

INVESTIGATION OF LIPOPHYLIC COMPOUNDS FROM NATIVE SPECIES OF *PORTULACA* L. (*PORTULACEAE*) GENUS

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Abstract

The species of *Portulaca* genus are little known, studied and are not used in Romania for therapy or food purposes. In the world, though, *Portulaca oleracea* is known for its therapeutic and nutritive value. This research aimed the investigation of some lipophilic compounds from two Romanian native species, *P. oleracea* and *P. grandiflora*. The analysis of volatile compounds was carried out by GC-MS and the total carotenoids were spectrophotometrically assayed. In *P. oleracea* 17 volatile compounds were identified, among which the most important were linalool (33.18%) and salicylaldehyde (21.23%). Their presence might justify the use of the species in the therapy of diabetes and its complications, as well as in inflammatory states. The total carotenoid content was higher for *P. oleracea* (between 281.3 - 361.9 mg carotene per 100 grams of dried herbal product) than for *P. grandiflora* (between 6.4 - 6.6). The results are in agreement with the antioxidant activity reported for the herbal product in the literature.

Rezumat

Speciile genului *Portulaca* sunt puțin cunoscute, studiate și sunt neutilizate în terapie sau alimentație la noi în țară. În lume însă, *Portulaca oleracea* este cunoscută pentru valoarea sa nutritivă și terapeutică. Cercetarea de față urmărește investigarea unor compuși lipofili din două specii autohtone, *P. oleracea* și *P. grandiflora*. Analiza compușilor volatili a fost realizată prin GC-MS, iar carotenoidele totale au fost determinate spectrofotometric. În *P. oleracea* au fost identificați 17 compuși volatili, dintre care cei mai importanți au fost linaloolul (33,18%) și salicilaldehida (21,23%). Prin prezența acestor compuși, specia poate fi utilă în terapia diabetului și a complicațiilor sale, precum și în diferite stări inflamatorii. Conținutul în carotenoide totale a fost mai mare pentru *P. oleracea* (între 281,3 - 361,9 mg caroten/100 g produs vegetal uscat) față de *P. grandiflora* (între 6,4 - 6,6 mg caroten/100 g produs vegetal uscat). Rezultatele sunt în concordanță cu acțiunea antioxidantă, menționată în literatura de specialitate.

Keywords: *Portulaca oleracea*, *P. grandiflora*, antioxidant, caroten

Introduction

The genus *Portulaca* (*Portulacaceae* family) is represented in Romania by two species, *P. oleracea* L. and *P. grandiflora* Hook. *Portulaca oleracea* L. (common purslane) has been traditionally used as food and in the treatment of digestive conditions, as an anthelmintic. In the scientific literature, various reports are available claiming its use in a number of conditions, some of them supported by scientific data; data on its chemical composition have also been published [20]. The species growing in Romania has never been investigated up to now, although it is widespread, even invasive in some cultures, producing a large number of seeds and being little-demanding. *Portulaca grandiflora* Hook (moss-rose purslane) is a species cultivated for its beautifully coloured flowers and its resistance to environmental conditions. Little

research has been carried out on the chemical composition and pharmacognosy of *Portulaca grandiflora* [5, 6]. The studies published by now have reported analgesic, antimutagenic and lymphocyte stimulating activities [3, 24, 35].

Essential oils are a complex class of secondary metabolites synthesized by plants usually in very low amounts (< 1%). The composition of secondary metabolites varies with the season, day time, environmental conditions, as well as the genetic material of the plant [4, 7, 29].

The essential oils from *Portulaca* genus have been little investigated by now. We only identified two reports on the chemical composition of essential oils of some species from China [25, 36].

Carotenoids are compounds universally widespread, being found both in lower organisms such as bacteria,

algae, fungi, lichens or ferns, as well as in higher organisms [9, 26].

Previous phytochemical studies have investigated carotenoids only in the species *P. oleracea*, while for *P. grandiflora* no reports have been identified in this sense in the literature. Varieties of *P. oleracea* growing in Europe have been checked for their β -carotene contents in experiments focused on this species only or in comparison with other species [17]. The content in carotenoidic pigments seems to depend on a series of environmental factors, such as soil composition, temperature, light (intensity, illumination time). These factors are different from those affecting the varieties which have been investigated in previous research on *P. oleracea*. Besides, *P. grandiflora* has not been investigated at all from this perspective.

In a previous study we have reported on the phytochemical screening of the two species, which *inter alia*, by specific chemical reactions, signalled the presence of lipophilic compounds: sterols, carotenoids [5] and flavonoid aglycons. In addition, as stated above, the literature mentions the presence of essential oils in *Portulaca* species [25, 36]. We have therefore proceeded to qualitatively and quantitatively assess the essential oils and total carotenoids in the two species of the *Portulaca* genus.

Materials and Methods

Both species have been harvested fresh from Bucharest, Romania in September. A voucher specimen was deposited in the Herbarium of the Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania. The purity and quality of the plant material were determined using the relevant methods described in the European Pharmacopoeia, 7th Edition. The above-ground parts of *P. oleracea* and *P. grandiflora* were subjected to distillation for 4 hours. The ratio herbal product - water was 1/5 (g/mL) for *P. oleracea* and 1/7 (g/mL) for *P. grandiflora*. The hydrodistillate obtained was analysed by GC-MS.

Chemicals

Dichloromethane Suprasolv for gas-chromatography, methanol and C₁₈ cartridge (500 mg) were purchased from Merck, Darmstadt, Germany. The n-alkanes C8 - C20 solutions in hexane used for the determination of Kovats indices were also purchased from Merck.

Volatile compounds extraction

Fresh plant material and water 1:5 g/mL was hydro-distilled in a Neo Clevenger apparatus for 4 h. The hydrodistillate obtained was passed through a conditioned 500 mg C₁₈ cartridge according to a method published earlier [32]. The compounds were eluted with 2 mL dichloromethane and 1 μ L of eluate were injected in the system for the GC-MS analysis.

Gas-chromatography – mass spectrometry assay

GC-MS analysis of dichloromethane extract of *Portulaca oleracea* was carried out using a Fisons Instruments GC 8000 with an electron impact quadrupole, MD 800 mass spectrometer detector. The electron ionisation energy was 70 eV. A fused silica column 5% phenyl-poly(dimethylsiloxane) (SLB - 5 ms, 30 m x 0.32 mm i.d., film thickness = 0.25 μ m) was employed. The operating conditions were the following: a split-splitless injector (split ratio, 1/30) at 280°C, ion-source temperature 200°C and the interface temperature 280°C; initial column temperature, 40°C for 3 min, raised at 5°/min to 250°C and finally held isothermally for 10 min; the carrier gas (helium) flow rate was 2 mL/min; sample volume injected, 1 μ L. Data acquisition was performed with MassLab Software for the mass range 30-600 u with a scan speed of 1 scan/s. The identity of dichloromethane extract components was established from their GC Kovats retention indices and from mass spectra by computer matching with mass spectra library (NIST, WILEY and a personal library of 600 spectra). The linear retention indices were determined in relation to a homologous series of n-alkanes (C8 - C20). The experimental value of Kovats indices were compared with those reported in literature [2]. Component relative concentrations were calculated from GC peak areas without using correction factors.

Carotenoids assay

For the total carotenoid assay, the dried, comminuted herbal product from the two species was used. It was assessed the intensity of colour of an etheric solution obtained after carotenoid saponification with KOH 10% *versus* a reference substance (β -carotene).

Preparation of extractive solutions

The extraction was carried out with a Soxhlet apparatus using ethyl ether as a solvent. 2 g of herbal product were extracted until the solution became colourless. The ether solution was concentrated to 10 - 12 mL.

Saponification was performed with 10 mL KOH 10% (in methanol) for 1 - 2 hours. By saponification, chlorophyll was removed and carotenoid esters were hydrolysed. After cooling, to the obtained sample, 2 - 3 volumes of water were added and it was subjected to 5 - 6 successive extractions with ethyl ether. The pooled etheric solutions were passed through anhydrous sodium sulphate and brought in a volumetric flask of 100 mL [12, 27].

The assay of total carotenoids of the obtained samples was carried out using a calibration curve prepared from a reference solution (stock) of β -carotene (Merk) 2.5 mg/100 mL in benzene. The calibration curve included concentrations ranging between 2.5 and 17.5 μ g/100 mL was prepared. The determination coefficient (r^2) was 0.9998 and the regression equation, $y = 0.0185x - 0.0013$.

Results and Discussion

The hydrodistillation of *P. grandiflora* herbal product did not manage to separate any essential oil and the analysis of the sample obtained by washing the

apparatus with 2 mL CH₂Cl₂ did not allow obtaining a chromatographic fingerprint for this species.

Table I shows the relative content of *Portulaca oleracea* dichloromethane extract components, expressed as percentage from total area.

Table I

The chemical composition of *Portulaca oleracea* dichloromethane extract

No.	Compound	RT (min)	KI _{exp}	KI _{lit}	Area (%)
1	4-hexen-1-ol	4.45	873	879	4.27
2	heptanal	5.71	904	908	8.25
3	2,4-hexadienal	6.03	917	916	0.80
4	benzaldehyde	7.50	965	965	1.34
5	1-heptanol	8.01	980	978	0.30
6	1-octen-3-ol	8.25	986	986	0.37
7	6-methyl-5-hepten-2-one	8.39	990	994	0.38
8	3-octanol	8.80	1001	1001	0.64
9	2,4-heptadienal	9.22	1015	1016	2.14
10	salicylaldehyde	10.15	1046	1049	21.23
11	linalool	12.10	1102	1101	33.18
12	α -terpineol	14.99	1195	1195	2.18
13	safranal	15.09	1198	1198	0.60
14	geraniol	16.75	1256	1255	1.33
15	2-methoxy-4-vinylphenol	18.40	1311	1313	6.55
16	β -damascenone	20.28	1380	1384	3.47
17	cis-nerolidol	25.04	1563	1565	0.41
TOTAL					87.44

A total of 17 compounds were identified from the dichloromethane extract accounting for 87.44% of the total area. Most of the compounds identified belong to either alcohols (42.62%) or carbonyl compounds (38.21%) class. Among these compounds only a phenolic ether, 2-methoxy-4-vinylphenol was found in a percentage of 6.55%. The most abundant compounds were linalool (33.18%) and salicylaldehyde (21.23%). Among the identified compounds, benzaldehyde and 2-methoxy-4-vinylphenol along with linalool derivatives were found in *Portulaca oleracea* L. from China [36].

Salicylaldehyde has been identified in buckwheat, being one of the constituents giving its specific flavour [21]. It also represents up to 68% of the essential oils extracted from the leaves of *Filipendula vulgaris* [33].

Linalool is a monoterpene frequently found in essential oils obtained from aromatic plants. It is known as an additive in the processing of food, drinks and as an ingredient in cosmetic products. It has also repellent, anxiolytic and antinociceptive activity, being potentially

useful in the therapy of persistent pain and as an anti-inflammatory agent [22, 10, 28]. Some studies have shown that linalool may attenuate the risk of diabetic nephropathy occurrence, being known that it has hypoglycaemic effects. [1, 16].

Another monoterpene identified in the essential oil of *P. oleracea*, also found in essential oils from other plants, is α -terpineol. Among its pharmacological activities we mention the anticonvulsant, antihypertensive, antinociceptive and antiseptic ones [14, 15, 34, 31].

Another compound found in a larger proportion in the essential oil analysed is β -damascenone. This may be found in the essential oils of roses and in certain fruits, such as grapes, being responsible for the flavour of the red wine and it has spasmolytic activity [13, 30].

Safranal, known as a volatile compound from *Crocus sativus*, has been shown to have anticonvulsant and antioxidant activities [8, 19].

The results of total carotenoid assays for the two species are shown in Table II.

Table II

Total carotenoids in the aerial parts of *P. oleracea* and *P. grandiflora*

No.	Sample	M (mg/100 g on a dry basis) \pm SD
1.	<i>P. grandiflora</i>	6.5 \pm 0.0001
2.	<i>P. oleracea</i>	321.5 \pm 0.0404

M = mean, DS = standard deviation

The total carotenoid contents, expressed in mg carotenoids per 100 grams of dried herbal product is 6.5 in *P. grandiflora* and considerably higher, 321.5

for *P. oleracea*. These results are different from those reported in the literature (22 - 30 mg/g of fresh leaves) [23], probably due to the methods used for

extraction and quantification, as well as to the differences in environmental conditions. The differences are also likely to be to a good extent attributable to the fact that in the literature assay data were reported for fresh leaves, while we have used dry herbal products consisting in the aerial parts. The results found by us are close to those reported in the literature for other species, known for their hypolipidemic activity (e.g. *Zingiber officinale* 0.64; *Anethum graveolens* 0.64 mg % carotenoids [11], but also similar to those reported in species known for their nutritive value and taxonomically related to *Portulacaceae* (*Chenopodium bonus henricus* L., 344.1 mg total carotenoids/100 g dry weight) [18].

Conclusions

P. oleracea, by its content in linalool (38%) and safranal (0,6%) (although the latter is in rather modest amounts) might be useful in the treatment of diabetes and its complications; by its content in salicylaldehyde (21%), it might have anti-inflammatory activity, while through the damascenone (3,47%), might have spasmolytic properties. However, the relatively small amount of essential oil in the species implies the use of considerable amounts of herbal product, which might be impractical.

The carotenoids content is higher in the wild species *P. oleracea* than in the cultivated species *P. grandiflora*, and is in agreement with the reported antioxidant activity for this species.

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