International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.2, pp 267-272, 2015

In vitro anticancer activity of some Egyptian plant extracts against different human cancer cell lines

Salwa M. El-Hallouty[1]*, Walid Fayad [1], Nefissa H. Meky [2], Bassem S. EL-Menshawi[1], Gamila M. Wassel[1], Ahmed A. Hasabo[1].

[1] Drug Bioassay-Cell Culture Laboratory, Pharmacognosy Department, National Research Center, Dokki, Giza, 12622, Egypt,
[2]Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt.

Abstract: Plants have shown to be valuable source of a variety of drugs for human ailments including cancer. Egypt is rich in plant species most of which have not been investigated for their biological activities. In the continuing effort to screen Egyptian plants for anticancer activity, 20 plants belonging to 5 families were collected from different areas in Egypt. These plants yielded 25 extracts and were tested for their anticancer activity using four human cancer cell lines, namely HepG2 (hepatocellular carcinoma), MCF-7 (breast adeno carcinoma), A549 (lung carcinoma) and HCT-116 (colorectal carcinoma). The selectivity index (SI) was evaluated for the promising extracts using human normal immortalized skin cell line (BJ-1). Out of the 25 methanol extracts tested, 7 demonstrated potential cytotoxic activities on the cancer cells. Leaves extract of *Harpophyllum caffrum* was the most promising as it had an exceptionally high activity on all cancer cell lines with IC50 ranged from (21-29µg/ml), with high selectivity index SI= 4.5 against breast cancer cell line (MCF-7) and showed relatively high selectivity index SI=3.3, 3, 3.3against HepG2, A549 and HCT-116 respectively. Further studies are also in process to evaluate the anticancer efficacy of *Harpophyllum caffrum* extract in animal models.
Keywords: *In vitro*; anti-cancer; Egyptian plants; cell lines.

Introduction

Cancer is a major cause of mortality and morbidity globally. According to recent estimates by the World Health Organization[1, 2], annual cancer incidence in sub-Sahara in Africa is 551 200 with a mortality of 421 000 per year (2008)[3, 4]. About 70% of all cancer deaths occurred in lowand middleincome countries[3, 4].

The available treatment methods include surgery, chemotherapy, and radiation[5]. The current available methods of treatment mostly induce significant side effects and their efficacy is still below expectation. Therefore, the need for alternative therapies has arisen [6]. Natural products are extremely an important source of medicinal agents. Although there are some new approachesin drug discovery, such as combinatorial chemistry and computer based molecular modeling design, none of them can replace the importance of natural products in drug discovery and development[7, 8]. Many synthetic drugs cause severe side effects that are intolerable and the metabolites discovered in medicinal plants may avoid the side effect of synthetic drugs[9].Medicinal plants have played an important role in the discovery of anticancer drugs[10,11].Medicinal plants -used traditionally-are being investigated *in vitro* for their anticancer activity. The potential of natural products as anticancer agents was recognized in the 1950s by workers at the U.S. National Cancer Institute, who established a large scale screening program using leukemic mice[12] and later (1985) set up an *in vitro* screening panel consisting of more

than 60 different human tumor cell lines[13].Indeed, most of the new clinical applications of plant secondary metabolites and their derivatives, over the last half century, have been in the treatment of cancer [14].

There are four main classes of plant-derived anticancer agents in current clinical use; the Catharanthus alkaloids, the epipodophyllotoxins, the taxanes and the camptothecines. The Catharanthus alkaloids and several of their semi-synthetic derivatives induce metaphase arrest and thereby inhibit mitosis by binding specifically to tubulin and causing its de- polymerization[15]. Vinblastine and vincristine were isolated from C. roseus (L.) G. Don (Apocynaceae), formerly *Vincarosea* L and has been used clinically for over 40 years[16]. The epipodo phyllotoxins bind to tubulin, causing DNA strand breaks during the G2 phase of the cell cycle by irreversibly inhibiting DNA topoisomerase II[17]. Podophyllotoxin was isolated from the resin of Podophyllum peltatum L. (Berberidaceae)[18] but was found to be too toxic in mice, so derivatives were made; the first podophyllotoxin-derived drug approved for clinical use was etoposide[19].

Ancient herbal medicines may have some advantages over single purified chemicals [20, 21]. Often the different components in an herb have synergistic activities or buffer toxic effects[21]. This study, therefor, aimed to determine the in vitro anticancer potential of some Egyptian plants, as alternative medicine in the treatment of cancer.

Materials and methods

Plant material: 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], bark [B], herb [H], and weed [W]) 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 then dried in a Rotavapour under a vacuum at 40 °C. The extract was then freeze dried (lyophilized) .The extracts were placed in glass vials and stored at -20 ºC.

Cell culture:

Culture was maintained in DMEM medium (in case of A549), RPMI medium (in case of HCT-116, HepG2 and MCF-7), DMEM F12 medium(in case of BJ-1) and supplemented with 10% foetal bovine serum 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 kindly provided by Professor Stig Linder, Oncology andPathology department, Karolinska Institute, Stockholm, Sweden. Other cell lines were obtained 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 [22].

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 IC_{50} values

In case of highly active extracts possessing ≥ 75 % cytotoxicity on different cancer cell lines and human normal cell line, different concentrations were prepared for dose response studies. 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) [23].

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

Results

The anticancer effect of methanolic extracts of 25 plant parts against the human cancer cells HepG2, MCF-7 A549 and HCT-116 were investigated. Cultures of different cell lines were treated with extracts first at one concentration of 100µg/ml and the results showed that 7 plant extracts possessed high activity (75-100%) against at least one cancer cell line (table 1). The anticancer activity profile of the active extracts is summarized in table 2. These plant extracts have been further tested for cytotoxicity on human normal cell line (BJ-1) to calculate their SI values (table 3).

Table 1: Cytotoxicity of methanolic plant extracts (100µg/ml) on four human tumor cell lines: hepatocellular carcinoma (HepG2), breast carcinoma (MCF-7), lung carcinoma (A549) and colon carcinoma (HCT-116).

Cytotoxicity (%)				Part	Plant name	Family	No
HCT-116	A549	MCF-7	HepG2				
100	100	100	100	Br	*Anacardiumoccidentale*	Anacardiaceae	1
0	75	86	75	Bark	*Pleiogynumsolandri*	Anacardiaceae	2
22	43	97	97	L,Br	*Pistachia terebinthus*	Anacardiaceae	3
14	41	70	30	Bark	*Shinopsisbalansea*	Anacardiaceae	4
16	3	1	0	Fr	*Pleiogynumsolandri*	Anacardiaceae	5
10	30	40	61	L	*Spondiaslutea*	Anacardiaceae	6
3	40	44	46	Bark	*Spondiaslutea*	Anacardiaceae	7
8	53	54	55	Br	*Harpophyllum caffrum*	Anacardiaceae	8
79	75	75	81	L	*Harpophyllumcaffrum*	Anacardiaceae	9
12	47	9	17	L	*Aberiasp*	Flacourtiaceae	10
20	89	30	30	L	*Albizziastipulat*	Leguminosae	11
25	76	46	0	Br	*Derris robusta*	Leguminosae	12
15	70	83	35	Bark	*Bauhinia variegate*	Leguminosae	13
57	22	66	54	L&Fl	*Adenantherapavonina*	Leguminosae	14
22	69	30	60	Br	*Caesalpiniaferrae*	Leguminosae	15
20	24	12	7	L& Br	*Adenantherapavonina*	Leguminosae	16
0	18	0	11	L& Br	*Acacia seyal*	Leguminosae	17
3	46	19	28	Fl	*Delonixregia*	Leguminosae	18
13	41	65	40	L, Fl	*Derris robusta*	Leguminosae	19
8	12	0	0	Herb	*Abutilonefruticosum*	Malvaceae	20
8	26	0	0	Weed	*Malvaparviflora*	Malvaceae	21
0	26	19	9	L	*Feroniaelephantum*	Rutaceae	22
18	40	21	41	L	*Glycosmispentaphylla*	Rutaceae	23
38	39	13	38	Fl	*Rutagraveolens*	Rutaceae	24
0	10	0	0	Br	*Murraea exotica*	Rutaceae	25

Parts: Ap: Br: branch; B: bark; FL: Flowers; Fr: Fruits; L: Leaves; H= herb: weed

Table 2: *In vitro* cytotoxic activity (IC $_{50}$ µg/ml) of crude extracts tested against different cancer cell lines after24 hours.

IC_{50}				Part	Plant name	No
HCT-116	A549	MCF-7	HepG2			
46.6±5	44.6±3.7	41.4±2.6	35±4.1	Br	*Anacardiumoccidentale*	1
-	28.8±3.5	37.3±3	31±6.2	B	*Pleiogynumsolandri*	2
-	43 ±13.8	-	-	L	*Albizzia stipulate*	3
-	31±12.1	-	-	Br	*Derris robusta*	4
29±1.9	28.8±1.3	21±1.2	29±2.2	L	*Harpophyllum caffrum*	5
-	-	23±0.52	37±4.5	Br	*Pistachia terebinthus*	6
-	-	31±4.6	-	B	*Bauhinia variegate*	7
37.6±1.5	28.3±1.7	26.1±1.3	21.6±1.2		Doxorubicin	Positivecontrol

Results are represented by means of three replicates. Br: Branch. B: Bark. L: leaves. (-) not tested

Table 3: The selectivity index (SI) values of the seven active plant extracts.

SI				Part	Plant name	NO.
HCt-116	A549	MCF-7	HepG2			
0.7	0.7	0.9	0.8	Br	*Anacardiumoccidentale*	1
-	0.9	0.6	0.7	Bark	*Pleiogynumsolandri*	2
3.3	3	4.5	3.3	L	*Harpophyllum caffrum*	3
-	-	2.2	1.3	L&Br	*Pistachia terebinthus*	4
-	2.3	-	-	L	*Albizzia stipulate*	5
-	2	-	-	Br	*Derris robusta*	6
-	-	1.6	-	Bark	*Bauhinia variegate*	7

(-) not tested

Discussion

Medicinal plants constitute a common alternative for cancer prevention and treatment in many countries around the world[24, 25]. Approximately, 60% of the anticancer drugs currently used have been isolated from natural products from the plants. At this time, more than 3000 plants worldwide have been reported to possess anticancer properties[25].

In continuation of our search for substances of plant origin with pharmacological effects, we have screened 25 plant extracts collected from the different regions of Egypt, for there cytotoxic activity against four cancer cell lines, namely; liver cancer cell line (HepG2), breast cancer (MCf-7), lung cancer (A549) and colon cancer (HCT-116). Out of 25 screened plant extracts, seven plant extracts (*Anacardiumoccidentale*, *Pleiogynumsolandri*, *Harpophyllum caffrum*, *Pistachia terebinthus, Albizzia stipulate*, *Derris robusta*, *Bauhinia variegate*) showed potent cytotoxic activity (≥ 75 % cytotoxicity) on the different studied cell lines. These extracts were subjected to further bioassaying at lower concentration to calculate their IC_{50} values and explore their cytotoxicity on BJ-1 normal human cell line to evaluate their SI values. The United States National Cancer Institute plant screening program, a crude extract is generally considered to have promising*in vitro* cytotoxic activity if the IC_{50} is <30–40 µg/mL[26]. Based on this criteria, only the extract from *Harpophyllum caffrum* is considered highly active (Table 2), with wide selectivity index on breast cancer cell (SI=4.5) and showed relatively high selectivity index against liver, lung and colon cancer cells (SI= 3, 3.3 & 3.3 respectively) (Table 3). The selectivity index of this plant extract is firstly to be reported. The leaves of ethanol extract of *H. caffrum* is reported to exhibit variable anti-inflammatory, analgesic, and antipyretic activities, besides the hepato-protective, *in vitro* cytotoxic and anti-microbial activities[27]. Extracts from various morphological parts of *H. Caffrum* have been reported to contain numerous polyphenolic compounds, protocatechuic acid, kaempferol and other flavonoids[28]. *Albizzia stipulate* and *Derris robusta* extracts possessed *in vitro* cytotoxicity on lung cancer cell line (A549) (Table 2), but no activity was noticed against other types of cancer cell lines (HepG2, MCF-7and HCT-116), with lower selectivity index against lung cancer cell line (SI= 2.3 and 2 respectively) (Table 3). To our knowledge, this is the first time to report the *in virto* cytotoxic activity of *A. stipulate* and *D. robusta* extracts against the four cancer cell lines under investigation

and to detect their selectivity indices. *Bauhinia variegate* also showed significant activity against MCF-7 (Table 2), with lower SI against breast cancer cell line (SI= 1.6) (Table 3). In this connection, B. Rajkapoor et al [29] revealed a significant cytotoxic activity of the ethanol extract of *Bauhinia variegate* against human epithelial larynx cancer (Hep 2) and human breast cancer (HBL-100) cells. *Pistachio terebinthus* extract possessed *in vitro* cytotoxicity on liver and breast cancer cell lines (Table 2), with maximum selectivity regarding breast carcinoma (SI= 2.2) and then liver carcinoma (SI= 1.3). From the literature, it was found that the leaves extract of *Pistachio terebinthus* has high antioxidant activity[30]. The extracts of *Anacardium occidentale* and *Pleiogynum solandri* showed the selectivity index less than 1 against different cancer cell lines (Table 3) which means that these extracts have high cytotoxicity effect on both cancer cells and normal cells (non-selective). Al-Rashidi *et al* [31] noted that low toxicity towards normal cells and high toxicity towards cancer cells indicates that a plant extract has promising anti-cancer constituents.

It can be seen that 7extracts (28 %) have shown potential activity on the cancer cell lines. Out of the 7 extracts, the leaves extract of *Harpophyllum caffrum* was the most active with IC50= 29, 21, 28.8 & 29 µg/ml, against HepG2, MCF-7, A549 and HCT-116, respectively. This plant extract showed high selectivity index against different cancer cell lines under investigation. This is an important observation, given that effective anticancer drug should demonstrate tumor specificity. Further studies are also in process to evaluate the efficacy of this active plant extract as an anticancer agent in animal models.

Acknowledgements

This study was carried out at the Department of Pharmacognosy, National Research Centre, Dokki – Cairo, under project in title:

"Evaluation of some Egyptian plant extracts with preliminary antitumour activity (Comprehensive Biological and phytochemical studies)", funded by Science & Technology Development Fund (STDF), Academy of Scientific Research and Technology, Egypt, (2013-Current) grant number 1206.

References

1. World Health Organization. GLOBOCAN 2008: cancer incidence and mortality worldwide. Geneva: World Health Organization; 2010. [Online] Availablefrom: http://www.iarc.fr/en/media-centre/iarcnews/2010/globocan2008.php [Accessed on 10th September, 2014].
2. Atwood S.E., Nigerian foodstuffs with prostate cancer chemo preventive polyphenols. Infect Agent Cancer 2011; 6(Suppl2): S9.
3. Ferlay J., Shin HR., Bray F., Forman D, Mathers C., Parkin DM., Cancer incidence and mortality worldwide,IARC Press,Lyon, 2010,10.
4. Latosińska JN., Latosińska M. Anticancer drug discoveryfrom Serendipity to rational design,Drug Discovery. Poland: Intact, 2013; 35-74.
5. Wamidh HT. Anticancer and antimicrobial potential of plantderivednatural products. In: Rasooli I., editor. Photochemical - bioactivities and impact on health. Croatia: InTech; 2011.
6. Mishra S., Ahmad S., Kumar N., Sharma BK. Annonamuricata(thecancer killer), J Pharm Res.,2013; 2; 1613-1618.
7. Heinrich M., Bremner P. Ethanobotany and ethanopharmacy-their role for anticancer drug development. Curr Drug Targets,2006; 7; 239-245.
8. Thirumal M., Kishore G., Prithika R., Das S., Nithya G. In vitro anticancer activity of Tecomastans(L) ethanolic leaf extract on human breast cancer cell line (MCF-7). Int. J Pharm Chem.Biol.Sci.,2012; 2; 488-493.
9. Valko M., Leibfritz D., Moncol J., Cronin MT., Mazur M., Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol,2007; 39: 44-84.
10. Balunas, M.J., Kinghorn, A.D. Drug discovery from medicinal plants. Life Sciences, 2005; 78: 431–441.
11. ItharataA.,HoughtonbP.J.,Eno-Amooquayec E.,Burkec P.J., Sampsonb J.H.,Ramanb A., Invitro cytotoxic activity of the medicinal plant sused traditionally to treat cancer. Journal of Ethno pharmacology, 2004; 90: 33–38.
12. CraggG.M., Newman D.J.Plants as a source of anti-cancer agents. Journal of Ethno pharmacology, 2005; 100: 72–79.
13. Boyd M.R., Paull K., D. Some practical considerations and applications of the National Cancer Institute *in vitro* anticancer drug discovery screen. Drug Development Research, 1995; 34: 91–109.

14. Newman D.J., Cragg G.M., Snader K.M. The influence of natural productsupon drug discovery. Natural Product Reports, 2000; 17: 215–234.
15. Okouneva T., Hill B.T., Wilson L., Jordan M.A. The effects of vinflunine, vinorelbine, and vinblastine on centromere dynamics. Molecular Cancer Therapeutics, 2003; 2: 427–436.
16. Van D., Heijden R., Jacobs D.I., Snoeijer W., Hallard D.,Verpoorte R. The Catharanthus alkaloids: pharmacognosy and biotechnology. Current Medicinal Chemistry, 2004; 11: 607–628.
17. Damayanthi Y., Lown J.W. Podophyllotoxins: current status and recent developments. Current Medicinal Chemistry, 1998; 5: 205– 252.
18. Gordaliza M.,Garcil´a., P.A.,MigueldelCorralJ.M.,CastroM.A.,Goo mez-Zurita M.A.Podophyllotoxin: distribution, sources, applications and new cytotoxic. Derivatives.Toxicon, 2004; 44: 441–459.
19. Damayanthi Y., Lown J.W. Podophyllotoxins: current status and recent developments. Current Medicinal Chemistry, 1998; 5: 205–252.
20. Vickers A. Botanical medicines for the treatment of cancer: rationale, overview of current data, and methodological considerations for phase I and II trials. Cancer Invest,2002; 20: 1069-1079.
21. Nassr-Allah AA, Aboul-Enein AM, Aboul-Enein KM, Lightfoot DA, Cocchetto A, El-Shemy HA. Anticancer and antioxidant activity of some Egyptian medicinal plants.J Med Plants Res.,2009; 3(10): 799-808.
22. Mosmann T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of immunological methods, 1983; 65(1): 55-63.
23. Prayong P., Barusrux S., Weerapreeyakul N., Cytotoxic activity screening of some indigenous Thai plants. Fitoterapia, 2008; 79(7): 598-601.
24. Mehta R.G., Murillo G., Naithani R. and Peng X. Cancer chemoprevention by natural products: how far have we come? *Pharm. Res*., 2010; 27:950-61.
25. Soobrattee M.A., Bahorun T. &Aruoma O.I. Chemopreventive actions of polyphenolic compounds in cancer. Biofactors.2006; 27: 19-35.
26. Oskoueian E., Abdullah N., Saad W.Z., Omar A.R., Kuan W.B., Zolkifli N.A., Hendra R., Ho Y.W. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas*Linn. *J. Med. Plants Res.*2011; *5*: 49–57.
27. EISherbeiny A E, EI Ansari MA. The phenolic and flavonoids of Harpephyllumcaffrum.Planta Med. 1976; 29: 129-132.
28. Van wyk B.E.,B.vanoudtshoorn and N.Gericke. Medicinal plants of South Africa. 2nd Edn.,Briza publications, Pretoria (south Africa), 2002; 146 – 147.
29. B.Rajkapoor,B. Jayakar, N. murugesh, D. sakthisekaran. Chemoprevention cytotoxic of Bauhinia variegate against N-nitrosodiethylamin induced liver tumors and human cancer cell lines. Journal of Ethno pharmacology, 2006; 104: 407 – 409.
30. D.D.Kavak, E. Altiok, O. Bayraktar, and S. ¨ Ulk¨ u,. "Pistaciaterebinthus extract: as a potential antioxidant, antimicrobial and possible β-glucuronidase inhibitor," Journal of Molecular Catalysis B.2010; 64:167–171.
31. Al-Rashidi W., Mat Supri N.N., Manshoor N. Cytotoxic activity of crude extract from *Costus malortieanus* (Costaceae). *Am.-Eurasian J. Toxicol. Sci.* (2011**)**, 3: 63–66.

*****