Antitumor Evaluation of Alhagi Maurorum Extracts and Flavonoids

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ABSTRACT

Alhagi maurorum is a wild Egyptian plant of various traditional uses. Six flavonoids of flavonol type have been isolated from the butanol fraction of the air dried herb of Alhagi maurorum. These compounds were identified as kaempferol (1), quercetin (2), Quercetin 3-O-α-thamnoside (3), kaempferol-3-O-β-glucoside (4), Quercetin 3-O-β-glucoside (5), Isorhamnetine 3-O-β-rutinoside (6). The antitumor activity of Alhagi maurorum extracts was studied on inhibition of cell proliferation in human cancer cell lines Lung large cell carcinoma COR-L23, Amelanotic melanoma C32, Renal cell adenocarcinoma ACHN, Breast cancer cell line MCF-7 and Hormone dependent prostate carcinoma LNCaP), evaluated in comparison with one normal cell line 142BR. A dose-response relationship was observed for all tested samples and the IC_{50} values were determined. Petroleum ether extract showed interesting effect against LNCaP, ACHN, COR -L23, with IC_{50} of 16.68, 10.20, and 14.56μg/mL respectively, chloroform extract showed significant activity against LNCaP with IC_{50} 6.25μg/mL. Kaempferol showed the most significant activity against COR-L23, followed by LNCaP with IC_{50} 10 and 5%, respectively of the IC_{50} of the control drugs. Quercetin showed significant activity against COR-L23 and MCF-7.

Key words: Alhagi maurorum, flavonoids, evaluation, extracts

Introduction

Recently It is well known that cancer with its different types is the second cause of death just after heart diseases, around six million people pass away every year due to cancer diseases. Natural products are considered to be a good source of different chemical compounds with wide range of biological activities including anti-cancer activity (Cragg and Newman, 2005).

This led to investigate the potential of different plant extracts and isolated compound as anticancer therapeutics specially some of these compounds are already used in chemotherapy (Arcamone et al. 1980).

Alhagi maurorum which belongs to family Leguminosae is native to Mediterranean and central Asia, it is known as camel thorn and always grows in salty soils and dry environment. It has been used in folk medicine as laxative, purgative, diaphoretic, expectorant and diuretic (Boulos, 1966 and Idem, 1970). Oil of Alhagi is used in the treatment of rheumatism and flowers are used to treat piles, migraine and warts (brown, 1995). Topically water extract of its roots is used to relax the ureter, remove kidney stones and also used as indicator for soil salinity (Habib, 1971).

Other Alhagi species are known to provide flavonoids glycosides, catechines and proanthocyani-dines. (Eskalieva and Burasheva, 2002) (Alimova et al., 2010) (Yang et al., 1996).

Previous researches showed that Alhagi maurorum posess anti-inflammatory, anti-oxidant and hepatoprotective effects (Laghari et al., 2011 and Alqasoum et al., 2008). The study aimed to investigation and identification of the flavonoidal constituents of Alhagi maurorum as well as the antitumor activity of its extracts and isolated flavonoids.

Material and Methods

General experimental procedure:

Nuclear magnetic resonance was performed using NMR Bruker AMXn500, Varian Inova n500, unity plus 300 NMR spectrometer apparatus using DMSO-d6 as solvent and TMS as internal standard and DMSO-d6. The data are expressed in δ-values in ppm and J-values in Hz.

UV invisible spectrophotometer Beckman DU7 and Shimadzu UV 240 (PIN 204-5800) were used for recording UV spectra and measuring the absorbance in UV and visible range.TLC Readymade chromatographic plates (20 x 20 cm) coated with silica gel F254 or polyamide were used for analytical separation. All chemicals

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used in this study were purchased from Sigma Aldrich Chemical Co.Ltd (Milan, Italy) and VWR International (Milan, Italy). Cell culture materials were obtained from Sigma-Aldrich Chemical Co.Ltd (Milan, Italy).

**Plant material:**

Samples of the herb *Alhagi maurorum* L. family Fabaceae were collected from the sides of train lines at Giza, Egypt in Feb. 2008, and were kindly authenticated by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC). The collected plant herb was air-dried, powdered and kept in tightly-closed containers.

**Extraction and isolation:**

Air-dried powdered herb of *Alhagi maurorum* (500 gm.) was exhaustively extracted with 70% methanol. The methanolic extract was evaporated under vacuum; the crude extract (165gm) was dissolved in distilled water. The aqueous solution was successively extracted with petroleum ether, diethyl ether, chloroform (to remove the pigment and fatty materials), ethyl acetate, and finally with *n*-butanol, respectively. The butanol extract was examined by TLC. The butanol fraction was evaporated till dryness, and then subjected to silica gel column chromatography (3×60 cm, Davisil LC60 A, 60-200 μm). The column eluted with CHCl3/MeOH mixtures of increasing polarity. Ten ml fractions were collected and checked with TLC, using (*n*-butanol: acetic acid: water, 4: 1: 5, v/v/v) and S4 (ethyl acetate: methanol: H2O, 77: 15: 8, v/v/v). The spots were visualized under 366 nm UV light and with exposure to NH3 vapor. Fractions showing similar TLC profiles were combined to give six sub fractions (I-VI). The sub fraction II was further purified using small Sephadex LH-20 column eluted with 70% MeOH solution to yield compounds 1 and 2. While, the subfraction III gave compounds 3 and 4 then sub fraction VI gave compounds 5 and 6 after purification on Sephadex LH-20 column eluted with 70% MeOH solution. The collected fractions were examined by TLC using the previously mentioned solvent systems and detection by examining under UV light at 366 nm and with exposure to NH3 vapor.

**Cell culture and in-vitro cytotoxicity assay:**

Protein-staining sulforodamine B (SRB) assay was used for measurement of cell proliferation. The test is based on the estimation of cell number indirectly by providing a sensitive index of total cellular protein content which is linear to cell density (Loizzo et al., 2005). SRB is an anionic protein stain containing sulphonic groups that bind electrostatically to basic amino acid residues of cellular protein, under mildly acid conditions, the bound dye can be quantitatively extracted from cells by weak bases and solubilized for spectrophotometry, the cells were trypsinized, count and placed in 96-well plates at optimal plating density of each cell line was determined over a range from 5 x 104 to 15x104 to ensure exponential growth throughout the experimental period and to ensure a linear relationship between absorbance at λ= 490nm and cell number where analyzed by the SRB assay, and incubated to allow for cell attachment. After 24h the cells were treated with serial dilutions of the samples. Each sample was initially dissolved in DMSO and further diluted in medium to produce different concentrations.

One hundred ml/well of each dilution were added to the plates in six replicates to obtain the final concentrations ranging from 2.5 to100 mg/ml for extract, and from 0.0625 to 50 mg/mL, for phenolics, the final mixture used for treating the cells contained not more than 0.5% of the solvent (DMSO), the same as in the solvent-control wells. After 48h of exposure 100 mL of ice-cold 40% trichloroacetic acid (TCA) was added to each well, left for 1h at 4°C, and washed with distilled water. The TCA –fixed cells were stained for 30 min with 50 mL of 0.4% SRB in 1% acetic acid. The plates were washed with 1% HCl and air dried overnight. For reading plate, the bound dye was solubilized with 100 ml of 10mM tris base (tris [hydroxymethyl]aminomethane). The absorbance of each well was read on a Molecular Devices Spectra Max Plus Plate Reader (Molecular Devices, CELBIO, Milan,Italy) at λ=490nm.

Cells survival was measured as the percentage absorbance compared to the untreated control. Vinblastine sulfate salt and taxol were used as positive controls. Cytotoxicity of *Alhagi maurorum* extracts were expressed in terms of their IC50.

**Results and Discussion**

The *n*-butanol fraction of *Alhagi maurorum* herb was chromatographed on silica gel column (3×60 cm, Davisil LC60 A, 60-200 μm) Eluted with CHCl3/MeOH mixtures of increasing polarity yielded six known flavonoids of flavonol type (1) kampferol (2) Querectin (3) Quercetin 3-O-α-rhamnoside (4)Kaempferol 3-O-β-glucoside (5) Quercetin 3-O-β-glucoside (6) Isorhamnetine 3-O-β-rutinoside Compounds (2)and(3) were...
isolated here for the first time from *Alhagi maurorum*, their structures were identified by using UV, NMR spectral data which were identical to the previously reported data (Mabry, *et al.* 1970), (Agrawal, 1989).

**Antiproliferative activity of *Alhagi maurorum***:

The antitumor activity of *Alhagi maurorum* extracts on inhibition of cell proliferation in human cancer cell lines (Lung large cell carcinoma) COR-L23, (Amelanotic melanoma) C32, (Renal cell adenocarcinoma) ACHN, (Breast cancer cell line) MCF-7 and (Hormone dependent prostate carcinoma) LNCaP was evaluated in comparison with one normal cell line 142BR. A dose-response relationship was observed for all tested samples. The with IC<sub>50</sub> values are reported in Table (1).

The petroleum ether extract showed interesting effect against LNCaP, ACHN, COR-L23, with IC<sub>50</sub> of 15.85 μg/mL and mild inhibition against C32, ACHN with IC<sub>50</sub> of 33.16, 38.18 μg/mL, respectively. The chloroform extract shows significant activity against LNCaP with IC<sub>50</sub> of 6.25 μg/mL and mild activity against COR-L23 with IC<sub>50</sub> of 41.66μg/mL. The methanolic extract showed no activity against the human cancer cell lines. Quercetin has a very interesting activity against most tested cell lines. The most significant activity was observed against Lung large cell carcinoma (COR-L23) and Breast cancer cell line (MCF-7) followed by Hormone dependent prostate carcinoma (LNCaP) then Amelanotic melanoma (C32), while its least activity was observed against Renal cell adenocarcinoma (ACHN).

From the results illustrated in Table (1) and Fig (4), it is clear that quercetin showed highly significant activity against most of the tested cell lines with very small IC<sub>50</sub> comparing with the control drugs (Vinblastine and taxol). The highly significant results of quercetin may be attributed to the ability of quercetin to interact with some receptors, particularly the ary hydrocarbon receptor, which is involved in the development of cancers induced by certain chemicals. Quercetin aglycone has also been shown to modulate several signal transduction pathways, which are associated with the processes of inflammation and carcinogenesis.

Rodent studies have demonstrated that dietary administration of this flavonol prevents chemically induced carcinogenesis, therefore dietary quercetin is a promising agent for cancer prevention (Murakami *et al.*, 2009). Quercetin also exerts a preferential cytotoxic effect on dividing colon carcinoma HT29 and CaCO<sub>2</sub> cells (Agullo *et al.* 1994) The results of Table (1) and the histogram of Fig (5) indicate that Kaempferol has a very significant activity against certain cell line as it shows the best results against Lung large cell carcinoma (COR-L23), followed by (LNCaP) with IC<sub>50</sub> equals to 10 and 5%, respectively of the IC50 of the control drugs. It shows also a significant activity against Breast cancer cell line (MCF-7) and moderate activity against Amelanotic melanoma (C32), while the least activity was observed against Renal cell adenocarcinoma (ACHN). Recent studies explained that Kaempferol and quercetin inhibited the proliferation of the human cancer cells CaCO<sub>2</sub> and HT29 using MTT in vitro assay (Agullo *et al.* 1994).

Also Kaempferol shows a significant activity against Ovarian cancer cell line, Ovarian cancer is one of the most significant malignancies in the western world. Studies showed that ovarian cancers tend to grow resistance to cisplatin treatment.

Therefore, new approaches are needed in ovarian cancer treatment. Kaempferol is a dietary flavonoid that is widely distributed in fruits and vegetables, and epidemiology studies have revealed a protective effect of kaempferol against Ovarian cancer risk. Early studies also found that kaempferol is effective in reducing vascular endothelial growth factor (VEGF) expression in Ovarian cancer cells. Investigation of kaempferol’s effects on sensitizing ovarian cancer cell growth in response to cisplatin treatment shows that synergistic interaction with cisplatin and found that kaempferol works synergistically with cisplatin in inhibiting Ovarian cancer cell viability. Their inhibition on cell viabilities was induced through inhibiting gene transcription.
Apoptosis assay showed that the addition of 20 μM kaempferol to the cisplatin treatment induces the apoptosis of the cancer cells (Haitao Luo et al., 2010). It is of interest to mention that no reports were traced in the literature on the inhibition activity of Alhagi species on the cell lines, therefore this is the first report on the significant cell lines inhibition activities of A. maurorum extracts.

Kaempferol

(1)

Quercetin

(2)

Quercetin 3-O-α-rhamnoside (3)

Kaempferol 3-O-β-glucoside

(4)

Quercetin 3-O-β-glucoside

(5)

Isorhamnetine 3-O-β-rutinoside (6)
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