American Journal of Ethnomedicine, 2014, Vol. 1, No. 1, 056-063
Available online at http://www.ajethno.com

Biological Activity of the Egyptian Medicinal Plants: Part 4 Cytotoxicity of 50 Egyptian Plants and Spices Against Hepatocellular Carcinoma

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ABSTRACT

In Egypt, liver diseases represent a national endemic crises, annually increasing to affect younger people. About 25% of population are sharing at least one of the liver problems; cirrhosis, hepatitis C virus HCV and/or hepatocellular carcinoma HCC with 100,000 death rate annually. Screening of 50 Egyptian spices, vegetables and wild plants for cytotoxicity against HepG2, successfully uncovered the presence of 13 potent cytotoxic plant extracts with IC_{50} ranging from 2.5 - 20 µg/ml and 20 extracts with moderate activities with IC_{50} 20-50 µg/ml. The rest of extracts showed week cytotoxic activities (IC_{50} > 50µg/ml, compared with Doxorubicin (IC_{50} 21.4 µg/ml).

Keywords- Liver diseases, Egyptian victims; Hepatocellular carcinoma HCC, Egyptian plants, Spices, 13 Potent anti-hepG2.

INTRODUCTION

At present, enthusiasm remains high among scientists involved in the discovery of new potential anticancer agents from plants since the approval of taxol; the first novel antimicrotubule agent approved for clinical use in the treatment of ovarian, breast, lung, head and neck cancers (Goodman & Walsh 2001). In this concern, many medical reports showed that the major cause of death in most developing and 3rd world countries are cardiovascular diseases and cancer with a latency of many years before clinical diagnosis (Gescher *et al* 2001).

In Egypt, liver diseases are the most agonizing endemic health problem especially in the rural governorates. About 20-25% of populations are suffering from hepatitis C virus, liver cirrhosis and /or hepatocellular carcinoma HCC (Frank *et al* 2000, Aboul-Enein *et al* 2012, Amara *et al* 2008, Khafagi *et al* 2001, Husein *et al* 2001, El-Sadawi *et al* 1996, Mostafa *et al* 2002). Unfortunately, incidence of hepatic diseases

in Egypt is escalating and progressively affecting younger persons.

This encouraged us to screening the Egyptian wild and cultivated plants as well as traditionally consumed spices for their activity against hepatocellular carcinoma.

MATERIALS AND METHODS

Plant Material

Wild plants and weeds were collected from Zagazig farms, irrigation canals and desert vicinity of Sharkia governorate area (60 kilometers west to Sues Canal, Egypt) in March-April 2010-2011. The collected samples were kindly identified by Dr. Abd-Elhalim Abdel-Mogly, Prof. of Taxonomy, Flora Department, Agricultural Research Institute, Ministry of Agriculture, Cairo, Egypt. The fruits, spices and seeds were purchased from the local central markets and herbalists. Voucher specimens are kept in the Pharmacognosy Department, Faculty of Pharmacy, University of Zagazig, Egypt.

Preparation of Extracts

The whole plant, fresh fruits, seeds or spices (50 g each) were washed thoroughly with fresh water - to remove dust and insecticides remainders-then extracted by cold percolation using ethyl alcohol 70% (500 ml x 2). The combined extract then concentrated under reduced pressure using vacuum rotatory evaporator at a temperature not exceeding 45^0C. The resulting aqueous residues were lyophilized into powder and submitted and /or kept in freezer at -20 ^{0}C for biological assay.

Biological Study (Hansen *et al* 1989)

Cell Culture

Human hepatocarcinoma cell line (HepG2), purchased from ATCC, USA, were used to evaluate the cytotoxic effect of the tested extracts. Cells were routinely cultured in DMEM (Dulbeco's Modified Eagle's Medium) which was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100 units/ml streptomycin sulphate, and 250 ng/ml amphotericin B. Cells were maintained at sub-confluence at 37ºC in humidified air containing 5% CO_2. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested extracts were dissolved in dimethyl sulphoxide (DMSO), and then diluted thousand times in the assay. All cell culture material was obtained from Cambrex Bio Science (Copenhagen, Denmark). All chemicals were from Sigma/Aldrich, USA, except mentioned. All experiments were repeated three times, unless mentioned.

Cytotoxicity

Cytotoxicity was measured against HepG2 cells using the MTT Cell Viability Assay. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals which is largely impermeable to cell membranes, resulting in its accumulation within healthy cells. Solubilization of the cells results in the liberation of crystals, which are then solubilized. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm.

Procedure

Cells (0.5X105 cells/well), in serum-free media, were plated in a flat bottom 96-well micro plate, and treated with 20µl of different concentrations of each tested extracts for 48 h at 37ºC, in a humidified 5%

CO_2 atmosphere. After incubation, media were removed and 40 µl MTT solution/well were added and Incubated for an additional 4 h. MTT crystals were solubilized by adding 180 µl of acidified isopropanol / well and plate was shacked at room temperature, followed by photometric determination of the absorbance at 570 nm using micro plate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability.

Calculation

Percentage of relative viability was calculated using the following equation:

[Absorbance of treated cells/ Absorbance of control cells)] X 100.

Then the half maximal inhibitory concentration (IC_{50}) was calculated from the equation of the dose response curve and the standard used was Doxorubicin (IC_{50} 21.4 µg/ml).The results are presented in tables 1-3.

RESULTS AND DISCUSSION

Plants from the local farms or wild flora and traditional spices, which are daily used by the rural citizens and most of the other cities were collected and properly identified. The plant samples were cold extracted lyophilized and the resulting residues were submitted to *in-vitro* bioassay.

The obtained results expressed strong-(less than IC_{50} 20 µg/ml , table 1) -to moderate activities(IC_{50} 21 -50 µg/ml, table 2) according to the previous protocols of the American National Cancer Institute NCI (Boyd 1997). The most interesting of all tested samples are : the unorganized oleo-gum-resin asafoetida (*Ferula foetida*), the seeds of *Erysimum corinthium*, *Ammi visnaga* and *Nigella sativa* (black seed) with IC_{50} 3.2, 2.5, 9.6 and 10.5 µg/ml, respectively. These promising results (IC_{50} 3.2 and 2.5 µg/ml, respectively) require further investigation. In this concern, previous researchers confirmed that the cytotoxic or anti-carcinogenic activity of many brassicaceae plants are most probably ascribed to their contents of the allyl isothiocyanates and sulphurated compounds (Al-Gendy *et al* 2010, Nastruzzi *et al* 1996,). They also revealed that the hydrolysis products of some brassica glycosides showed a marked *in vitro* cytotoxicity against human erythroleukaemic K562 cells (Schonberg *et al* 1953). Considering the visnaga (IC_{50} 16.2 µg/ml), although, the chemical constituents are well known in the middle east many years ago (Mahran *et al* 1954) yet none of its chemical contents have been tested or claimed to be active against HCC.

Among the other strong active extracts comes the black seed (*Nigella sativa*) although, to-our-knowledge, no previous reports on testing the seed extract or the oil as anti-HCC cells, yet in our study it showed very promising result with IC_{50} 10.5 µg/ml. Previous report on the black seed indicated that, it only induces liver protection, while the results obtained by other authors indicated that both TQ and DIM were cytotoxic for several cell lines with very poor effect (IC_{50}'s 78 to 393 µg/ml) (Badary 1999, Haq *et al* 1999, Nagi *et al* 1999, Hassan *et al* 1998, Worthen *et al* 1998). Also, from spices tested in our study with remarkable strong activity are curcuma, long pepper and nutmeg with IC_{50} 11.6, 10.5, 18.4 µg/ml, respectively. This is the first report on the activity of long pepper and nutmeg against HCC (Chan *et al* 1998, Jacob *et al* 2012, Sohn *et al* 2007).

Most interestingly, the results observed from three herbal extracts with strong unexpected results against HCC, these are the Egyptian tomato herb (*Solanum*

lycopersicum), *Hibiscus trionum* and *Hibiscus rosa-sinensis* with IC_{50} 9.6, 12.43 and 13.90 µg/ml, respectively. Concerning the herb of tomato which is generally used as animal feed, previous reports on its chemistry (Ripperger & Himmeireich 1994, Kumari *et al* 1985) indicated the presence of steroidal alkaloids, flavonoids and coumarins without any mention on cytotoxicity. In addition, previous chemical studies on Hibiscus plants showed high anthocyanin contents together with several flavonoidal compounds (Ateya *et al* 2012, Abdel Latif 2010). This is the first report on testing these Egyptian agricultural waste plants as active against HCC.

Concerning the vegetables in this study, although the Brassica and Cruciferous vegetables are well known -for long time- to contain the isothiocyanate sulforaphane well known inhibitors of carcinogen-induced mammary tumorigenesis (Marion 1997), unfortunately the Egyptian vegetables showed weak to very weak activities against HCC. In Egypt, rural areas consume one or more locally grown vegetables daily, a cultural dietary habit which could have greatly helped in reducing the incidence of hepatic diseases. An exception of these results was the aqueous extract of Broccoli which exhibited strong activity with IC_{50} 16.2 µg/ml (table 1). A previously impressive report showed that, rats fed a broccoli diet showed a significant increase in colon and hepatic ubiquinone -reductase (Keck *et al* 2003).

ACKNOWLEDGEMENT

This work was kindly fully supported by the grant No. 2413 / 4 from the vice president sector for graduate studies and researches, University of Zagazig. Authors appreciate the help of Dr. Khaled Mahmoud, Cell Culture Laboratory, National Research Center, Cairo, Egypt for *In-vitro* assay.

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Table 1. Strongly active anti-HepG2 extracts (< 20 µg/ml)

Serial #	Plant name	Part used	Family	IC_{50} (µg/ml)
1	Wall flower, *Erysimum corinthium*	Seed	Brassicaceae	2.6
2	Asafetida, *Ferula foetidae*	oleo-gum-resin	Leguminosae	3.2
3	Tomato, *Solanum lycopersicum*	whole Herb	Solanaceae	9.6
4	Long pepper, *Piper longum*	Fruit	Piperaceae	10.5
5	Black Seed, *Nigella sativa*	Seed	Ranunculaceae	10.5
6	Turmeric, *Curcuma longa*	Rhizome	Zingberaceae	11.6
7	*Hibiscus trionum*	Petals	Malvaceae	12.43
8	Curry powder	Powder	Mixture	12.6
9	*Hibiscus,Hibiscus rosa-sinensis*	Petals	Malvaceae	13.90
10	Broccoli, *Brassica oleracea var. pompejana*	Whole plant	Brassicaceae	16.2
11	Visnaga, *Ammi visnaga*	Fruit	Apiacae	16.3
12	Nutmeg, *Myristica fragrans*	Seed	Myristicaceae	18.4
13	Milk thistle, *Silybum marianum*	Whole herb	Compositae	18.5

Table 2. Moderately active anti-HepG2 extracts (between 20-50µg/ml)

Serial #	Plant Name	Part used	Family	IC_{50} (µg/ml)	% Inhibition
14	Purslane, *Portulaca oleraceae*	Seed	Portulaceae	23.9	100
15	Plum, *Prunus domestica*	Fruit Peel	Rosaceae	23.2	100
16	Cassia, *Cinnamomum cassia*	Bark	Lauraceae	25.2	100
17	Neem, *Azadirachta indica*	Fruit	Meliaceae	9	100
18	Boswellia, *Boswellia serrata*	True frankincense (Resin)	Burseraceae	0	100
19	Neem, *Azadirachta indica*	leaves	Meliaceae	39.3	92
20	Cardamom, *Elettaria cardamomum*	Seed	Zingiberaceae	26	100
21	Pomegranate, *Punica granatum*	Fruit Peel	Punicaceae	28.7	100
22	Visnaga, *Ammi visnaga*	Fruit	Apiaceae	29.1	100
23	Tomato, *Solanum lycopersicum*	Root	Solanaceae	31.8	100
24	False Daisy, *Eclipta prostrata*	Leaves	Compositae	32.3	100
25	Egg Plant, *Solanum melongena*	Fruit Peel	Solanaceae	34.9	100
26	Coriander, *Coriandrum sativum*	Fruit	Apiaceae	35.3	100
27	Pomegranate, *Punica granatum*	Fruit (edible red part)	Punicaceae	36.7	100
28	Hopbush, *Dodonaea viscosa*	Herb	Sapindaceae	38.5	100
29	Cumin, *Cuminum cyminum*	Fruits	Apiaceae	42.9	98
30	Liqorice, *Glycyrrhiza glabra*	Root and rhizome	Leguminosae	45.5	100
31	Common, fig *Ficus carica*	Bark	Moraceae	47.7	97.1
32	Belladona, *Atropa belladonna*	Leaves	Solanaceae	48.9	85.2
33	Lemon grass, *Cymbopogon proximus*	Whole plant	Poaceae	50.34	80.4
34	Royal Poinciana, *Delonix regia*	Fruit	Leguminosae	50.30	85.3
35	Bauhinia, *Bauhinia variegatae var, candida*	Aerial parts	Leguminosae	50.8	78.0

Table 3. Weakly active anti-HepG2 (IC_{50} > 50µg/ml)

Serial #	scientific name	Part used	Family	IC_{50} /ml)	% Inhibition
36	Ginger, *Zingiber officinale*	Root and rhizome	Zingiberaceae	59.5	98.4
37	Sage, *Salvia officinalis*	Leaves	Labiateae	64.5	75.5
38	Eucalyptus, *Eucalyptus obliqua*	Leaves	Lauraceae	-----	36.6
39	Cuban laurel, *Ficus retusa*	Areal parts	Moraceae	68.4	85
40	Table sugar, *Beta vulgaris*	Leaves	Chenopodiaceae	68.4	100
41	Nerium, *Nerium oleander*	Areal parts	Apocynaceae	66	81.5
42	Banana, *Musa paradisiace*	Leaves	Musaceae	------	17
43	Plum, Prunus *domestica*	Dried (desiccated) fruits	Rosaceae	------	74
44	Squirting cucumber, *Ecballium elaterium*	Whole plant	Cucurbitaceae	------	57.3
45	Egyptian leek, *Allium ampeloprasum var. kurrat*	Leaves	Amaryllidaceae	-----	50
46	Sumac, *Rhus coriaria*	Fruit	Anacardiaceae	-----	66
47	Anise , *Pempinella anisum*	Fruit	Apiaceae	------	56.6
48	Henna, *Lawsonia inermis*	Leaf	Lythraceae	------	59.5
49	Radish, *Raphanus sativum*	Seed	Bassicaceae	------	22.1
50	Tomato, *Solanum lycopersicum*	Leaves	Solanaceae	64.4	68.2
* NCI protocols (Ref .8)					