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Evaluation of anticancer activity of some Egyptian plants showed free radical scavenging activity

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Abstract: Twenty plants belonging to 6 families were collected from different areas in Egypt. These plants yielded 25 methanol extracts and were tested for their free radical scavenging activity by using DPPH assay. Thirteen plant extracts showed potent free radical scavenging activity. The extractswere investigated for theiranticancer activity in vitro using four human cancer cell lines, namely, A549 (lung carcinoma) and HCT-116 (colorectal carcinoma), HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma). Cell viability was determined using the MTT assay. Results showed the methanol extracts of four plants possessed both high cytotoxicity against human cancer cells and free radical scavenging activities but showed no cytotoxic effect on human normal immortalized fibroblast cells (BJ-1). These extracts were: bark of*Adenanthera pavonina*, branch of *Albizzia stipulate*, leaves of *Derris elliptica* and bark of *Hyphaenethebaica*. The study identified four plant extracts possessing both antioxidant and cancer cytotoxicity activities and at the same time to be safe on normal cells. The purpose of this approach is to provide candidate drugs to be used for cancer chemoprevention by preventing cancer initiation and eradicating any newly formed microscopic cancer cells and to behighly safe to healthy cells.Further studies are recommended to evaluate theantioxidant,anticancer efficacy and general toxicity of the active plant extracts in animal models.
Keywords: free radical scavenging; anti-cancer; Egyptian plants, cancer chemoprevention.

Introduction

The worldwide use of natural products including medicinal plants has become more and more important in primary health care especially in developing countries. Many pharmacognostical and pharmacological investigations are carried out to identify new drugs or to find new lead structures for the development of novel therapeutic agents for the treatment of human diseases such as cancer and infectious diseases [1].

Cancer is the leading cause of mortality worldwide. According to the cancer reports published by the World Health Organization (WHO) and the World Cancer Research Fund, the incidence of cancer is still increasing especially due to diet, environment and carcinogenicvirus infections[2,3]. In hospitals, conventional

drugs are commonly prescribed to cancer patients. However, due toless toxic and adverse effects of phytochemicals, the research on medicinal plants and cancer has been intensified[4].

The oxidative stressis defined as the imbalance between oxidants and antioxidants in favor of the oxidants potentially leading to damage in human cells. Physiologically, antioxidants play major role in preventing the formation of free radicals, which are responsible for many harmful oxidative processes. Antioxidants may be synthetic or of natural source [5].

Free radicals can react with biomolecules, causing extensive damage to DNA, protein, and lipid, which are considered to be related to aging [6], degenerative diseases of aging [7], cancer [8]. Antioxidants play an important role in the later stages of cancer development. There is increasing evidence that oxidative processes promote carcinogenesis, although the mechanisms for this are not well understood. The antioxidants may be able to cause the regression of premalignant lesions and inhibit their development into cancer [9].The scope for this study is to identify agents for cancer chemoprevention. Indeed, 13 plant extracts were potent in free radical scavenging, however, the authors of this study contemplated that a cytotoxic activity included in the cancer chemopreventive agent would be beneficial to eradicate any microscopic cancer lesions that might evolve in early stages. Additionally, the cytotoxic extracts were tested on normal human cells to select for extracts safe to normal cells and consequently are expected to be safe on prolonged usage.

2. Materials and methods

Plant material:

Table 1: Free radical scavenging activity of methanol plant extracts by using DPPH assay at concentration 100µg/ml.

DPPH scavenging % at 100 µg/ml	part	Plant name	Family	No
94 %	Br	*Spondiaslutea*	Anacardiaceae	1
90.5 %	Fr	*Spondiaslutea*	Anacardiaceae	2
27	L	*Spondiasspeciosa*	Anacardiaceae	3
81	Br	*Spondiasmangifera*	Anacardiaceae	4
91.2	Br	*Aberiasp*	Flacourtiaceae	5
91.6	Br	*Albizzia stipularis*	Leguminosae	6
90.2	L	*Derris elliptica*	Leguminosae	7
94	Bark	*Adenanthera pavonina*	Leguminosae	8
15	L,Br	*Erythrinaindica*	Leguminosae	9
13	Fl	*Tipuanatipu*	Leguminosae	10
14	Br	*CaesalpiniaCaffra*	Leguminosae	11
5	Fr	*Bauhinia variegate*	Leguminosae	12
93	Br	*Bauhinia junnejesii*	Leguminosae	13
59	Br	*Derris elliptica*	Leguminosae	14
8	Br	*Samaneasaman*	Leguminosae	15
88	L,Fl	*Caesalpiniacaffra*	Leguminosae	16
83	L,Br	*Cassia javanica*	Leguminosae	17
92.8	Br	*Adenanthera pavonina*	Leguminosae	18
69	L&Br	*Bauhinia hooker*	Leguminosae	19
90	B	*Gossypiumbarbadeinse*	Malvaceae	20
39	L	*Hibiscus esculentus*	Malvaceae	21
2	B	*Hibiscus esculentus*	Malvaceae	22
90.7	B	*Hyphaenethebaica*	Palmae	23
14	L&Fr	*Aeglemarmelos*	Rutaceae	24
48	Br	*Glycosmispentaphylla*	Rutaceae	25

Parts abbreviation; Br: branch; B: bark; Fr: Fruits; L: Leaves; FL: Flowers.

The plants for study were randomly collected from different areas in Egypt. Wild plants were collected by the help of Dr. Ashraf Soliman – Faculty of Science – Cairo University & authenticated by Prof. Dr. LotfyBoulos. Cultivated plants were collected and authenticated by Mrs. Teresa Labib - Head specialist for plant identification – El-Orman Garden – Giza. Theplant parts collected werebranches, barks, leaves, flowers, fruits, herbs and weeds. After collection, plant samples were dried and ground to coarse powder (Table 1).

Extraction:

Whole plant samples were divided into separate plant parts (leaves [L], branches [Br], fruits [Fr], flowers [Fl] and bark [B]) and dried in solar ovens at 50°C. After complete drying, the plant parts were grinded. Powdered plant parts were extracted with methanol at room temperature, using 450 ml methanol for 75 g powder. The powder was soaked in methanol at room temperature overnight. The filtrate was dried in a rotavapour under vacuum at 40 °C. The extract was then freeze dried (lyophilized) .The extracts were placed in glass vials and stored at -20 ºC. The freeze dried plant extracts were deposited at the Extract Bank of the In vitro Bioassay Laboratory, National Research Centre, Egypt.

DPPH method

The radical scavenging activity of plant extracts was evaluated by using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay[10]. Plant extracts were screened at 100 µg/ml using 0.1mM DPPH dissolved in methanol. After incubation for 30 min in the dark at room temperature, the absorbance was measured at 517 nm and a reference wavelength of 690 nm. Ascorbic acid (vitamin C) was used as positive control at different concentration ranging from 11 - 2.7 µg/ml. The DPPH / methanol mixture was used as a negative control. The DPPH scavenging activity of plant extracts was calculated according to the following equation:

Percentage reduction = (1-(X/ (av(NC)))* 100

Where x indicates the absorbance of sample and av(NC) indicates the average absorbance of the negative control.

Determination of antioxidant EC_{50} Values:

EC_{50} values were calculated for the most active extracts possessing ≥ 80% scavenging activityusing probit analysis and utilizing the SPSS computer program (SPSS for windows, statistical analysis softwarepackage /version 9/ 1989 SPSS Inc., Chicago, USA).

Cell culture:

A549 human lung carcinoma was maintained in DMEM medium,HCT-116 (colorectal carcinoma), HepG2 (hepatocellular carcinoma) and MCF-7 (breast adenocarcinoma) were maintained in RPMI. BJ-1 (human normal immortalized skin fibroblasts) was maintained in DMEM F12 medium. All media was supplemented with 10% foetal bovine serum and incubated at 37^{o}C in 5 %CO2 and 95% humidity.Cells were sub-cultured using trypsin versene 0.15 %.Skin normal human cell line (BJ-1) immortalizednormal foreskin fibroblast cell line was generously provided by Professor Stig Linder, Oncology andPathology department, Karolinska Institute, Stockholm, Sweden. Other cell lines were purchased fromVacsera (Giza, Egypt).

Cell viability assay

After 24 h of seeding 20000 cells per well in case of A-549, HCT-116 and BJ-1,10000 cells per well in case of HepG2 and MCF-7 cell lines (in 96 well plates), the medium was changed to serum-free medium containing a final concentration of the extracts of 100 µg/ml in triplicates. The cells were treated for 24 h. 100 µg/ml doxorubicin was used as positive control and 0.5 % DMSO was used as negative control. Cell viability was determined using the MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay as described by Mosmann 1983 [11].

The equation used for calculation of percentage cytotoxicity: (1- (av(x) / (av(NC))) 100*

Where Av: average, X: absorbance of sample well measured at 595 nm with reference 690 nm, NC: absorbance of negative control measured at 595 nm with reference 690.

Determination of cytotoxicity activity IC_{50} values

In case of highly active extracts possessing ≥ 75% cytotoxicity on different cancer cell lines, different concentrations were prepared dose response study. The results were used to calculate the IC_{50} values of each extract using probit analysis and utilizing the SPSS computer program (SPSS for windows, statistical analysis software package / version 9 / 1989 SPSS Inc., Chicago, USA).

Selectivity Index (SI)

The selectivity index (SI) indicates the cytotoxic selectivity (i.e. safety) of the crude extract against cancer cells versus normal cells (BJ-1, skin human normal immortalized cell line) [12].

SI= IC_{50} of plant extract in a normal cell line/ IC_{50} of the same plant extract in cancer cell line.

Results

Radical scavenging activity

Initially, 25 methanol plant extracts were examined by DPPH (2, 2-diphenyl-1-picrylhydrazyl) antioxidant assay. For the plant extracts that showed high free radical scavenging activity (≥ 80%) at one concentration (100µg/ml), further dilution were prepared to calculate their EC_{50} values and were chosen for anticancer activity in vitro investigation by using different human cancer cell lines. The data in table 1: shows the free radical scavenging activity of the 25 plant extracts. Out of them, 13 plant extracts possessed high free radical scavenging activity (≥ 80%). These are the extracts of the following species: (branch extract of *Spondias lutea* (94 %), bark extract of *Adenanthera pavonina* (94 %), *Bauhinia variegate* (93 %), branch extract of *Adenanthera pavonina* (92.8 %),*Albizzia stipulata* (91.6 %), *Aberia sp* (91.2 %), *Hyphaenethebaica (90.7 %), Spondias lutea* (fr) (90.5%), *Derris elliptic*s (90.2 %),*Gossypiumbarbadeinse (90 %),Caesalpiniacaffra* (88 %), *Cassia javanica* (83 %) and *Spondias mangifera*(81 %). According to the obtained results, a dose response study was performed on these plant extractsat concentrations50, 25, 12 and 5 µg/ml to calculate their EC_{50} values (Table 2).

Table 2: EC_{50} (the concentration required to scavenge 50% of the DPPH)

NO.	Family	*Plant name*	Part	EC_{50} (µg/ml)
1	Anacardiaceae	*Spondias lutea*	Br	43.6±1.3
2	Leguminosae	*Adenanthera pavonina*	B	41.6±1.2
3	Leguminosae	*Bauhinia junnejesii*	Br	46±1.8
4	Leguminosae	*Adenanthera pavonina*	Br	43±1.4
5	Leguminosae	*Albizzia stipulata*	Br	43±1.3
6	Flacourtiaceae	*Aberia sp*	Br	39±1.7
7	Anacardiaceae	*Spondias lutea*	Fr	43±1.7
8	Leguminosae	*Derris elliptica*	L	36±1.6
9	Malvaceae	*Gossypiumbarbadeinse*	B	25±0.9
10	Leguminosae	*Caesalpiniacaffra*	L&br	39±1.7
11	Palmae	*Hyphaenethebaica*	B	44±1.5
12	Leguminosae	*Cassia javanica*	L&br	34±1.2
13	Anacardiaceae	*Spondias mangifera*	Br	20±0.8
Positive control	Vitamin C			4.3±05

Parts abbreviation; Br: branch; B: bark; Fr: Fruits; L: Leaves.

Anticancer and safety to normal cells activities

The 13 methanol plant extracts which possessed high free radical scavenging activity were investigated to evaluate their Cytotoxicity effect against four human cancer cell lines, namely lung cancer cell line (A549), Colon cancer (HCT-116), hepatocellular carcinoma (HepG2) and breast cancer cell line (MCF-7) (table 3). The plant extracts were also tested at 100 µg/ml. The data in table 3 showed four plant extracts with high cytotoxic

activity (≥ 75 %) against at least one cancer cell line. These included: bark extract of *Adenanthera pavonina* with Cytotoxicity against HepG2 and MCF-7 (75 % and 91 % respectively), *Albizzia stipulate* showed cytotoxicity against HepG2 and MCF-7 (75 % and 86 % respectively), *Derris elliptica* showed cytotoxicity against HCT-116, HepG2 and MCF-7 (93 %, 76 % & 90 % respectively) and *Hyphaenethebaica* showed cytotoxicity against A549 and MCF-7 (87 % and 89 % respectively). For the most active plant extracts a dose response study was made to calculate their IC_{50} values (Table 4). These plant extracts have been further tested for cytotoxicity on human normal cell line (BJ-1) to calculate their SI values (table 5). The concentration range was 50, 25, 12 and 5 µg/ml for all the tested cell lines.

Table 3: Cytotoxicity of methanol plant extracts (100µg/ml) on four human tumor cell lines.

Cytotoxicity (%)				Part	Plant name	Family	No
MCF-7	HepG2	HCT-116	A549				
56 %	14 %	16 %	69%	Br	*Spondias lutea*	Anacardiaceae	1
65 %	25 %	10 %	52 %	Fr	*Spondias lutea*	Anacardiaceae	2
16 %	12 %	2 %	13 %	Br	*Spondiasmangifera*	Anacardiaceae	3
10 %	13 %	31 %	48 %	Br	*Aberia sp*	Flacourtiaceae	4
91 %	75 %	51 %	45 %	Bark	*Adenantherapavonina*	Leguminosae	5
60 %	52 %	0 %	40 %	Br	*Bauhinia junnejesii*	Leguminosae	6
13 %	14 %	0%	44 %	Br	*Adenanthera pavonina*	Leguminosae	7
86 %	75 %	30 %	13 %	Br	*Albizzia stipulate*	Leguminosae	8
90 %	76 %	93 %	41 %	L	*Derris elliptica*	Leguminosae	9
15 %	20 %	37 %	25 %	L&br	*Caesalpiniacaffra*	Leguminosae	10
35 %	16 %	23 %	44 %	L&br	*Cassia javanica*	Leguminosae	11
10 %	4 %	4 %	15 %	B	*Gossypiumbarbadein se*	Malvaceae	12
89%	70 %	28 %	87 %	B	*Hyphaenethebaica*	Palmae	

Parts abbreviation; Br: branch; B: bark; Fr: Fruits; L: Leaves.

Table 4: IC_{50} values for the active anticancer plant extracts.

IC_{50}µg/ml				Part	Plant name	No
MCF-7	HepG2	HCT-116	A549			
37±1.3	28±0.9	-	-	B	*Adenanthera pavonina*	1
29±1.2	24±1.1	-	-	Br	*Albizzia stipulate*	2
34±0.8	-	37±1.5	-	L	*Derris elliptica*	3
38±1.2	-	-	32±0.9	B	*Hyphaenethebaica*	4
26.1±1.3	21.6±1.2	37.6±1.5	28.3±1.7		Doxorubicin	Positive control

Parts abbreviation; Br: branch; B: bark; Fr: Fruits; L: Leaves.(-) not active on the cancer cell line.

Table 5: The selectivity index (SI) values of the seven active plant extracts.

SI				Part	Plant name	NO.
MCF-7	**HepG2**	**HCT-116**	**A549**			
1.2	1.6	-	-	B	*Adenanthera pavonina*	1
6.8	8.2	-	-	Br	*Albizzia stipulate*	2
3.4	-	3.1	-	L	*Derris elliptica*	3
3.8	-	-	4.6	B	*Hyphaenethebaica*	4

Parts abbreviation; Br: branch; B: bark; Fr: Fruits; L: Leaves.(-) not active on the cancer cell line.

Discussion

In the etiology of cancer, free radicals are one of themajor factors necessary to cause DNA mutation, which inturn triggers the initiation stage of carcinogenesis[4]. Exogenous antioxidants from naturalsources can improve the function of the endogenousantioxidant system which is responsible for preventing the formation of free radicals in the body [13]. Unlike cytotoxic agents that damage tumor cells,antioxidants act by preventing the onset of cancer duringcarcinogenesis, and they are generally beneficial to cells[14]. In recent years, interest has grown in the use of natural antioxidants for the prevention or treatment of cancer and different diseases[15]. In our research for anticancer drugs from plant extracts which possess remarkable antioxidant activity, four plant extracts showed significant cytotoxic effect against at least one of the tested cancer cell lines. These included:bark extract of*Adenanthera pavonina* possessed cytotoxicity on liver cancer cell line (HepG2) and breast cancer cell line (MCF-7) (table 3) and antioxidant activity (table 1). Reports indicated that the bark extract of *Adenanthera pavonina*had antioxidant activity [16] and antitumor activity on Dalton's ascitic lymphoma in male Swiss albino mice[17]. Bark extract of *Hyphaenethebaica* possessed antioxidant activity (table 1) and cytotoxic activity against lung cancer (A549) and breast cancer cell line (MCF-7) (table 3). Others reported that the fruit extract of *Hyphaenethebaica* had antioxidant and anticancer activities against acute myeloid leukemia [18]. Leaves extract of *Derris elliptica* possessed antioxidant activity (table 1) and cytotoxic effect on the colon cancer cells and breast cancer cell line (table 3). However, other researchers revealed antioxidant activity [19]and antimicrobial activity [20] of *Derris elliptica* extract. The branch extract of *Albizzia stipulate* showed high antioxidant activity (table 1) and high cytotoxic activity against hepatocellular carcinoma (HepG2) and breast cancer cell line (MCF-7) (table 3). It is worth to note that it is the first time to report the in vitro cytotoxic activity of *Albizzia stipulate* branch extract against the four cancer cell lines underinvestigation. The results of the cytotoxicity on human normal cells (BJ-1)of these four plant extracts indicated avery high selectivity of the extracts for cancer cells, as theyshowed no cytotoxicity on the normal cells throughout therange of concentrations tested (Table 5). In the view of the authors, a plant extract which combines antioxidant and anticancer activities and at the same time safe to healthy cells is a promising cancer chemopreventive candidate. The logic behind this, is that the antioxidant will reduce, if not prevent, the DNA mutations and adducts caused by cytosolic free radicals and consequently prevent the initiation of cancer through induction of mutations. The anticancer activity will be useful in early eliminating any newly formed neoplastic cells that are not clinically detectable. However, these cancer cytotoxic agents should be with minor or no side effects as they are planned to be used for prolonged time preventing cancer formation. That's why experiments in this study were designed to select for plant extracts that are safe to normal cells. Thus, the four pre-mentioned identified plant extracts in this study could be promising cancer chemopreventive agents. Being from a natural source, there is a high probability to be tolerated for prolonged administration. Further studies are recommended to evaluate the anticancer and antioxidant efficacy of the active plant extracts and also their general toxicity profile in animal models.

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