PRELIMINARY RESEARCH ON *PORTULACA GRANDIFLORA* HOOK. SPECIES (*PORTULACACEAE*) FOR THERAPEUTIC USE

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Abstract

Portulaca grandiflora Hook. has been used in eastern traditional medicine for alleviating sore throat, for skin rashes and for detoxification. No research has been published correlating these actions with the active substances of the plant. For this reason, we decided to study the chemical composition of the species grown in Romania, in order to determine its therapeutic potential. The species was identified by comparing its morphological characteristics with those described in the literature. The chemical composition was assesed through specific chemical reactions and thin layer chromatography (TLC). The total phenols, polyphenol-carboxylic and flavonoid compounds have been determined quantitatively through spectrophotometric methods (Folin–Ciocalteu, nitric acid and sodium molibdate, and chelation with $AlCl_3$, respectively).

There were identified: sterols, carotenoids – in the etheric extract (Pe); phenolic acids – in the ethanolic extract (Pa); polysaccharides, reducing agents in the aqueous one (Paq). The presence of the sterols, of caffeic and chlorogenic acids, of quercetol and its heterosides and of kaempferol, was confirmed by TLC. In alcohol flavonoids had the highest extraction yield (0.2519g%), while in water this was the case for the phenolic-carboxylic acids and total polyphenols (0.1874, and 0.6110g%, respectively; all percentage are calculated on dry basis).

Rezumat

Portulaca grandiflora este utilizată în medicina tradiţională orientală pentru ameliorarea durerii în gât, în erupţii cutanate şi în detoxifiere. Cercetări care să justifice aceste acţiuni prin principii active nu sunt întâlnite în literatură. De aceea, ne-am propus cercetarea compoziţiei chimice a speciei cultivate în România, în vederea determinării eventualului potenţial terapeutic. Identificarea speciei s-a efectuat pe baza caracterelor morfologice descrise în literatura de specialitate. Compoziţia chimică a fost explorată prin reacţii chimice specifice şi

cromatografie în strat subţire (CSS). Compuşii flavonici au fost determinaţi cantitativ printr-o metodă spectrofotometrică.

S-au identificat: steroli, carotenoide - în extractul eteric (Pe); acizi fenolici - în extractul alcoolic (Pa); poliholozide, compuşi reducători în extractul apos (Paq). Prezenţa sterolilor, a acizilor cafeic şi clorogenic, kaempferolului, quercetolului şi a heterozidelor acestuia a fost confirmată prin CSS. În alcool, se extrag în cantitate mai mare flavonele (0,2519g%), iar în apă acizii fenol-carboxilici şi polifenolii totali (0,1874 respectiv 0,6110g% de produs vegetal uscat).

Keywords: *Portulaca*, flavonoids, phenolic-carboxylic acids, polyphenols

Introduction

The genus *Portulaca* is represented in Romania by the species *Portulaca grandiflora* Hook. and *Portulaca oleracea* L. Known as moss-rose in English and as "the stone flower" in Romanian, *Portulaca grandiflora* is often cultivated in urban or rural places, on sandy soil, in sunny areas, for its big and beautifully coloured flowers. Besides being a decorative plant this species seems to have no other uses described in literature. It spreads easily, which is why it is widely sought by gardeners [3].

Our research aims to put into value the *Portulaca grandiflora* species, based on the phylogenetic kinship to *Portulaca oleracea*, which is traditionally used as an anti-inflammatory, analgesic, anti-ulcer, anti-diabetic and diuretic drug by different people. In eastern traditional medicine, *Portulaca grandiflora* is used for soothing the sore throat, for skin rushes and also for "detoxification" reason. These claims seem to have never been assessed in non-clinical models or clinical trials. There is, however, a small number of scientific studies on the anti-mutagenic actions, *in vitro* lymphocytic stimulation and on the analgesic actions of *Portulaca grandiflora* [11]. The published literature contains few data regarding the chemical composition of the species.

The significant influence of the soil and climate on the chemical composition of the herbal species, the small number of data in this area and the scientific interest in assessing the traditional uses have determined us to study the contents of the main potential active substances of *Portulaca grandiflora*. We have started with the research of the phenolic compounds (phenolic acids, coumarins) and flavonoidic compounds.

Materials and Methods

The plant material used in the study was the fructified aerial part of the plant, harvested from Bucharest (several private gardens) in October

2010. This material was identified by macroscopic and microscopic examinations, and the results were previously published [1]. The herbal product was subsequently extracted with solvents of different polarities and the extracted solutions were coded as follows: ether (Pe), ethanol (Pa), water (Paq). Half of the Pa and Paq solutions were refluxated for 45 minutes with HCl 1N. After cooling, the aglycons were extracted with ether. The newly obtained extractes (coded with Pah and Paqh, respectivelly) were used to identify the aglycons coming from the hydrolysis of the heterosides. The identification of the chemical compounds was undergone by specific chemical reactions mentioned in the literature [4, 5] and by thin layer chromatography (TLC).

TLC conditions

- Stationary phase: GF_{254} Silica gel, precoated TLC plates Merck (20cm x 10cm);
- Mobile phase: petroleum ether, ethyl-acetate, formic acid (40:60:1) [12] (MPh1) for aglycons, phenolic acids, coumarins; ethyl-acetate – distilled water – formic acid – acetic acid (72:14:7:7) [10] (MPh 2) for phenolic acids and flavonosides; chloroform – acetone (80:20) for sterols (MPh3) [8];
- Reference substances: quercetin, kaempferin, myricetin, resveratrol, caffeic acid, chlorogenic acid, rutin (alcoholic solutions 1.0mg/mL), hyperoside (alcoholic solutions 0.2mg/mL), isoquercetin (alcoholic solutions 0.3mg /mL), umbelliferone, *beta* sitosterol (alcoholic solutions 1.0mg /mL);
- Reagents: diphenyl boryloxy ethylamine (reagent 1) – for phenolic compounds [9]; acetic anhydride (reagent 2), sulfuric acid:ethanol, 1:1 (reagent 3) – for sterols, triterpenes. The chromatograms were examined in visible spectrum and in UV spectrum at 254 and 366nm.

The quantitative determination of the polyphenolcarboxylic acids was crried out spectrophotometrically according to *Fraxini folium* monography from EP VII. The measurements were performed at λ=525 nm with Jasco V 530 UV/VIS spectrophotometer [6].

The content in total phenolic components of the samples was determined using the Folin-Ciocalteu reagent solution with a few adaptations [13]. The determinations were made in a 96-well microplate and the absorbances of the samples were determined using a microplate reader. The reaction mixture consisted in 20 µL of test sample solution, distilled water, Folin–Ciocalteu reagent and 20% sodium carbonate solution (w/v).

The microplate was covered and incubated in a thermo-mixer for 2h at 25°C. The absorbance was measured at 760 nm. The blank solution contained 20 µL ethanol instead of the test solution. Every assay was carried out in triplicate. The total phenolic content was estimated using the calibration curve of standard gallic acid (GAE), r^2=0.9982, concentration range 1–10 µg/mL, detection limit (DL) = 0,0565 µg/mL, quantification limit (QL)= 0,0716 µg/mL (Figure 1).

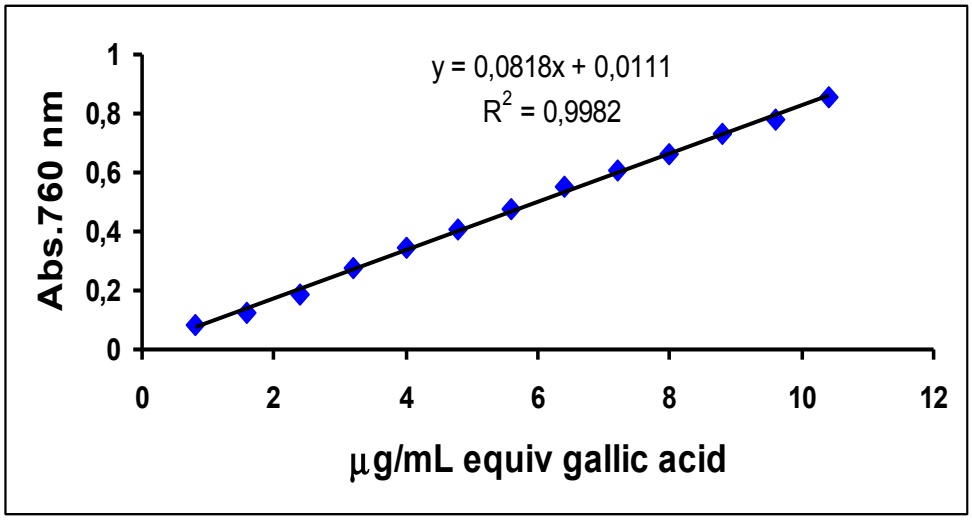

Figure 1
Calibration curve for gallic acid

The total flavonoids' content was determined using the method described by Woisky and Salatino employing $AlCl_3$ to form a complex, which was measured spectrophotometrically at λ=425nm. The determinations were made in a 96-well microplate and the absorbances of samples were determined using a microplate reader. The reaction mixture consisted in 25 µL of the test sample solution, $AlCl_3$ 10%, 1M potassium acetate solution and distilled water - also 25 µL. The microplate was covered and incubated in a thermo-mixer for 30 minutes at 25° C. The blank sample contained the same reagents, with exceptions of $AlCl_3$, which was replaced by ethanol. Every assay was carried out in triplicate. The total flavonoids content was estimated using the calibration curve of standard rutin (r^2=0.9985), concentration range 2.5-55 µg/mL, DL= 0,0502 µg/mL, QL=0,1670 µg/mL [2, 7] (Figure 2).

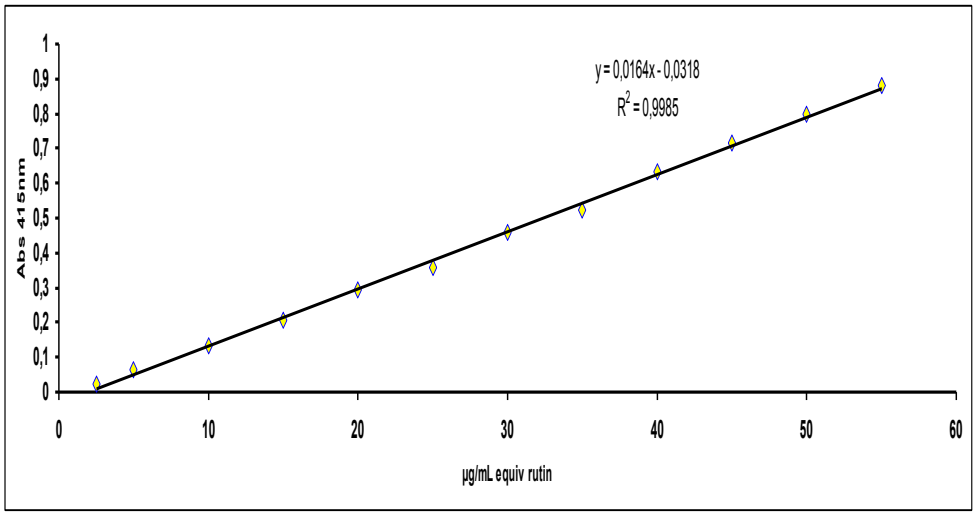

Figure 2
Calibration curve for rutin

Results and Discussion

By characteristic chemical reactions were revealed: sterols, carotenoids, phenolcarboxylic acids, polysaccharides, reducing agents (Table I).

Table I
Results of the chemical screening

No.	Studied compound	Reaction	Etheric solution	Alcoholic solution	Aqueous solution
1	Sterols	Libermann-Burchard	+++	++	-
2	Phenolcarboxylic acids	Arnow	-	+	++
3	Flavones	Shibata	+	+	+
4	Oses	Molisch	-	++	+
5	Polysaccharydes	Precipitation in acetone: methanol	-	-	++
6	Starch	Lugol	-	-	-
5	Coumarins	UV fluorescence	-	-	-
7	Tannins	$FeCl_3$	-	+	+
8	Carotenoides	Carr-Price	+++	++	+
9	Alkaloids	Mayer Bertrand	- -	- -	- +
10	Reducing agents	Fehling	-	++	++

Legend: reaction + + +strongly positive , ++ positive, + weakly positive, - negative

By TLC numerous spots were separated; based on their reaction with the reagents and on their Rf values, quercetin and caffeic acid were identified in Paq, MPh1 (table II, fig 3,4), rutin, isoquercetin, caffeic and chlorogenic acids in Pa and Paq, MPh2 (table III, fig. 3), several sterolic compounds were detected in Pe, Pa, Pah and Paqh, MPh 3 (Table IV, Figure 4).

Table II

Rf values of the separated spots by TLC in MPh1

No.	Sample / Reference substance	Rf sample std.	Fluorescence of the spot			Identified compound
			UV 366nm	UV 254nm	Visible	
1	Paqh	0.45	Orange	-	Green	Quercetin
		0.50	Bright blue	Blue	-	Coumarin
		0.52	Yellowish-green	-	-	Caffeic acid
2	Quercetin	0.46	Orange	Violet	Green	-
3	Kaempferin	0.61	Yellow	Reddish-orange	Greenish-brown	-
4	Miricetin	0.43	Dark orange	Orange	Greenish-brown	-
5	Resveratrol	0.61	Violet	Violet	-	

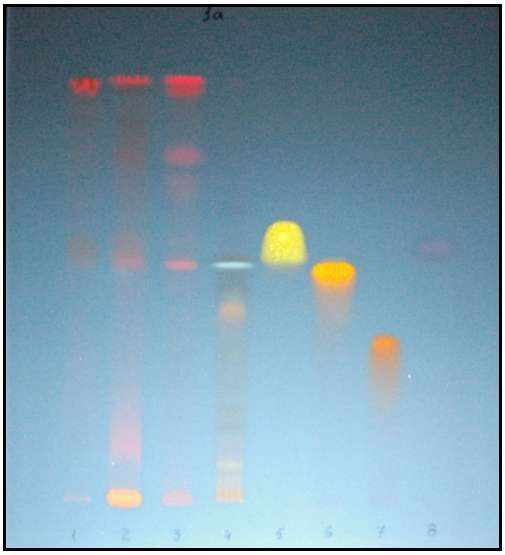

Figure 3

TLC separation of flavones (MPh1, reagent 1,UV 366 nm). Start: 1- Pe, 2-Pa, 3-Pah, 4-Paqh,5 – kaempferin, 6-quercetin, 7-myricetin, 8-resveratrol

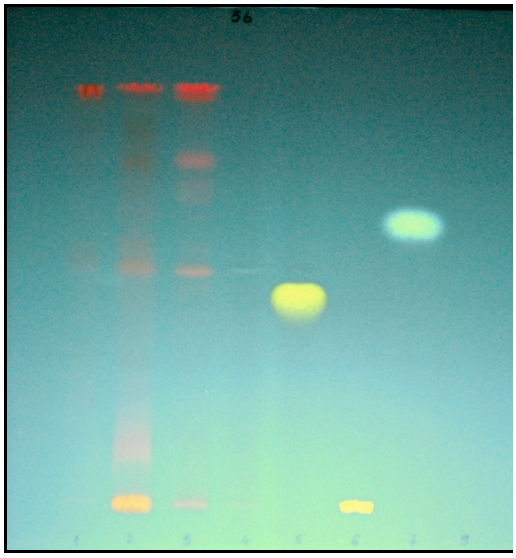

Figure 4

TLC separation of polyphenol carboxilic acids (MPh1, reagent1, UV 366 nm). Start: 1 – Pe, 2 – Pa, 3 – Pah, 4 – Paqh, 5 – caffeic acid, 6 – chlorogenic acid, 7-umbelliferone

Table III

Rf values of the separated spots by TLC in MPh2

Sample / Reference substance	Rf	Fluorescence of the spot			Identified compound
		UV (366)	UV (254)	VIS	
Paq	0.36	Light orange	Greenish-blue	-	Rutin
	0.39	Light blue	Greenish-blue	-	Chlorogenic acid
	0.53	Orange	Greenish-blue	-	Isoquercetin
Pa	0.31	Light orange	Greenish-blue	-	Rutin
	0.39	Blue	Greenish-blue	-	Chlorogenic acid
	0.53	Orange	Greenish-blue	-	Isoquercetin
	0.95	Blue	Greenish-blue	Yellow	Caffeic acid
	0.95	Red	Greenish-blue	Green	Chlorophyll
Isoquercetin	0.53	Orange	Greenish-blue	Yellowish-green	
Hyperoside	0.48	Orange	Greenish-blue	-	
Rutin	0.36	Orange	Greenish-blue	Yellowish-green	
Caffeic acid	0.95	Blue	Greenish-blue	Yellowish-green	
Chlorogenic acid	0.39	Blue	Greenish-blue	Yellowish-green	

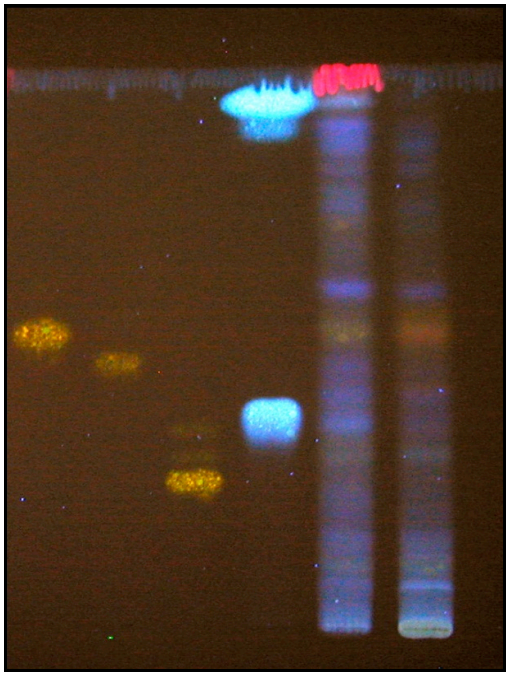

Figure 5

TLC separation of flavones (MPh 2 , reagent 1, UV 366 nm). Start: 1-isoquercetin, 2-hyperoside, 3-rutin, 4-caffeic + chlorogenic acids, 5- Pa, 6- Paq

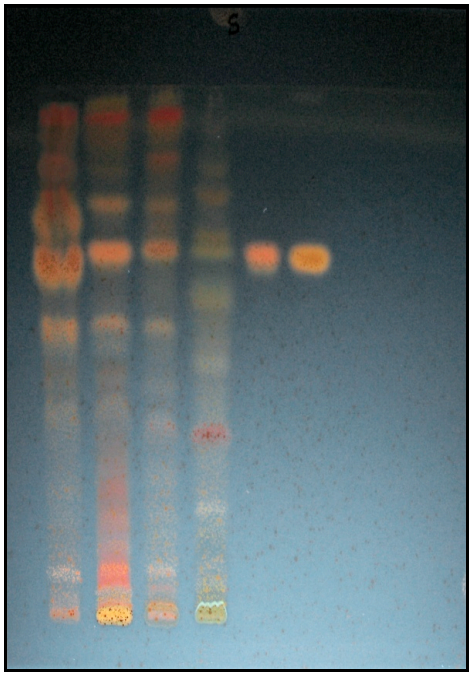

Figure 6

TLC separation of sterols (MPh3 , reagent 2,3, UV 366 nm). Start: 1-Pe, 2-Pa,3-Pah, 4-Paqh, 5-beta sitosterol, 6-stigma sterol

Table IV

Rf values of the separated spots by TLC in MPh3

Sample / Reference substance	Rf	Fluorescence of the spot			Identified compound
		UV (366 nm)	UV (254 nm)	VIS	MPh 3
Pe	0.66	Orange	brown	Dark pink	Sterolic compound
Pa	0.68	Pinkish-orange	Brown	Pink	Sterolic compound
Pah	0.69	Orange	Brown	Pink	Sterolic compound
Paqh	0.69	Light orangel	Brown	Pink	Sterolic compound
Sitosterol	0.68	Pinkish-orange	Brown	Pink	
Stigmasterol	0.68	Orange	Brown	Pink	

Table V

Polyphenols quantitative analysis results

Sample	Phenolcarboxylic acids (g/100g herbal product)			Flavonoids (g/100g herbal product)			Polyphenols (g/100g herbal product)		
	M	SD	CI	M	SD	CI	M	SD	CI
Aqueous solution	0.1874	0.0074	0.1874± 0.0074	0.0981	0.0274	0.0981± 0.0547	0.6110	0.0440	0.6110± 0.088
Alcoholic solution	0.1757	0.0017	0.1757± 0.0017	0.2519	0.0749	0.2519± 0.1499	0.2407	0.0069	0.2407± 0.014

M = average of the 3 determinations; SD = standard deviation; 95%CI = confidence interval

Conclusions

By the chemical analysis of the *Portulaca grandiflora herba* the following groups of phytochemicals were identified: sterols, carotenoids, polyphenolic acids, flavonoids, polysaccharides, reducing agents. The aqueous solutions obtained from the herbal material are relatively rich in polyphenols (0.61±0.04UM) and poor in flavonoids (0.10±0.03UM). In comparison, the alcoholic solutions are rather high in flavonoids (0.25±0.07UM) and lower in polyphenols (0.24±0.01UM). The amount of polyphenolcarboxilic acids does not differ considerably between the two solvents (0.19±0.01 and 0.18±0.00UM, respectively).

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Manuscript received: January 12th 2012