

Bioactive Phenolics from *Carthamus lanatus* L.

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Two flavonoid aglycons, eight flavonoid glycosides, chlorogenic acid and syringin were isolated from aerial parts of *Carthamus lanatus*. Isorhamnetin 3-*O*- β -D-glucoside and chlorogenic acid were found for the first time in the genus *Carthamus* and respectively, quercitrin, astragalin, kaempferol 3-*O*- β -D-sophoroside and syringin in the species. The ethyl acetate fraction of the methanol extract exhibited a higher antioxidant activity than the butanol fraction measured by the α,α -diphenyl- β -picrazyldrazyl (DPPH) free radical scavenging assay. Cytotoxicity and antioxidant activities of the main constituent, luteolin 7-*O*- β -D-glucoside, were evaluated.

Key words: *Carthamus lanatus*, Phenolics, Biological Activity

Introduction

Recently, the physiological potential of naturally occurring plant phenolics has attracted considerable attention. Their important role as antioxidants in protecting cell membranes against pathological conditions such as carcinogenesis, atherosclerosis, aging and mutagenesis has been proven. Significant efforts have been made to find new sources of natural antioxidants.

Carthamus lanatus L. (Asteraceae) is recommended as an important flavonoid-bearing plant of phytopharmaceutical importance (El-Shaer *et al.*, 1998). Until now, eight flavonoids and one serotonin have been found in the species. Apigenin, quercitrin (**5**) and *N*-(*p*-methoxycinnamoyl)-serotonin mono- β -D-glucopyranoside were isolated from seeds (Lahloub *et al.*, 1993). Five flavonoid glycosides were found in flowers: isoquercitrin (**6**), quercitin 3-*O*- α -arabinoside 7-*O*- β -glucoside, kaempferol 3-*O*- β -glucoside, luteolin 7-*O*- β -glucoside (**3**) and carthamidin 5-*O*- β -glucoside (Novruzov and Shamsizade, 1998). The flavonoid aglycone luteolin (**1**) and three glycosides, luteolin 7-*O*-glucoside, luteolin 7-*O*-rutinoside and chrysoeriol 7-*O*-glucoside were isolated from aerial parts (El-Shaer *et al.*, 1998). Recently, the presence of the flavonoid aglycones, 5-hydroxy-6,7-dimethoxyflavone, 5-hydroxy-7-methoxyflavone and chrysin in aerial parts of *C. lanatus* was detected by GC/MS (Mitova *et al.*, 2003).

In the present paper, we report our studies on the composition of phenolics of *C. lanatus* aerial parts as a promising source of natural antioxidants. Antioxidant and cytotoxic activities were evaluated by the α,α -diphenyl- β -picrazyldrazyl (DPPH) radical-scavenging method and by the brine shrimp (*Artemia salina*) assay.

Experimental

Plant material

Aerial parts of *Carthamus lanatus* were collected in July 2001 at the Losen region, Sofia, Bulgaria and identified by Dr. Rilka Taskova. A voucher specimen (No 156639) is deposited in the Herbarium of the Institute of Botany, Bulgarian Academy of Sciences (SOM).

Extraction and isolation

Air-dried and powdered aerial parts (3 kg) were extracted twice with methanol (15 l) and the concentrated extract (322 g) partitioned in hexane-methanol-water (19:19:2 v/v/v). The lower layer was successively extracted with diethyl ether (35 g), ethyl acetate (9 g) and butanol (18 g); water soluble part (220 g).

1 g of the ethyl acetate fraction was separated by ascending DCCC (Büchi 670) with CHCl₃-MeOH-H₂O-PrOH (9:12:8:2 v/v/v/v) with a flow-

rate of 25 ml/h. Fractions of 10 ml of the mobile phase and 100 ml of the stationary phase were collected. Fractions 28–35 (10 mg) of the mobile phase and fractions 5–6 (23 mg) of the stationary phase were purified by preparative TLC (silica gel plates, Macherey-Nagel, Germany) eluted with 2-methoxy-2-methyl-propane-MeOH-H₂O (12:2:1) to give **1** (7.6 mg) and **2** (2.9 mg).

11 g of the butanol fraction were separated by ascending DCCC with CHCl₃-MeOH-H₂O-PrOH (9:12:8:2) with flow-rate of 30 ml/h. Fractions of 12 ml mobile phase were collected. Fraction 3 (1.2 g) was separated by silica gel column chromatography (Silica gel 60, Fluka, Switzerland) using 2-methoxy-2-methyl-propane-MeOH-H₂O (12:2:1) as eluant to give **4** (29 mg) and a mixture of **8** and **10** (23 mg). A crystalline substance **3** (480 mg) was separated from fraction 5 (959 mg). The methanol soluble part of this fraction was additionally purified by HPLC (SP 250/10 NUCLEOSIL 100-7 C₁₈, Macherey-Nagel), using a water/acetonitrile gradient 0–70% (flow: 3 ml/min; detection: UV at 280 nm) to yield pure **11** (4 mg) and **12** (11 mg). Fraction 7 (280 mg) was separated on a silica gel column with 2-methoxy-2-methyl-propane-MeOH-H₂O (12:2:1) and additionally purified by preparative TLC with the same mobile phase to afford pure **5** (4 mg) and **6** (3 mg). The same procedure was applied for purification of fraction 9 (236 mg) yielding pure **7** (1 mg), a mixture of **5** and **9** (5 mg).

DPPH radical-scavenging activity

2 mg of the studied samples and α -tocopherol (reference compound) were dissolved in DMSO (10 ml). 250 μ l of each solution was added to 1 ml DPPH/DMSO solution (6 mg/50 ml) and the total volume was adjusted to 3 ml with DMSO. After vortexing the mixture was incubated for 30 min at room temperature. Absorbance was measured at 517 nm (Hatano et al., 1988).

Cytotoxicity assay

Artemia salina lethality was determined according to Solis et al. (1993). Concentrations of 1, 0.1, 0.01 and 0.001 mg/ml in three replications, ten larvae per concentration and caffeic acid phenethyl ester (CAPE) as a reference substance were used.

The data was statistically analyzed by the Finney program (Finney, 1971).

Results and Discussion

The ethyl acetate and butanol fractions of the methanolic extract from aerial parts of *Carthamus lanatus* were separated using a combination of chromatographic methods (DCCC, CC on silica gel, HPLC RP-18 and PTLC) to give twelve compounds. Compounds **1**–**12** (Fig. 1) were identified by means of spectral data (1D and 2D NMR, ESI-MS) and comparison with reference compounds. Ten of them were identified as the known flavonoids, the aglycones luteolin (**1**) and quercetin (**2**) and eight glycosides, the main constituent luteolin 7-*O*- β -D-glucoside (**3**) along with quercimeritrin (**4**), quercitrin (**5**), isoquercitrin (**6**), isorhamnetin 3-*O*- β -D-glucoside (**7**), rutin (**8**), astragalins (**9**) and kaempferol 3-*O*- β -sophoroside (**10**). Another two compounds were identified as syringin (**11**) and chlorogenic acid (**12**). Compounds **4**, **9**, **10** and **11** were found for the first time in this species. To the best of our knowledge isorhamnetin 3-*O*- β -D-

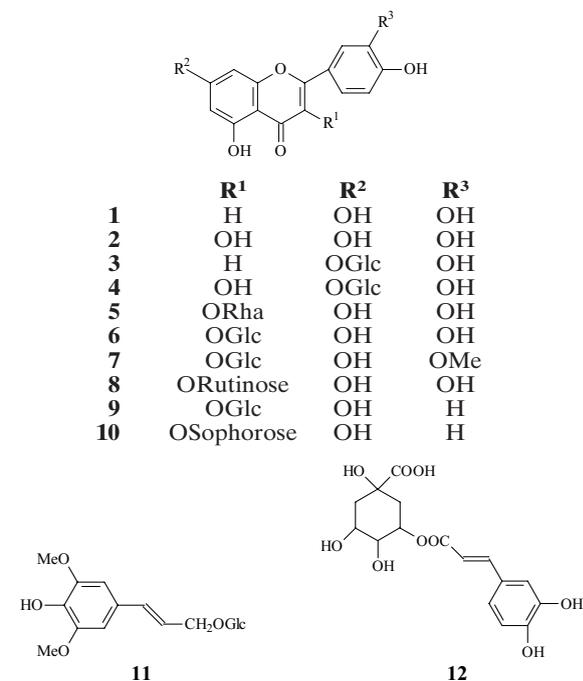


Fig. 1. Phenolic compounds isolated from aerial parts of *C. lanatus*.

glucoside (**7**) and chlorogenic acid (**12**) have not been found in the genus *Carthamus* until now.

All isolated phenolics are bioactive compounds. Antiinflammatory (compounds **1**, **2**), anti-tumor (**1**, **2**, **12**), antibacterial and antiviral (**1–6**, **8**, **12**), antiulcer and antihepatotoxic (**5**, **11**), allelopathic (**3–5**, **8**) and antioxidant (**1–6**, **8**, **12**) activities were found for these constituents (Pathak *et al.*, 1991; Wassel *et al.*, 1996; Harborne *et al.*, 1999; Kim *et al.*, 1999; Morishita and Ohnishi, 2001; Lee *et al.*, 2002).

The potential antioxidant activity of some fractions and flavonoid glycosides of *C. lanatus* was assessed by the DPPH free radical scavenging method. The activity was evaluated by measuring the decrease of absorption of the DPPH solution at 517 nm after addition of the antioxidant solution. The relative radical scavenging activities of the ethyl acetate and butanol fractions, of compounds **3–5** and of a mixture of **3** and **4** were presented in Table I. Analysis of the data revealed a higher activity of the ethyl acetate fraction than the butanol fraction. Flavonoids **3** and **5** showed considerable, close to α -tocopherol activities. The antioxidant activity of a mixture of **3** and **4** (1:1) was higher than the individual compounds and could be a result of synergism.

The cytotoxic activity of the main flavonoid constituent, luteolin 7-*O*-glucoside (**3**), was evaluated by using brine shrimp assay (*Artemia salina*). **3** showed significant cytotoxicity ($LD_{50} = 85 \pm 34 \mu\text{g/ml}$), however weaker than the active reference substance caffeic acid phenethyl ester

Table I. DPPH radical scavenging activity of *C. lanatus* extracts and constituents.

Sample	Relative radical scavenging activity (%)
EtOAc fraction	37.7
BuOH fraction	22.9
Luteolin 7- <i>O</i> - β -D-glucoside (3)	82.8
Quercimeritrin (4)	59.3
Quercitrin (5)	84.2
3 + 4 (1:1)	89.5
α -Tocopherol*	100

* α -Tocopherol was used as the positive reference.

(CAPE) ($LD_{50} = 0.45 \pm 0.05 \mu\text{g/ml}$). In our previous study (Taskova *et al.*, 2002) a higher cytotoxic activity of the ethyl acetate fraction than the butanol fraction was found. Interestingly, the present investigation revealed the same effect for the antioxidant activity of these fractions.

In conclusion, *Carthamus lanatus* is a flavonoid-bearing plant with a pharmaceutical potential. The ethyl acetate fraction of the methanol extract appeared to possess promising natural antioxidant and cytotoxic activities.

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