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Screening of some Plants in Egypt for their Cytotoxicity against four Human Cancer cell lines

Seham MA Moustafa[1]*, Bassem M. Menshawi[2], Gamila M. WASSEL[2], Khaled Mahmoud[2], Marwa, M. Mounier[2]

[1]Department of Botany, Faculty of Science, Ain Shams University, Cairo, Egypt.
[2]Department of Pharmacognosy, National Research Centre, Dokki, Cairo 12622, Egypt.

***Corres.author: sehammoustafa@yahoo.com**

Abstract: Two hundred wild and cultivated plants growing in Egypt, belonging to seventy four families, have been randomly collected from different localities. The methanol extracts of these plants have been preliminary screened for their cytotoxicity against four human cancer cell lines namely breast (MCF-7), colon (HCT-116), hepatocellular (HepG 2) and lung (A-549) using the in vitro (MTT) test. Remarkable cytotoxic activities were obtained with fifteen of the tested extracts (at 100ppm) against the four tumor cell lines mentioned above. Four of these selected plants possessed high cytotoxicity against the majority of the tested tumor cell lines. The selectivity index (SI) was also estimated for these four plant extracts using human normal skin cell line (BJ-1). The four plants that exhibited maximum cytotoxic activities were: *Dovyalis caffra*, *Gingko biloba*, *Ipomoea carnea* and *Lonchocarpus speciosus.* On the bases of SI values, the branch extract of *Dovyalis caffra* showed relatively high selectivity to the lung tumor cell line. The methanol extracts of leaves of *Gingko biloba and Ipomoea carnea* and the bark extract of *Lonchocarpus speciosus* exhibited markedly high SI values with colon cancer. However, further work is running on to identify the compound(s) responsible for antitumor activity in each extract.
Keywords: Cytotoxicity, MCF-7, HCT116, HepG2, A549, BJ-1, Cancer, LC_{50}, SI.

Introduction

Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012[1]. The first written records on the medicinal uses of plants appeared in about 2600 BC from the Sumerians and Akkaidians and the Ebers Papyrus, the best known Egyptian pharmaceutical record which documented over 700 drugs, represented the history of Egyptian medicine dated from 1500 BC[2,3] mentioned that the National Cancer Institute has screened about 35,000 higher plant species for activity against cancer, where about 3,000 of these plants had demonstrated reproducible activity. As mentioned above, the exploration of nature as a source of new active agents is needed for discovering bioactive chemo- types from natural product for the development and novel molecular diversity of efficacious drugs. In this respect, natural products from plants, used either alone or with combinatorial synthetic methodologies, constitute a multidisciplinary approach to the current drug productivity[4,5]. This has opened up new fields of investigation of potential antitumor compounds, some of which are already widely used in cancer chemotherapy which act through different pathways in activation of apoptosis in cancer cells leading to cell cytotoxicity[6].

According to the above mentioned, screening of new natural compounds against cancer is required for many reasons: a. with time, some plant species with anticancer activity may suffer extinction before they are ever studied, b. New products are required to counteract many difficulties associated with cancer treatment; the

most common of which include drug resistance, toxicity, excessive hair loss, nausea and loss of appetite and the low specificity of currently available cytotoxic drugs[7], c. with the development of new technologies, a revival of collections of plants and high throughput screening for their anticancer potential is urgent where molecules isolated from plants are proving to be an important source of novel inhibitors of the action of key proteins that have regulatory effects on tumor cell cycle progression[8], and d. the majority of plant species have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compound(s).

Thus, the present work intended to screen extracts of 200 plants growing in Egypt for testing their potential to inhibit the viability of cells in four human cancer cell lines namely breast, colon, hepatic and lung carcinomas for their possible further lead in drug design. In vitro MTT assay methods have been developed to measure the efficiency of the plants under investigation against cancer cells as described in the materials and methods.

Materials and Methods

Plant Materials

Two hundred cultivated and wild plant species were collected from different localities in Egypt. The plant material was identified according to[9] as illustrated in Table 1. A voucher specimen representing each collection was kept in the herbarium of the National Research Centre (NRC), Cairo, Egypt.

Preparation of Plant Extracts

Plant extracts were prepared according to the procedure described by[10] with slight modification. The plant parts under investigation (75g) were dried in a solar oven at 40°C followed by grinding and percolation in 450 ml methanol and then fully extracted by percolation at ambient temperature. The extracts were filtered using Whatman No.1 paper and then dried by high vacuum and stored at -70°C in glass vials, ready for use.

Cell lines

Human lung carcinoma (A-549 cell line) and skin normal human cell line (BJ-1) "A telomerase immortalized normal foreskin fibroblast cell line "were obtained from Karolinska Center, Department of Oncology and Pathology, Karolinska Institute and Hospital, Stockholm, Sweden. Human breast carcinoma (MCF-7 cell line), colon carcinoma (HCT-116 cell line), and hepatocellular carcinoma (HepG2 cell line) were obtained from Vacsera (Giza, Egypt).

Cell culture

The procedure was done in a sterile area using a laminar air flow cabinet biosafety class II level.

Culture was maintained in DMEM medium (in case of A-549, BJ-1), RPMI 1640 medium (in case of MCF-7, HCT-116, HepG2) and with 1% antibiotic-antimycotic mixture (10,000U/ml potassium penicillin, 10,000µg/ml streptomycin sulfate and 25µg/ml amphotericin B), 1% L-glutamine, and supplemented with 10% heat inactivated fetal bovine serum. Culturing and subculturing were carried out according to Thabrew M. et al [11]. Doxorubicin was used as a positive control. A negative control composed of DMSO was also used.

Cell viability assay

This was done according to El-Menshawi B.et al[12] as described by Mosmann T. et al. Following culturing for 10 days, the cells were seeded at concentration of $20x10^3$cells per well in case of A-549 and HCT-116, $10x10^3$ cells/well in a fresh complete growth medium in case of MCF-7 and HepG2 cell lines using 96-well microtiter plastic plates at 37 ºC for 24 hours under 5% CO_2, in a water jacketed carbon dioxide incubator. Fresh medium (without serum) was added and cells were incubated either alone (negative control) or with plant extracts to give a final concentration of 100µg/ml. After 24 hours incubation, the medium was aspirated and then 40µl MTT salt (2.5mg/ml) were added to each well and incubated for further four hours at 37ºC under 5% CO_2. To stop the reaction and dissolve the formed crystals, 200µl 10% sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37ºC. The absorbance was measured using a microplate multi-well reader at 595nm and a reference wavelength of 690nm. Cell viability was assessed

according to the mitochondrial- dependent reduction of yellow MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide) to purple formazan.

Determination of LC50 values

LC_{50} values were calculated for the promising active extracts possessing 75≥ % cytotoxicity 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 cell line)[14].

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

Results

The result of preliminary screening at 100 ppm showed that 15, 13,12,8 plant extracts out of 200 extracts gave remarkable cytotoxic (≥75%) effect on MCF-7, HCT-116, HepG2 and A-549 respectively (Table 1), whereas the cytotoxicity of the other plant extract ranged from moderate (75- 40%) to weak (<40%) effects. Table 2 shows the LC_{50} values of the extracts on the four cancer cell lines under investigation. The most promising plant extracts that possessed the least LC_{50} values corresponded to *Ipomoea carnea, Ginkgo biloba, Dovyalis caffra* and *Lonchocarpus speciosus* (LC_{50} ranged from 11 to 37.3, 9.7 to 25.7, 12.6 to 43.3 and 15.5 to 31.9 on breast, colon, liver and lung respectively). These four plant extracts have been further subjected to human normal cell line (BJ-1) to calculate their SI values (Table 3). *Dovyalis caffra* branches exhibits relatively high cytotoxic selectivity on lung cancer (SI=3) more than other tumor cell lines. The extract of *Ginkgo biloba* leaves, *Ipomoea carnea* leaves and *Lonchocarpus speciosus* bark showed relatively high cytotoxic selectivity (SI= 3.9, 3 and 3.9 respectively) on colon cancer cells, as compared to the remaining cell lines.

Table1.: List of selected plants collected from different localities in Egypt, their scientific names, families and their parts used for screening the cytotoxic activities of methanolic extracts, in vitro, against four human tumor cell lines: breast carcinoma (MCF-7), colon carcinoma (HCT-116), hepatocellular carcinoma (HepG2) and lung carcinoma (A-549). In each case, 10mg plant extract were dissolved in 500 µl DMSO to make 20000 ppm (µg/ml). Preliminary concentration for screening was 100ppm

No.	Species	Part used	Family	Cytotoxicity (%)			
				MCF-7	HCT-116	HepG 2	A-549
1*	*Abutilon hybridum* Hort.	Br	Malvaceae	68.9	25.1	19.6	6.2
2**	*Acacia nilotica* (L.) Delile	Br, L, Fr	Leguminosae	44.7	23.8	1.8	28
3**	*Acacia saligna* H.L.Wendl.	H	Leguminosae	10.3	29.2	0	0
4*	*Acokanthera oblongifolia* (Hochst.) Codd	L	Apocynaceae	14	26.9	13.6	0
5*	*Acrocarpus fraxinifolius* Arn.	L	Leguminosae	0	0	0	5.5
6**	*Adonis dentata* Delile	H	Ranunculaceae	30.1	46.9	38.5	26.9
7**	*Aegilops ventricosa* Tausch	H	Gramineae	34.3	6	0	4.8
8*	*Agave filifera* Salm-Dyck	L	Agavaceae	68.7	49.8	42.7	36.8
9*	*Agave* L.	L	Agavaceae	49.5	50.1	44.6	44.8
10*	*Agave macroacantha* Zucc.	L	Agavaceae	75.6	56.1	75.5	38.3
11**	*Agropyron junceum* (L.) P. Beauv.	H	Gramineae	24.2	13.3	0	0
12*	*Ailanthus altissima* (Mill)Swingle	B	Simaroubaceae	99	91.8	99.8	4.5
13*	*Aloe grandidentata* Salm-Dyck	L	Liliaceae	31.4	6	0	1.5
14*	*Aloe mitriformis* Mill.	L	Liliaceae	0	19.1	4.9	0
15*	*Alpinia nutans* K.Schum.	Fl	Zingiberaceae	30.8	35	9.5	1.9
16**	*Anarrhinum pubescens* Fresen.	H	Scrophulariaceae	7.1	14.8	10.1	1.9
17*	*Anisacanthus virgularis* Nees	L, Br	Acanthaceae	0	1.9	0	0

18*	*Antirrhinum majus* L.	Shoot system	Scrophulariaceae	13.4	7.9	2.3	0
19**	*Apium graveolens* L.	W	Umbelliferae	19.8	14.9	0	1.2
20**	*Artemisia monosperma* Del.	H	Asteraceae	48.6	52.5	46.4	17.7
21**	*Asparagus stipularis* Forssk.	R	Liliaceae	26.7	19.6	43.4	30.4
22**	*Asphodelus ramosus* Gouan ex Willk. & Lange	Root system	Liliaceae	10.4	30.6	5.3	0
23**	*Atractylis carduus* C.Chr.	H	Asteraceae	0	0	0	0
24**	*Atriplex lindleyi* Moq. subsp. inflata (F. Muell.) P. G. Wilson	H	Chenopodiaceae	30	13.7	24.1	20.6
25*	*Balanites aegyptiaca* (L.) Delile	L	Balanitaceae	37.8	10.3	11.9	3.1
26*	*Barleria cristata* Lam.	L	Acanthaceae	16.7	16.7	3.5	0
27**	*Bassia scoparia* (L.)A.J.scott.	H	Chenopodiaceae	41.2	22.2	11.7	30.9
28**	*Beta vulgaris* L.	H	Chenopodiaceae	24.5	13.7	6.9	0.5
29*	*Brachychiton australis* (Schott & Endl.) Terrac.	L	Sterculiaceae	18.8	29.5	9.6	0
30**	*Brachypodium distachyon* (L.) P. Beauv.	H	Gramineae	3.5	16	12.8	7.2
31*	*Brahea armata* S.Watson	L, Br	Arecaceae	8.3	2.1	0	0
32**	*Brassica nigra* (L.) W.D.J.Koch	H, Fl, Fr	Cruciferae	41	35.8	51.4	14.8
33*	*Butea frondosa Roxb. ex Willd.*	L	Leguminosae	16.9	14.6	27	3.4
34*	*Caesalpinia ferrea* Mart.	Fr, S	Leguminosae	14.6	12.7	8.4	8
35*	*Caesalpinia pulcherrima* (L.) Sw.	L ,Fl	Leguminosae	49.5	24.1	5.1	4.6
36**	*Capparis sinaica* Veill.	H	Capparaceae	6.9	13.2	11.4	0
37*	*Carica papaya* L.	Br	Caricaceae	29.4	15.8	10.4	12.7
38*	*Carissa carandas* L.	L, Br	Apocynaceae	16	21.1	17.3	0
39**	*Carrichtera annua* L.(DC.)	L, B, Fl	Cruciferae	10.5	6.8	21.8	0
40*	*Carya illinoinensis* (Wangenh.) K.Koch	L	Juglandaceae	13.8	22.9	3	0
41*	*Casimiroa edulis* La Llave	L	Rutaceae	15.9	33.3	15.5	37.1
42*	*Cassia candolleana* Vogel.	L, Br, Fl, Fr	Leguminosae	32.5	14	3.3	2.2
43*	*Cassia fistula* L.	Fl	Leguminosae	4	13.2	1.3	0
44*	*Cassia grandis* L.f.	L	Leguminosae	0	15.2	0	9.6
45*	*Cassia nodosa* Buch,-Ham-ex Roxb.	B	Leguminosae	23.8	43.7	4	0.8
46*	*Casuarina equisetifolia* L.	L, Br	Casuarinaceae	2.5	0	9.4	14.9
47*	*Catalpa bignonioides* Walter	L, Fl	Bignoniaceae	19.6	22.4	15	0
48*	*Cedrela odorata* L.	B	Meliaceae	0	3.3	0	0
49*	*Cedrela toona* Roxb. ex Rottler & Willd.	S	Meliaceae	98.4	94.3	99.5	53.2
50**	*Cenchrus biflorus* Roxb.	H	Gramineae	21	2.3	0	0
51**	*Centaurea calcitrapa* L.	H	Asteraceae	18.4	18.4	11.6	0
52*	*Chorisia insignis* Kunth	Br	Bombacaceae	12	9.1	37.5	0
53*	*Chrysalidocarpus lutescens* H.Wendl.	L	Arecaceae	18.5	27.2	20	0
54*	*Cinnamomum zeylanicum* Breyne	Br	Lauraceae	0	11.4	0	0
55*	*Citharexylum quadrangulare* Moc. & Sessé ex D.Don	L	Verbenaceae	0	16.5	6.4	0
56*	*Citrus sinensis* (L.) Osbeck	L	Rutaceae	0	25.9	0.5	0
57**	*Cleome chrysantha* Decne.	H	Cleomaceae	8.5	46.2	15.5	0
58*	*Clerodendrum trichotomum* Thunb.	L, Br	Labiatae	16.3	23.9	18.8	0
59**	*Corchorus olitorius* L.	L	Tiliaceae	4.6	25.2	0	0
60*	*Cordia myxa* L.	L	Boraginaceae	3.6	18	3.6	0
61**	*Coronopus niloticus* Spreng.	W	Cruciferae	10.7	4.1	0	2.4

62*	*Cryptostegia grandiflora* R.Br.	L	Apocynaceae	52.8	21.8	34.9	0
63*	*Cycas revoluta* Thunb.	Br	Cycadaceae	10.7	7.6	0	10.6
64**	*Cynanchum acutum* L.	H	Asclepiadaceae	0	22.2	5.6	0
65**	*Cynara cornigera* Lindl.	H	Asteraceae	0	27.4	3.6	0
66*	Dalbergia sissoo *Roxb.*	L, Fr	Leguminosae	6	45.3	4.7	0
67*	*Dendrocalamus giganteus* Munro	Shoot system	Gramineae	36	6.5	6.7	25.3
68*	*Deutzia scabra* Siebold & Zucc.	L	Hydrangeaceae	0	25.1	1	0
69**	*Dichanthium annulatum* (Forssk.) Stapf	H	Gramineae	9.1	6.7	0	0
70*	*Diospyros kaki* Thunb.	L	Ebenaceae	51.5	48.6	34.6	7.1
71*	*Dovyalis caffra* (Hook.f. & Harv.) Warb.	Br	Flacourtiaceae	99.6	90.3	98.8	98.4
72**	*Echinops galalensis* Schweinf.	H	Asteraceae	5.8	18.9	0	0
73*	*Encephalartos villosus* Lem.	L	Zamiaceae	0	6.1	0	0
75*	*Eriobotrya japonica* (Thunb.) Lindl.	L	Rosaceae	44.1	25.3	31.3	0
76**	*Eryngium creticum* Lam.	H	Umbelliferae	4.8	24.5	3.2	0
77*	*Eucalyptus citriodora* Hook.	Br	Myrtaceae	54.9	53.9	51.9	35.1
78*	*Eugenia jambos* L.	Br	Myrtaceae	17.4	15.4	8.8	0
79*	*Euonymus japonicus* Thunb.	L	Celastraceae	20.3	23.2	0	0
80*	*Ficus afzelii* G.Don	L	Moraceae	8.6	0	0	17.1
81*	*Ficus obliqua* G.Forst.	L	Moraceae	25.7	14.2	9.2	0
82*	*Ficus pyriformis* Hook. & Arn.	L	Moraceae	20.2	30.2	13.9	10.7
83*	*Ficus trijuja* L.	B	Moraceae	5.5	0	18.4	0
84*	*Flacourtia cataphracta* Roxb. ex Willd.	L	Flacourtiaceae	30	13.7	6.3	14.3
85*	*Flacourtia rukam* Zoll. & Moritzi	L	Flacourtiaceae	51.3	35.3	38.5	15.2
86**	*Foeniculum vulgare* Mill.	H	Umbelliferae	45.1	20.8	37.6	3.3
87*	*Gingko biloba* L	L	Ginkgoaceae	99.5	99.4	98.3	97.2
88*	*Gleditsia caspica* Desf.	Br	Leguminosae	29.8	5.4	0.3	0
89*	*Gmelina arborea* Roxb.	L	Verbenaceae	24	42.1	17	0
90**	*Gnaphalium luteoalbum* L.	W	Asteraceae	16.8	16.1	10.5	7.5
91*	*Grewia occidentalis* L.	L	Tiliaceae	0	13.5	5.7	17
92**	*Gypsophila capillaris* C.Chr.	H	Caryophyllaceae	32.5	10.3	24	10.8
93**	*Halocnemum strobilaceum* M.Bieb.	H	Chenopodiaceae	17.7	44	25.4	0
94*	*Harpullia cupanioides* Roxb.	Br	Sapindaceae	59.8	23.6	55	27.7
95*	*Harpullia pendula* Planch. ex F.Muell.	L	Sapindaceae	17.4	3	0	20.1
96**	*Helianthemum vesicarium* Boiss.	H, Fl	Cistaceae	0	6	8.3	0

97**	*Herniaria hemistemon* J.Gay	H	Caryophyllaceae	20.7	30.2	0	0
98**	*Hyoscyamus boveanus* (Dunal) Asch. & Schweinf.	H	Solanaceae	27.9	28.9	2.6	0.4
99*	*Hyphaene thebaica* Mart.	Fr	Arecaceae	12.8	23.1	0	8.4
100**	*Inula crithmoides* L.	H	Asteraceae	3.2	29.2	7.4	0
101**	*Ipomoea carnea* Jacq.	L	Convolvulaceae	99.8	99.9	99.6	100.3
102*	*Jacaranda acutifolia* Humb. & Bonpl.	L, Fl, very Small Br.	Bignoniaceae	18.8	9.9	4.7	0
103*	*Jasminum primulinum* Hemsl. ex Baker	L	Oleaceae	0	4	0	0
104*	*Khaya grandifoliola* C.DC.	Fl	Meliaceae	19.1	8.8	0	0
105*	*Khaya senegalensis* A.Juss.	L	Meliaceae	0	1	0	0
106**	*Kickxia aegyptiaca* Nábělek	H	Scrophulariaceae	32.2	23.7	7.9	5
107*	*Koelreuteria elegans* (Seem.) A.C.Sm.	L	Sapindaceae	10	25.2	4.9	0
108*	*Koelreuteria paniculata* Laxm.	L	Sapindaceae	8.2	13.8	0.2	0
109*	*Lagerstroemia indica* L.	L	Lythraceae	5.4	20.1	0	0
110*	*Lagerstroemia speciosa* (L.) Pers.	L	Lythraceae	27.3	17.9	37.5	14
111**	*Lathyrus annuus* L.	H	Leguminosae	29.4	9.6	29.8	3.8
112**	*Leontodon hispidulus Boiss.*	H	Asteraceae	7.1	16.1	0	0
113**	*Limonium meyeri* (Boiss.) kuntze	Shoot System	Plumbaginaceae	22.6	16.3	0.2	11.3
114**	*Limonium pruinosum* Kuntze	H	Plumbaginaceae	34.5	45.1	31.4	5.3
115*	*Livistona decipiens* Becc.	B	Arecaceae	6.4	12.5	0	17.2
116**	*Lobularia libyca* Meisn.	H	Cruciferae	45.3	17.4	24.2	21.9
117*	*Lonchocarpus speciosus* Bolus	B	Leguminosae	94.7	94.7	89.2	88.6
118*	*Lonicera japonica* Thunb.	L, B	Caprifoliaceae	1.9	6.4	0	0
119*	*Macadamia integrifolia* Maiden & Betche	L	Proteaceae	2.7	29.3	2.6	4.3
120*	*Magnolia grandiflora* L.	B	Magnoliaceae	22.4	10.7	0	1
121**	*Malva neglecta* Wallr.	H	Malvaceae	27.4	16.3	22.8	0
122**	*Malvaviscus arboreus* Cav.	Br	Malvaceae	27.3	5.6	13.2	0
123*	*Mangifera indica* L.	L	Anacardiaceae	24	41.9	6.2	0
124**	*Marrubium vulgare* L	L, B, Fl	Labiatae	19.4	12.8	0	3.5
125**	*Matricaria aurea* (Loefl.) Sch. Bip.	H, Fl	Asteraceae	35.4	29.9	17.2	18.1
126**	*Matthiola arabica* Boiss.	H	Brassicaceae	4.1	9.5	0	0
127**	*Medicago intertexta* (L.) Mill.	H, Fl	Leguminosae	26.9	9.1	3.4	2.6
128**	*Medicago polymorpha L.*	W	Leguminosae	23.7	12	7.5	3
129*	*Melia indica* Brand.	L, B	Meliaceae	7.8	17.3	26	20.6
130**	*Moricandia nitens* E.Durand & Barratte	H	Cruciferae	6.6	13	10.1	0
131*	*Moringa peregrina* C.Chr.	L, B, Fr	Moringaceae	24.2	32.9	15.5	17.2
132*	*Morus rubra* L.	Br	Moraceae	89.7	82.9	41.5	68
133*	*Nephelium tomentosa* F.Muell.	L	Sapindaceae	2.6	29	0	4.8
134*	*Nerium oleander* L.	L	Apocynaceae	40.8	54.1	62.5	0
135**	*Neurada procumbens* L.	H	Neuradaceae	26.9	36.9	10.3	0
136**	*Nicotiana glauca* Graham	L, Fl	Solanaceae	25.5	34.2	24.3	1.6
137*	*Opuntia brasiliensis* (Willd.) Haw.	L	Cactaceae	7.5	5.2	9.8	0
138**	*Oryzopsis miliacea* (L.) Batt. & Trab.	H	Gramineae	33.4	45.4	19.7	0
139*	*Oscularia* Schwantes.	L, Br	Aizoaceae	33.6	32.4	7.7	5.6
140**	*Papaver rhoeas* L.	H	Papaveraceae	29	15.9	3.2	3
141*	*Passiflora edulis* Sims	L, Br, Fl	Passifloraceae	22.1	9.8	0	8.8
142*	*Paulownia tomentosa* (Thunb.) Steud.	L	Scrophulariaceae	0	0	0	0
143**	*Phlomis aurea* Decne.	H	Labitae	0	16.4	0	0
144*	*Phoenix dactylifera L.*	L	Arecaceae	18	32.3	4.9	9.7
145**	*Picris sprengeriana* Chaix ex Lapeyr.	H	Asteraceae	6.1	5	0	0
146*	*Pinus canariensis* C.Sm. ex DC.	B	Pinaceae	37.5	24.6	18.1	0
147*	*Pinus pinnae* L.	L	Pinaceae	22.5	35.8	11.4	3.5
148*	*Pistachia* Salisb.	B	Anacardiaceae	82.2	76	73.6	82.6
149*	*Platanus orientalis L.*	L	Platanaceae	81.7	92.1	87.8	83.9
150*	*Platycladus orientalis* (L.) Franco	L, B	Cupressaceae	23.5	21.6	12.4	0
151*	*Plumbago capensis* Thunb.	L	Plumbaginaceae	23	16.7	14.8	0
152*	*Plumeria acutifolia* Poir.	L	Apocynaceae	1.7	9	3.2	0
153*	*Podocarpus gracilior* Pilg.	L	Podocarpaceae	9.8	31.2	0	0
154**	*Polygonum salicifolium* Schur	H	Polygonaceae	48.6	61.9	35	0

155**	*Polypogon viridis* (Gouan) Breistr.	W	Gramineae	15	17.8	14.6	6
156**	*Prosopis juliflora* (Sw.) DC.	H	Leguminosae	95.4	99.1	98.7	89
157*	*Punica granatum* L.	Br	Punicaceae	0	2.5	0	8.2
158*	*Pyracantha fortuneana* (Maxim.) H.L.Li	L	Rosaceae	25.4	23	8.9	0
159*	*Ravenala madagascariensis* J.F.Gmel.	L , B	Strelitziaceae	7.7	16.1	0	10.8
160**	*Reseda muricata C.Presl*	L, Br	Resedaceae	34.9	29.9	12.4	9.8
161*	*Rhus* L.	L	Anacardiaceae	85.6	67.1	64.8	37.7
162*	*Robinia pseudoacacia* L.	L	Leguminosae	9.2	21.5	6.9	0
163*	*Ruprechtia salicifolia* L.	L	Polygonaceae	19.6	0	12.4	0
164**	*Salix mucronata* Thunb.	H	Salicaceae	47	8.5	3.8	6.7
165**	*Salix tetrasperma* Roxb.	L	Salicaceae	0	17.6	0	0
166*	*Saraca cauliflora* Baker	L	Leguminosae	6.3	18.8	5.5	0
167*	*Saraca indica* L.	L	Leguminosae	26.5	24.8	0.2	3.8
168*	*Schinopsis balansae* Engl.	B	Anacardiaceae	0	8.2	0	0
169**	*Schinus dependens* Ortega	Br	Anacardiaceae	7.5	0	0	12.3
170**	*Schinus Terebinthifolius* Raddi	L	Anacardiaceae	95.2	97.1	87	0
171**	*Scorpiurus muricatus* L.	H	Leguminosae	33.9	12.6	48	0
172**	*Scrophularia hypericifolia* Wydler	H	Scrophulariaceae	26.4	27.3	12.1	0
173*	*Senecio cineraria* DC.	L	Asteraceae	0.8	22.3	7.6	0
174*	*Senna surattensis* (Burm.f.) H.S.Irwin & Barneby	Br	Leguminosae	9.7	3.4	0	3.5
175*	*Sophora japonica* L.	L	Leguminosae	8.5	22.6	0	0
176**	*Sorghum halepense (L.) Pers.*	H	Graminae	44.4	36.9	34.6	25.5
177*	*Spathodea nilotica* Seem.	L, Br	Bignoniaceae	12.3	5.6	0	0.7
178**	*Sporobolus pungens* (Schreb.) Kunth B22	H	Gramineae	0	15.2	0	7.2
179*	*Sterculia foetida* L.	Br	Sterculiaceae	8.6	25.3	0	8.9
180*	*Sterculia lurida* F.Muell. ex Benth.	L, Br	Sterculiaceae	60.1	43.9	32.3	43.3
181**	*Suaeda vera* Forssk. ex J.F.Gmel.	H	Chenopodiaceae	26.6	26.6	18.6	7.1
182*	*Swietenia macrophylla* King	L	Meliaceae	0.3	16.9	0	0
183*	*Swietenia mahagoni* (L.) Jacq.	L	Meliaceae	0	7.4	0	0
184*	*Tabernaemontana coronaria* Willd.	L, Immune Fl	Apocynaceae	27.3	13.8	11.7	8.2
185*	*Tamarindus indica* L.	L, Br, Fr	Leguminosae	12	25.7	0.6	5
186*	*Tecoma radicans* (L.) DC.	L, Br	Bignoniaceae	15.2	2.4	0.8	0
187*	*Terminalia angustifolia* Sauvalle	L	Combretaceae	0	25.4	0	0
188*	*Terminalia arjuna* (Roxb. ex DC.) Wight & Arn.	B	Combretaceae	34.7	26	37.8	0
189**	*Teucrium leucocladum* Boiss.	H	Labiatae	82.5	81.1	26.3	53.2
190**	*Thymus capitatus* Hoffmanns. & Link	H	Labiatae	13.8	23.2	3.4	0
191**	*Trichodesma africanum* (L.) Lehm.	H	Boraginaceae	32.3	7.7	7.8	3.7
192**	*Triticum aestivum L.*	H	Graminae	17.2	6.5	0	3.2
193**	*Verbascum Fruticulosum post*	H	Scrophulariaceae	37.7	16.8	34.7	50.6
194**	*Verbesina encelioides Benth. & Hook.f. ex A.Gray*	H	Asteraceae	9.1	4.4	13.7	0
195*	*Vitex agnus castus L.*	Br	Verbenaceae	14.7	20.8	0	0
196*	*Washingtonia filifera (Linden ex André) H.Wendl.*	S	Arecaceae	9	13.1	0	0
197**	*Xanthium pungens Wallr.*	L , Br	Asteraceae	38.8	17.6	39.7	8.2
198**	*Zilla spinosa Prantl*	Shrub	Brassicaceae	25.8	24.1	0	0
199**	*Ziziphus spina-christi (L.) Willd.*	W	Rhamnaceae	13.9	25.3	18.1	0
200**	*Zygophyllum coccineum L.*	H	Zygophyllaceae	19.2	14.9	17.3	0

Br= branch, L= leaf, Fr= fruit, H= whole herb, B= bud.
* = cultivated, ** = wild

Table 2: LC_{50} (the concentration required to kill 50% of the cell population) of active plant extract exhibiting more than 75% cytotoxicity on cell lines of, breast carcinoma (MCF-7), colon carcinoma (HCT-116), hepatocellular carcinoma (HepG2) and lung carcinoma (A-549). The four human tumor cell lines under investigation. The serial numbers given herein are similar to those given for the plant species in Table 1.

No.	Species	Plant Part used	LC_{50}			
			MCF-7	HCT-116	HepG2	A-549
11	*Agave macroacantha* Zucc.	L	51.1(±3.5)		49.2(±3.4)	
13	*Ailanthus altissima* (Mill)Swingle	B	61.8(±3.6)	46(±3.3)	53.2(±3.1)	
49	*Cedrela toona* Roxb. ex Rottler & Willd.	S	62.8(±3.7)	61.5(±3.5)	68.8(±3.8)	
71	*Dovyalis caffra* (Hook.f. & Harv.) Warb.	Br	37.3(±2.8)	71.8(±6.7)	43.3(±3.9)	20.3(±1.2)
74	*Enterolobium timbouva Mart.*	B	57.1(±3.4)	39.6(±2.9)	52.7(±4.1)	49.5(±3.1)
87	*Gingko biloba* L	L	14.5(±2.8)	11.3(±3.7)	22.7(±2.8)	27.3(±1.5)
101	*Ipomoea carnea* Jacq.	L	11(±1.9)	9.7(±2.6)	12.6(±2.5)	15.5(±2.2)
117	*Lonchocarpus speciosus* Boulos	B	33.3(±4.1)	13.5(±2.9)	32(±3.3)	31.9(±4.2)
132	*Morus rubra* L.	Br	63.1(±4.3)	36.7(±4.1)		
148	*Pistachia* Salisb.	B	45.5(±1.9)	44.4(±4.9)	49.6(±3.8)	59.4(±5.3)
149	*Platanus orientalis L.*	L	71(±6.7)	44.1(±4.6)	73.1(±5.3)	60.7(±4.7)
156	*Prosopis juliflora* (Sw.) DC.	H	50.4(±3.4)	36.8(±2.1)	48.8(±4.4)	48.6(±3.3)
161	*Rhus* L.	L	47.2(±3.7)			
170	*Schinus Terebinthifolius* Raddi	L	47.3(±2.5)	25.7(±3.9)	66.5(±4.2)	
189	*Teucrium leucocladum* Boiss.	H	60(±4.6)	67.1(±5.1)		
Positive control	Doxorubicin		26.1(±1.3)	37.6(±1.5)	21.6(±1.2)	28.3(±1.7)

Table 3: The values of selectivity index (SI) that demonstrate the differential activity of the methanol extracts of the five plants exhibiting most cytotoxicity, among the 200 taxa under investigation, on cell lines of breast carcinoma (MCF-7), colon carcinoma (HCT-116), hepatocellular carcinoma (HepG2) and lung carcinoma (A-549). Normal human cell line (skin BJ-1) was used to estimate. The greater SI value corresponds to higher selective action. SI values lower than 1 indicate general (non specific) toxicity of the crude plant extract. The serial numbers given herein are similar to those given for the plant species in Table 1.

No.	Name	Plant part used	SI			
			MCF-7	HHCT-116	HepG2	A-549
71	*Dovyalis caffra* (Hook.f. & Harv.) Warb.	Br	1.6	0.9	1.4	3
87	*Gingko biloba* L	L	3	3.9	1.9	1.6
101	*Ipomoea carnea* Jacq.	L	2.6	3	2.3	1.9
117	*Lonchocarpus speciosus* Boulos	B	1.6	3.9	1.6	1.6

(-) = No activity

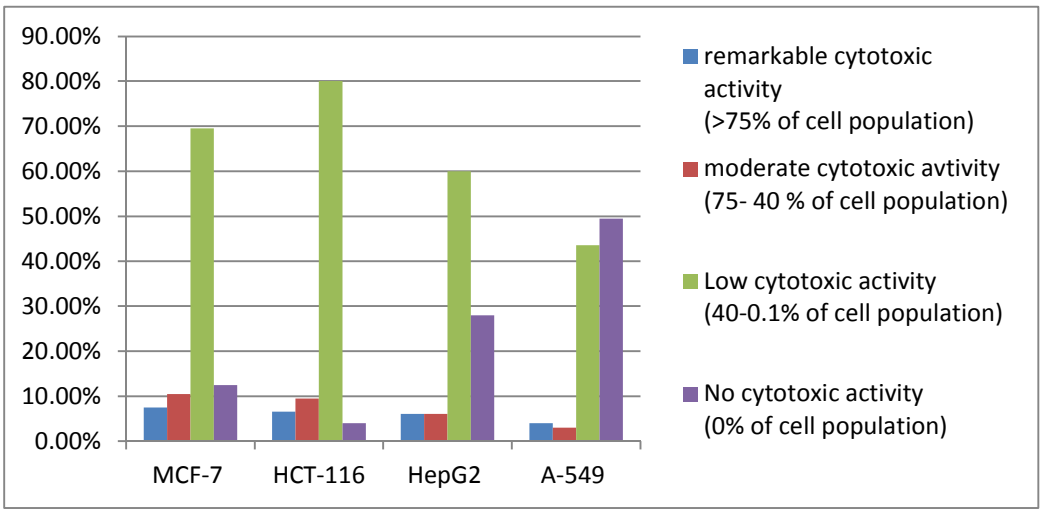

Figure1. Range of cytotoxicity effects of the methanol extracts of the 200 taxa under investigation on cell lines of breast carcinoma (MCF-7), colon carcinoma (HCT-116), hepatocellular carcinoma (HepG2) and lung carcinoma (A-549). The results are expressed as cytotoxic activity of cell population.

Discussion

The anticancer potentiality was tested for two hundred plants representing wild and cultivated taxa in Egypt, belonging to seventy four families. We followed a random approach[15] for plant collection; i.e. without phytochemical, ethno- directed, chemotaxonomic, or specific organ targeting. According to Khafagi I.K. et al[16], such a collection of the plants may be useful for the identification of the presence of bioactive compounds from plants with unknown folk medicinal use. Despite Egypt's long history of traditional medicine, few numbers of the plants used by Egyptian herbalists have had their medicinal properties investigated, using contemporary scientific methods. In particular, very little attention has been paid to the potential anticancer activity of Egyptian plants[17]. It is known that different carcinoma cell lines may exhibit different sensitivities towards cytotoxic agents, therefore the use of more than one cell line is necessary for experimentation[18]. In the present work, we selected the human tumor cell lines according to the criteria stated by Molnár L. et al[19] to be characterized as follows: (i) well-described human cell lines, (ii) proliferation is quickly, i.e., the duplication time of the cells should not exceed 30 h, and (iii) viable cell lines devoid of any phenotypical changes after several passages.

First, we carried out a preliminary screening for the in vitro anti-proliferative effect of the methanol extracts of the plant species under investigation against the studied breast (MCF-7), colon (HCT-116), liver (HepG2) and lung (A-549) human carcinoma cell lines. This led to the segregation of 15 extracts (at 100ppm) of the plant species that showed the most promising cytotoxicity effects (≥75% cytotoxicity) with the differently studied cell lines. These extracts were subjected to further bioassaying at lower concentrations to calculate their LC50 values. The most four potent extracts (possessed the least LC50) against each cell line were selected to explore their cytotoxicity on BJ-1 normal human cell line and evaluate their SI values. In this respect, maximum activities were shown by the extracts of *Dovyalis caffra branches*, leaves of *Ginko biloba* and *Ipomoea carnea* and bark of *Lonchocarpus speciosus* on the different cancer cell lines under study. *Dovyalis caffra* is used as fodder (bulk feed for livestock), the fruits are edible, the roots and thorns are used in African traditional medicine to treat amenorrhea and chest pain[20]. In the present work, *Dovyalis caffra* branch extract possessed in vitro cytotoxicity on the four tested tumor cell lines (Table 2), with a wide selectivity index on the lung tumor cell line (SI=3) (Table 3). The cytotoxic effect of the extract of this species on the liver tumor cell line HepG2 has been also investigated earlier by El-Menshawi B.S. et al[21]. The leaf extract of *Ginkgo biloba* possessed in *vitro* antiproliferative activity on the four tumor cell lines under study (Table 2) with relatively wide SI values on breast and colon tumor cell lines (3.0 and 3.9, respectively) and lower SI values against liver and lung carcinoma cell lines (1.9 and 1.6, respectively) (Table 3). However, other research workers revealed antiproliferative and apoptotic inducing activities of *Gingko biloba* on hepatocellular carcinoma cells[21-24] oral cavity cancer cells[25], ovarian adenocarcinoma cells[26], signaling pathway of antioxidants (Lui et al., 2007), and antiapoptotic mechanism[27,28] attributed the cytotoxicity of *Ginkgo biloba* to the occurrence of flavonoids as major constituents. The results of the present work also indicated cytotoxicity of the leaf methanol extract of *Ipomoea carnea* (Table 2) with a maximum selectivity regarding colon carcinoma (SI= 3.0), followed by breast and hepatic cell lines (SI values= 2.6, 2.3, respectively) and then lung carcinoma cell line (SI= 1.9). In this

connection, Anand G. et al[29] revealed a significant cytotoxic activity of the of the hydro- alcoholic extract of *Ipomoea carnea* leaf against Ehrlich Ascites carcinoma cell lines. Gupta R.K. et al[30] also reported that *Ipomoea carnea* extracts possessed hepatoprotective activity against the hepatotoxicity induced by the combination of three antitubercular drugs. Hepatoprotective activity of *Ipomoea carnea* has been also reported by Sharma A. et al[31]. We also found, in the present work, that the bark extract of *Lonchocarpus speciosus* showed promising cytotoxicity against the four carcinoma cell lines under study (Table 2). The highest SI value (3.0) was obtained with the colon cancer cell line, as compared with comparable SI values (1.6) with regard to the breast, hepatocellular and lung carcinoma cell lines (Table 3). As far as we are aware, this is the first time to refer to the in vitro cytotoxic activity of *Lonchocarpus speciosus* bark extract against the four cancer cell lines under investigation and to detect their concomitant selectivity indices.

Thus, it might be concluded that out of the two hundred plant methanol extracts screened in this work, those of *Dovyalis caffra branches, Ginko biloba* and *Ipomoea carnea* leaves, and *Lonchocarpus speciosus* stem bark were extremely promising to be further studied for the development of new effective anticancer drugs in future on the bases of their highly cytotoxicity against the four studied carcinoma cell lines (breast, colon, hepatocellular and lung). Highest SI index with the four carcinoma cell lines under study were obtained by the extracts of *Dovyalis caffra* in lung cancer, *Ginko biloba* and *Lonchocarpus speciosus* in colon cancer and *Ipomoea carnea* in hepatocellular carcinoma. However, further studies are needed to determine the active constituents responsible for cytotoxicity of these plants.

Note: Our Co-Author Prof. Dr. Gamila M. Wassel†**2** was deceased in *25 Nov, 2012.*
Our Co- Author Bassem El-menshawi †**2** was deceased in Nov, 2013.

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