded with 20µl extract

In conclusion, it is clear that in Egypt some wild plant types and folk herbs were widely used in developing countries for treatment of some danger diseases like cancer and should be considered as good sources for drugs discovery.

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النباتات المصرية كمصدر للمركبات ذات الفاعلية كمضادات للأكسدة والسرطانات

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تعتبر النباتات مصدرا غنيا بالمركبات الطبيعية التي استخدمت في معاملة العديد من الامراض بدلا من العلاج الكيماوي. كما ان العديد من العقاقير التي استخدمت كمضادات للسرطانات تم فصلها من النباتات خاصة تلك التي تنمو في المناطق الحارة مثل مصر. لذلك تم دراسة بعض مستخلصات النباتات المصرية (ورق اللوف ، الحرجل ، السنامكي ،الريحان ، القلقاس ، بنجر المائدة و ثمار الفلفل الاحمر الحار) كمضادات للاكسدة مستخدما طريقة DPPH radical وكمضادات للسرطان علي نوعين من الخلايا AML and ALL *in-vitro* و علي فئران عوملت بـ EACC *in-vivo* .

حيث اوضحت النتائج المتحصل عليها ان المستخلص المائي الساخن لورق نبات الحرجل استطاع تقليل نمو الورم داخل حيوانات التجارب المعاملة كما انة اخر موت الحيوانات ٢٩ يوم اكثر عندما قورنت بالحيوانات المعاملة بـ EACC فقطباستخدام تركيز ٢٠٠مليجرام / فأر/ يوم.

ايضا ادت هذه المستخلصات الي موت الخلايا السرطانية عند دراستها in-vitro بمعدل يصل الي (من ٦٦ الي ٩٠% موت) وكان اكثر المستخلصات تأثيرا ايضا المستخلص المائي الساخن لورق نبات الحرجل.

ايضا تم دراسة ميكانيكية موت هذة الخلايا بالمعاملة بهذه المستخلصات من خلال احداث موتا مبرمجا للخلية Apoptosis حيث اظهرت تكسير الحامض النووي الريبوزي لهذه الخلايا .

ايضا اظهرت النتائج مقدرة المستخلصات علي التخلص من الشقوق الحرة وفعليا كمضادات للاكسدة تصل الي ٩٢%.