

Cytotoxic Activity of Flavonoids of *Jasonia Montana* Vahl. (Botsch). (Astraceae) Growing in Egypt.

¹Fathy M. Soliman, ¹Mohamed Y. Moussa, ¹Hossam M. Abdallah, ²Samir M. Othman.

¹Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Kasr-el-Aini St., Cairo, 11562, Egypt.

²Department of Pharmacognosy, Faculty of Pharmacy, October 6th University, October 6th City, Egypt.

Abstract: Eighteen phenolic compounds were isolated from the chloroform, ethyl acetate and n-butanol fractions of *Jasonia montana* Vahl. (Botsch) growing in Egypt; eleven flavonoid aglycons (1-11) from chloroform fraction, three flavonoid glycosides from ethyl acetate fraction (12-14), while n-butanol fraction yielded two phenolic acids (15-16) and two flavonoid glycosides (17-18). The structures of the isolated compounds were established as 3,6,7,3',4'-pentamethoxy quercetin (artemitin) (1), 3,6,7,3'-tetramethoxy quercetin (chrysosplenetin) (2), 3,6,3',4'-tetramethoxy quercetin (3), 3,6,7-trimethoxy kaempferol (4), 3,6,3'-trimethoxy quercetin (jaceidin) (5), 3,6,4'-trimethoxy quercetin (centaureidin) (6), 3,3',4'-trimethoxy quercetin (7), 3,6-dimethoxy quercetin (8), 3,3'-dimethoxy quercetin (9), 7,4'-dimethoxy quercetin (10) and quercetin (11) from the chloroform fraction, quercetin-3-O-β-D-4C1 galactouronopyranoside (12), quercetin-3-O-β-D-4C1-glucopyranoside (13) and patuletin-7-O-β-D-4C1-glucopyranoside (14) from ethyl acetate fraction and 3,5-dicaffeoyl-quinic acid (15), caffeic acid (16), quercetin-3-O-L-1C4-rhamnopyranoside (Quercitrin) (17) and quercetin-3-O-β-D-4C1 glucuronopyranoside (18) from n-butanol fraction. Compounds 8, 10-12, 15 and 17 were isolated for the first time from the genus *Jasonia*. The major isolated flavonoid; 2, 6 & 13, as well as, the aqueous extract were found to have a moderate anti-tumor activity against human cervix carcinoma (HELA) cell line.

Key words: *Jasonia montana*, *Varthemia montana*, Anti-tumor, Cervix carcinoma, Flavonoids.

INTRODUCTION

Jasonia montana [= *Chiliadenus montana* (Vahl.) Brullo, *Chrysocoma montana* (Vahl.) Symb., *Varthemia montana* (Vahl.) Boiss.] belongs to family Compositae (Asteraceae) which comprises about 25000 species in 1300 genus (Bhattacharyya and Johri, 1998). In Egypt, the genus *Jasonia* comprises three species (Täckholm, 1974); *J. montana* (Vahl.) Botsch which is common in Sinai Peninsula. It is locally known as Haneida (Täckholm, 1974). Haneida has a strong pleasant aromatic odour. It is used in folk medicine for diarrhea, stomach-ache and chest diseases (Täckholm, 1969). Haneida is used by Bedouins of St. Catherine in Sinai as a herbal tea for treatment of renal troubles. Many reports in the literature confirmed that *J. montana* is rich in methoxylated flavonoids (Ahmed *et al.*, 1989). Methoxylated flavonoids were reported to have a cytotoxic activity (Harborne and Baxter, 1999). The above mentioned medicinal activities encouraged us to subject *J. montana* herb for further studies.

MATERIALS AND METHODS

UV spectra were measured on a Shimadzu UV 240 (P/N 204-58000) spectrophotometer. NMR spectra were recorded on Varian Mercury (¹H-NMR, 300 MHz, ¹³C, 75 MHz) and Jeol JNM ECA 500 instrument (¹H-NMR, 500 MHz, ¹³C, 125 MHz). NMR spectra were recorded in DMSO, CD₃OD and CDCl₃. Chemical shifts were recorded in δ (ppm) relative to TMS as internal standard. For column chromatography, silica gel (E-Merck) 70- 230, sephadex LH-20 (Pharmacia, Uppsala, Sweden) and polyamide S (Fluka) were used. For paper chromatography, sheets of Whatmann No. 1 and No. 3. (Whatmann, Ltd., Maidstone, Kent, England) were used. Silica F₂₅₄ (Merck, Germany) were used for TLC. For vacuum liquid chromatography (VLC), silica gel-60 (E-Merck) was used.

Corresponding Author: Samir M. Othman, Department of Pharmacognosy, Faculty of Pharmacy, October 6th University, October 6th City, Egypt.

Plant Material:

The aerial parts of *J. montana*, were collected from hilly areas from El-Arbaeen valley Saint Catherine, South Sinai, Egypt, in November 2003. The taxonomical features of the plant were kindly confirmed by late Prof. Dr. M. N. El-Hadidi, Prof. of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University. Voucher specimens were kept in the Museum of the Department of Pharmacognosy, Faculty of Pharmacy, Cairo University.

Extraction and Isolation:

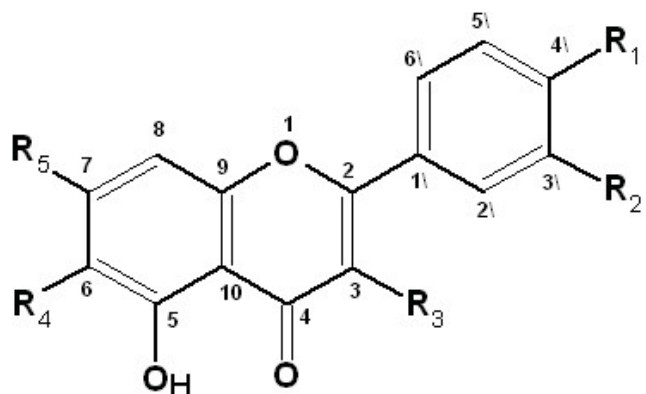
Two kg of the air dried powdered *J. montana* herb was exhaustively extracted by percolation in ethanol (70%) at room temperature. The ethanolic extract was evaporated under reduced pressure at low temperature (40 °C) to yield 200g dried extract. The residue was suspended in water (400ml), defatted with petroleum ether (40-60 °C). The suspension was partitioned successively with chloroform, ethyl acetate and n-butanol (each of 6 x 500 ml). The chloroform, ethyl acetate and n-butanol fractions were evaporated to yield 40, 10 and 13 g respectively. Twenty g of the chloroform fraction was chromatographed over a vacuum liquid chromatographic column (VLC, 15 cm length, 10 cm diameter) packed with silica gel H (100 g.) for VLC. Gradient elution was carried out with petroleum ether, chloroform, ethyl acetate, ethanol and their mixtures with an increased polarity pattern. Fractions, 200 ml each, were collected and monitored by TLC under UV light at λ_{max} 365 nm and 254 nm with $AlCl_3$ and p-anisaldehyde spray reagents (Stahl, 1969 and Markham, 1982). Similar fractions were pooled together to yield 13 collective fractions (I – XIII). Flavonoid rich fractions were fractions III – X. Fractions III, [Pet. ether / $CHCl_3$ (50:50)], IV, [Pet. ether / $CHCl_3$ (25:75)] and V, ($CHCl_3$ 100%) each contained one major spot. Each was purified on a silica gel column with chloroform / ethanol (99 : 1) as an eluent to yield compounds 1 (10 mg), 2 (30 mg) and 3 (15 mg), respectively. Fractions VI, [$CHCl_3$ / EtOAc (75:25)] and VII, [$CHCl_3$ / EtOAc (50:50)], each contained two major spots. Each was chromatographed on a silica gel column with chloroform / ethanol (95 : 5) as an eluent to yield compounds 4 (30 mg), 5 (35 mg), 6 (60 mg) and 7 (10 mg) respectively. Fraction VIII, [$CHCl_3$ / EtOAc (25:75)], contained two major spots. It was chromatographed on a silica gel column with chloroform / ethanol (9 : 1) as an eluent to yield compounds 8 (10 mg) and 9 (17 mg) respectively. Fractions IX, (EtOAc 100%) and X, [EtOAc / EtOH (75:25)], each contained one major spot. Each was purified on a silica gel column with chloroform / ethanol (9 : 1) as an eluent to yield compounds 10 (7 mg) and 11 (30 mg).

Ethyl acetate fraction (10 g) was subjected to a polyamide column (250 g, 120 x 5 cm) eluted using water - ethanol mixtures starting with 100% water up to pure ethanol. Fractions (250 ml) each were collected and monitored on PC developed with 15% acetic acid and n-butanol / acetic acid / water (BAW, 4:1:5, upper layer) as solvent systems. Identical fractions were pooled together to yield 5 collective fractions (I – V). Flavonoid rich fractions were fractions IV and V. Fraction IV, [H_2O / EtOH (25 / 75)], contained one major spot. It was purified on a sephadex LH-20 column (40 x 1cm), using ethanol / water (70:30) as eluent to yield compound 12 (10 mg). Fraction V, (EtOH 100%), contained two major spots. It was subjected to preparative PC on Whatman 3MM using BAW (4:1:5 upper layer) as solvent system followed by sephadex LH-20 column using 90% ethanol as eluent for further purification to yield compounds 13 and 14, (60 and 10 mg, respectively).

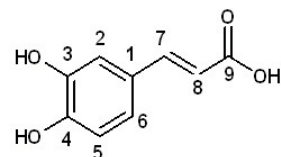
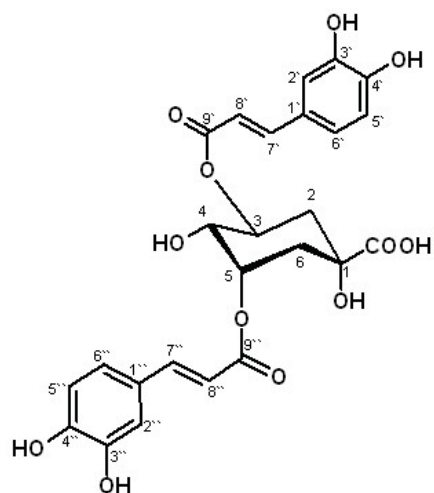
The n- butanol fraction (13 g) was found to contain four major spots ($R_f = 0.83, 0.71, 0.55, 0.41$) on PC using 15% acetic acid as solvent system. It was subjected to a polyamide column (250 g, 120 x 5 cm) using water / ethanol mixtures starting with 100% water and decreasing polarity with ethanol till 100% ethanol. Fractions were collected and monitored on PC as mentioned above. Similar fractions were pooled together to yield 5 collective fractions (I – V). Fractions rich in phenolic compounds were fractions III – V. Fractions III, [H_2O / EtOH (50/50)] and IV, [H_2O / EtOH (25/75)], each contained one major spot. Each was purified on a sephadex LH-20 column (40 x 1cm), using ethanol / water (50:50) as eluent to yield compounds 15 (7 mg) and 16 (10 mg), respectively. Fraction V, (EtOH 100%), contained two major spots. It was subjected to preparative PC (Whatman 3MM) developed with (acetic acid 15%) as solvent system followed by sephadex LH-20 column eluted by 90% ethanol for further purification to yield compounds 17 and 18, (20 and 8 mg, respectively). Chemical structures of the eighteen compounds, isolated from aerial parts of *Jasania montana* were shown in Figure 1.

Cells for Testing Cytotoxic Activity:

Anti-tumor activity was screened in vitro on five cell lines, namely: U251 (brain cancer cell line), MCF7 (breast cancer cell line), HELA (cervix cancer cell line), HCT116 (colon carcinoma cell line), and HEPG2 (liver cancer cell line) at Tumor Biology Department, National Cancer Institute, Cairo University, Cairo, Egypt. The potential cytotoxicity of the compounds (2, 5, 6 and 13), aqueous and 70% ethanolic extracts were tested (Skehan and Storeng, 1990).



Compounds	R ₁	R ₂	R ₃	R ₄	R ₅
1	OMe	OMe	OMe	OMe	OMe
2	OH	OMe	OMe	OMe	OMe
3	OMe	OMe	OMe	OMe	OH
4	OH	H	OMe	OMe	OMe
5	OH	OMe	OMe	OMe	OH
6	OMe	OH	OMe	OMe	OH
7	OMe	OMe	OMe	H	OH
8	OH	OH	OMe	OMe	OH
9	OH	OMe	OMe	H	OH
10	OMe	OH	OH	H	OMe
11	OH	OH	OH	H	OH
12	OH	OH	β- D-Galacturonic acid	H	OH
13	OH	OH	β- D-Glucopyranose	H	OH
14	OH	OH	OH	OMe	β- D-Glucopyranose
17	OH	OH	α- L-rhamnopyranose	H	OH
18	OH	OH	β- D-Glucuronic acid	H	OH



Caffeic acid, (16)

3, 5- dicaffeoyl quinic acid, (15)

Fig. 1: Chemical structures of the isolated compounds from aerial parts of Jasonia montana.

Determination of LD₅₀:

LD₅₀ of aqueous and (70%) ethanolic extracts of J. montana herb was determined according to the procedures developed by Karber (1931).

Tumor Cell Line Preparation:

The five cell line cells were planted in 96-multiwell plate (10⁴ cells/well) for 24 hours to allow attachment of cells to the wall of the plate before treatment with components under investigation.

Study of Cell Growth Inhibition:

From each sample different concentrations of the tested materials (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the tested sample for 48 hours at 37°C in an atmosphere of 5% CO₂. After 48 hours, cells were fixed, washed and stained with Sulphorhodamine-B stain (Skehan and Storeng, 1990). Excess stain was washed with acetic acid and adsorbed stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA reader.

The relation between surviving fraction and drug concentration was plotted to get the survival curve of each tested material. The results were tabulated in Table 1 & shown in Figure 2.

Table 1: Cytotoxic activity of compounds 2, 6 and 13 and aqueous extract of *J. montana* herb on HELA carcinoma cell line

Concentration in µg	Surviving Fraction			
	Compound 2	Compound 6	Compound 13	Aqueous extract
00.00	1.000	1.000	1.000	1.000
01.00	0.966	0.921	0.898	0.812
02.50	0.712	0.825	0.857	0.735
05.00	0.603	0.608	0.612	0.608
10.00	0.576	0.594	0.580	0.590

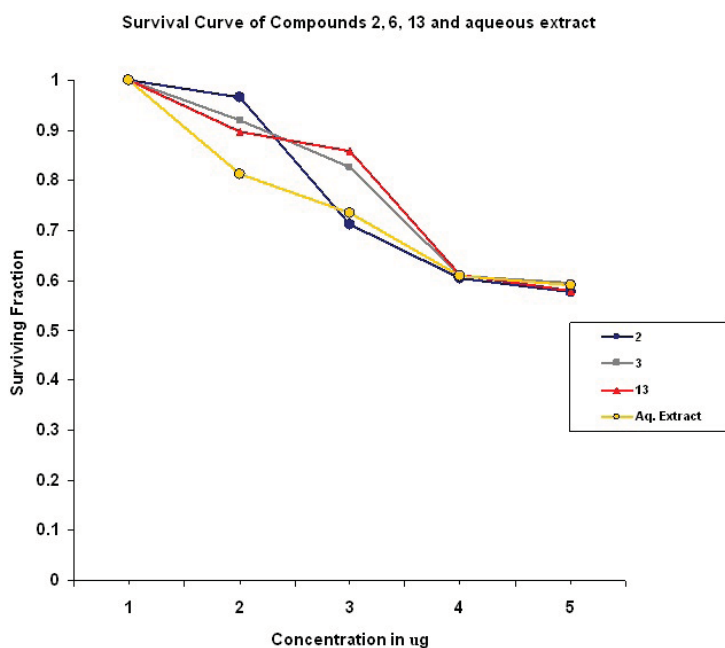


Fig. 2: Cytotoxic activity of compounds 2, 6, 13 and aqueous extract of *J. montana* herb on cervix (HELA) carcinoma cell line

RESULTS AND DISCUSSION

General:

Eleven flavonoid aglycons obtained from the chloroform fraction, all were quercetin derivatives except 4 which was a kaempferol type. Three flavonoid glycosides from ethyl acetate fraction and two phenolic acids and three flavonoid glycosides from n-butanol fraction were isolated. Their structures were confirmed by comparison of their chromatographic properties, chemical and spectroscopic data (UV, ¹H and ¹³C NMR) with those reported in the literature (Mabry *et al.*, 1970; Agrawal and Bansal, 1989; Markham 1982).

Biological Study:

The tested extracts were safe under the experimental conditions as their LD₅₀ were (8.5 g/kg, equivalent to 95.2 g of dry herb) and (8.2 g/kg, equivalent to 82 g of dry herb) respectively.

All tested compounds and extracts showed no effect against U251, MCF7, HCT116, and HEPG2 at the tested concentration.

A concentration of 2.5 mg/ml of the compounds 2, 6 and 13 and the aqueous extract showed inhibition in the surviving fraction of HELA cervix carcinoma cell line by (29, 17, 14 and 26 %, respectively).

When the concentration was increased to 5 mg/ml, the percentages of inhibition in survival fraction were 40, 39, 39 and 39%, respectively. While 10 mg/ml of the same previous components showed higher percentages of inhibition calculated as 42, 40, 42 and 41%, respectively.

Discussion:

The antiproliferative assay of flavonoids; 2, 5, 6 and 13, as well as, the aqueous and ethanolic extracts of *J. montana* herb showed that the aqueous extract, compounds 2, 6 and 13 had a promising cytotoxic effect against HELA cervix carcinoma cell line in the concentration of 10 mg/ml.

On the other hand the tested samples had no effect on the other cell lines giving indication that cytotoxic activity of these components depends on the type of the cell line, suggesting further investigations on these compounds.

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