SHORT REPORT

A C G publications

Rec. Nat. Prod. 6:1 (2012) 62-66

records of natural products

Antioxidant and Cytotoxic Effects of *Moltkia aurea* Boiss

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(Received October 26, 2010; Revised March 28, 2011; Accepted April 1, 2011)

Abstract: The water extract of *M. aurea* exhibited strong scavenging effect on 2,2-diphenyl-1-picrylhydrazil (DPPH), nitric oxide (NO) and superoxide (SO) radicals. The free radical scavenging effect of the extract was found comparable to that of reference antioxidants, 3-*t*-butyl-4-hydroxyanizole (BHA) and ascorbic acid (AA, vitamin C). Cytotoxic activity of the extract was also investigated against three different cancer cell lines, Hep-2 (human larynx epidermoid carcinoma), RD (human rhabdomyosarcoma), L-20B (transgenic murine L-cells) and one non-cancerous cell line (VERO- African green monkey kidney epithelial cell) using 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) assay. While dose dependent cytotoxic activity was observed against cancer cell lines, no cytotoxic effect on VERO cell line was found in the tested experiments. In addition, phytochemical investigations to identify chemical content of the plant were resulted to the isolation of (+)-syringaresinol-4′-*O*-β-glucopyranoside (**1**), p-hydroxybenzaldehyde (**2**), quercetin-3-*O*-rutinoside (Rutine, **3**) and isorhamnetin-3-*O*-rutinoside (**4**) on the basis of different spectroscopic techniques (UV, IR, 1D and 2D NMR, HR ESI-MS).

Keywords: *Moltkia*; Boraginaceae; Radical scavenging effect; Cytotoxicity; Secondary metabolites.

1. Plant Source

The aerial parts of the plant were collected from the METU (Middle East Technical University) forest in June, 2004. A voucher specimen [HUEF 00013] has been deposited in the Herbarium of the Faculty of Pharmacy, Hacettepe University, Ankara, Turkey.

2. Previous Studies

Moltkia aurea L. is an endemic species for Turkey and we have found its traditional usage for different kidney disorders in Ankara region during our fieldwork [1]. There have been no phytochemical or biological reports in the literature about *M. aurea* up to now. Only the seed oil contents of some Boraginaceae species were researched and 7.4 % γ-linolenic acid, 14.2 % linoleic acid and 32.2 % α-linolenic acid contents were detected for *M. aurea* seed oil by gas chromatographic

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The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 08/05//2011 EISSN:1307-6167

analysis [2]. It is also mentioned about the presence of trace amount of alkaloids in *Moltkia* species growing in Armenian flora [3]. Recently, antioxidant and antimicrobial properties of *M. petraea* flower, leaf and stem infusions were reported in Croatia. While the *M. petraea* extract showed strong radical scavenging activity, it did not show antimicrobial activity against bacteria and fungi tested in the diffusion and dilution assay [4].

3. Present study

Cancer which is known as uncontrolled cell proliferation is a serious diseases that threaten to human health. Free radicals which are originated from cigarette smoking, air pollution, UV radiation, malnutrition and normal body functions are one of the important factor that cause cancer. For these reasons, researches on the anticancer and antioxidant compounds from natural sources are getting important for drug discovery from the nature. In the present study, the air-dried aerial parts of the plant (400 g) were extracted with MeOH at 40^{o}C for 12 h (3 x 2 L). The combined MeOH extracts were evaporated under vacuum to give crude MeOH extract (56 g). MeOH extract was dissolved in water and partitioned with petroleum ether to remove chlorophylls. The water fraction was lyophilized to yield 36 g dry weight and used for the bioactivity and isolation studies. Antioxidative activities of the water extract against DPPH, NO and SO radicals were determined in addition to total phenolic content of the plant [5-7]. The results demonstrated that while *M. aurea* extract had strong DPPH scavenging activity, it showed moderate NO and SO scavenging effect, dose dependently and this effect was found comparable to that of reference antioxidants ascorbic acid and BHA. Inhibition ratios and IC_{50} values for the extract and the reference compounds were given in Table 1 and 2. These results indicated the presence of phenolic compounds in the extract such as flavonoids, phenylethanoids and lignans. Here, total phenolic compounds of the aqueous *M. aurea* extract was expressed as gallic acid equivalent in mg/g dry extract and the amount of total phenolics was found 85 mg/g extract using Folin-Ciocalteu reagent [8]. High phenolic content of the plant makes the plant interesting from the view point of antioxidative and cytotoxic activities. In addition to radical scavenging activity, cytotoxic activity was also tested for the extract using 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenytetrazolium bromide (MTT) assay [9,10].

Table 1. DPPH radical scavenging activity of *M. aurea,* ascorbic acid (AA) and BHA

Concentration	Inhibition of DPPH (%)		
(µg/mL)	*M. aurea*	AA	BHA
400	85.98±2.0	90.58±0.7	88.57±1.2
200	71.82±1.9	90.31±0.1	89.61±0.1
100	39.35±1.4	90.77±0.2	89.60±0.2
50	26.89±1.3	90.83±0.6	88.99±0.1
20	9.67±0.9	90.31±0.3	79.86±0.9
10	7.41±0.9	78.36±5.6	56.10±2.0
	IC_{50} (µg/mL)		
	132.59	< 10	< 10

Table 2. SO and NO radical scavenging activity of *M. aurea,* ascorbic acid (AA) and BHA, ([a]NA: No activity)

Concentration	Inhibition of Superoxide (%)			Inhibition of Nitric oxide (%)		
(µg/mL)	*M. aurea*	AA	BHA	*M. aurea*	AA	BHA
800	64.36±3.0	86.44±0.9	34.17±2.9	43.36 ±1.9	33.99±4.3	28.09±1.2
400	50.08±2.2	86.38±0.3	9.54±6.1	34.58±1.4	24.33±4.5	17.92±5.3
200	44.82±4.1	83.97±1.1	NA[a]	31.28±2.1	14.76±1.8	6.24±1.0
100	42.31±0.3	73.82±1.1	NA	17.38±2.7	10.85±4.3	3.91±7.6
25	36.13±2.0	NA	NA	10.63±1.4	9.53±0.7	NA
		IC_{50} (µg/mL)			IC_{50} (µg/mL)	
	527	659	1253	377.1	108.3	1011.1

Three cancer cell lines, Hep-2 (human larynx epidermoid carcinoma), RD (human rhabdomyosarcoma), L-20B (transgenic murine L-cells) and one non-cancerous cell line (VERO-African green monkey kidney epithelial cell) were used for the MTT assay and their concentrations were $1x10^5$ cells/mL cells for Hep-2, $2x10^5$ cells/mL for L-20B, RD and VERO cell lines. Viability was decreased almost 50% at 400 µg/mL concentration for L-20B and Hep-2 cell lines. Comparing to RD cells, extract showed more cytotoxicity to L-20B and Hep-2 cell lines, and maximum effect was observed in 800 µg/ml concentration of the extract for three of the tested cell lines (Fig 1). Their IC_{50} values were found 400 µg/mL for Hep-2, 409.8 µg/mL for L-20B and 591.6 µg/mL for RD cells. While aqueous *M. aurea* extract show moderate cytotoxic activity against Hep-2, RD and L-20B cell lines, it did not show any cytotoxicity against VERO cells even in the highest concentration, 800 µg/ml. This difference is important for the selective effect of extract between cancer cells and normal cells. Further studies need to clarify cytotoxic activity and selectivity of the extract against different cancer cell lines.

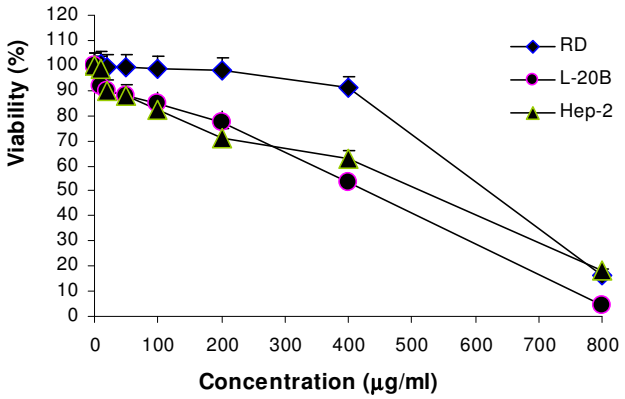

Figure 1. Cytotoxic activity of *M. aurea* water extract on RD, L-20B and Hep-2 cell lines*.

*Cells ($1x10^5$ cells/mL for Hep-2, $2x10^5$ cells/mL for L-20B and RD) were incubated for 48 h with various concentrations of the extract. After incubation, viability was determined by the MTT method.

To identify chemical content of the plant, water extract was subjected to different column chromatographies and 4 compounds were isolated. Their structures were determined as (+)-syringaresinol-4'-*O*-β-glucopyranoside **(1)**, p-hydroxybenzaldehyde **(2)**, quercetin-3-*O*-rutinoside

(Rutine, **(3)**) and isorhamnetin-3-*O*-rutinoside **(4)** on the basis of different spectroscopic techniques (UV, IR, 1D and 2D NMR, HR ESI-MS) and comparison with the data those reported in literature [11-14]. It is important to note that this study is the first evidence for the radical scavenging and cytotoxic properties of the aqueous *M. aurea* extract together with the phytochemical content of the plant.

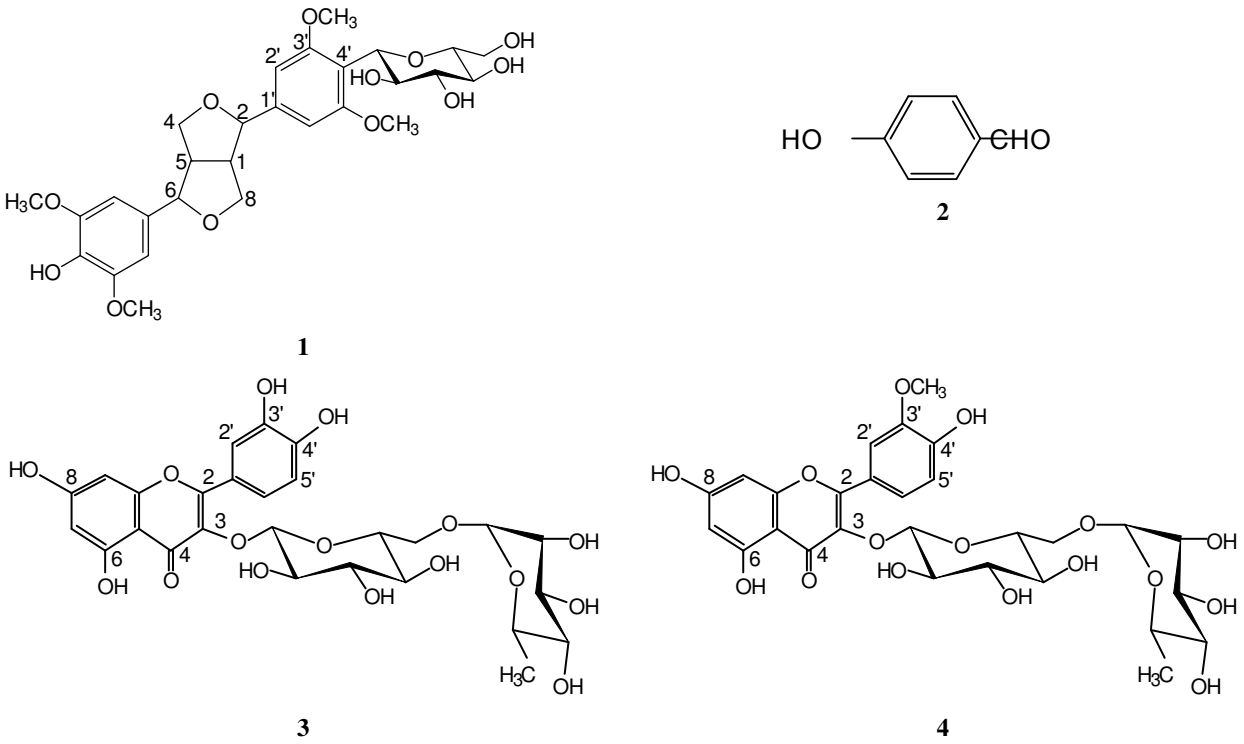

Figure 2. The isolated compounds from *M. aurea*

Acknowledgment

This study was financially assisted by Hacettepe University, Research Foundation (Project No: 0202301007; 0302301010) and The Scientific and Technological Research Council of Turkey (TUBITAK Project No: 108T518).

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