

INVESTIGATION OF ANTIOXIDANT ACTIVITY (*IN VITRO*) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY PROFILING OF *PORTULACA OLERACEA* L. AND *PORTULACA GRANDIFLORA* HOOK. EXTRACTS

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ABSTRACT

Objective: The aim of this research was to investigate the antioxidant activity (AOA) of the methanolic extracts of different parts of *Portulaca oleracea* and *Portulaca grandiflora*.

Methods: The plant different parts were extracted with methanol. The methanolic extracts of both species were screened for 2,2-diphenyl-1-picrylhydrazyl radical scavenging abilities and researched by gas chromatography-mass spectrometry (GC-MS) analysis.

Results: GC-MS analysis of *P. oleracea* root extract revealed the presence of 39 biologically active compounds. 32 biologically active compounds were identified in *P. grandiflora* root extract. Both extracts revealed the AOA.

Conclusion: The results of the investigation could be useful for further pharmacological and phytochemical researches to assess the beneficial properties of both these species.

Keywords: *Portulaca oleracea* L., *Portulaca grandiflora* Hook., 2,2-Diphenyl-1-picrylhydrazyl method, Gas chromatography-mass spectrometry analysis.

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INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomena of symbiosis. In the western world, as the people are becoming aware of the potency and side effect of synthetic drugs, there is an increasing interest in the natural product remedies with a basic approach toward the nature.

Natural products isolated from various sources, especially derived from plants, have long been used in the treatment of human ailments. Herbal medicines are also in great demand in the developed world for primary health care due to their efficacy, safety, and lesser side effects.

A number of scientific investigations have highlighted the importance and the contribution of many plant families used as medicinal plants. Plants have a large unexplored range of compounds, which is almost impossible to imitate, they will always remain a potential source of future drug discovery.

Search for new natural sources of biologically active compounds to obtain effective and safe drugs is one of the important problems of modern pharmacognosy. Accordingly, the source of good and safe natural raw materials can be genus *Portulaca* L. In Ukraine [1], the genus *Portulaca* L. is represented by two species such as *Portulaca oleracea* L. (Fig. 1) and *Portulaca grandiflora* Hook. (Fig. 2).

P. oleracea and *P. grandiflora* have been used in folk medicine since ancient times. *P. oleracea* possesses a wide spectrum of pharmacological properties such as neuroprotective, antimicrobial, antidiabetic, anti-inflammatory, antiulcerogenic, and anticancer activities [2-6]. *P. grandiflora* Hook. is a succulent plant. In oriental traditional medicine, *P. grandiflora* is used for the relief of a sore throat, skin rash, and detoxification [7].

The analysis of pharmacological and phytochemical researches for underground parts of the plants of genus *Portulaca* in Ukraine were not found to be reported earlier.

The aim of this investigation is to determine antioxidant activity (AOA) and to identify phytochemical compounds of underground parts of *P. oleracea* and *P. grandiflora*.

METHODS

Collection of specimen

The plants of *P. oleracea* and *P. grandiflora* were collected from Tomakivka district, Dnipropetrovs'k region, Ukraine. The plants were identified taxonomically and authenticated. The plants were dried in the shade. The dried plant material was powdered to a good powder using grinder mixer. The experimental samples taken for investigation were: *P. oleracea* root (POR), *P. grandiflora* root (PGR), *P. oleracea* herb, and *P. grandiflora* herb.

Extraction of parts plants

The dried plant parts were powdered to a good powder using grinder mixer. The plant powders were extracted with methanol (in the ratio of 1-10) at room temperature for about 3 days. The obtained mixture was filtered.

Antioxidant analysis

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), free radical scavenging activity of methanolic extracts of the two plants, was determined using DPPH [8,9]. 0.5 ml of different methanolic tinctures was mixed with 2.5 ml of 0.1 mM DPPH methanolic solution, and it was incubated for 30 min at the temperature of 25°C and optical density (A_d) was measured at 517 nm by a spectrophotometer "ULAB 108UV" Ascorbic acid was used as the reference standard. Methanol was used as a blank. The experiment was carried out in triplicates. Weighing reagents and

powdered samples of plants were conducted on electronic scales "ANG 200C."

AOA was calculated by the next formula:

$$\text{AOA}(\%) = \frac{(A_{\text{DPPH}} - A_d)}{A_{\text{DPPH}}} \times 100 \%$$

In case of a negative meaning, AOA in percentage was estimated like 0.

Gas chromatography-mass spectrometry (GC-MS) analysis

The methanolic extracts of different parts of the plants were performed on gas chromatograph "Agilent 7890B" with mass spectrometer detector 5977B. The capillary column was DB-5 ms 30 m in length, 250 μm inner diameter, and 0.25 μm film thickness. Helium was used as carrier gas at a constant flow rate of 1.3 ml/min with an injection volume of 0.5 μl with an injector temperature of 265°C. The oven temperature was programmed from an initial temperature of 70°C (isothermal for 1 min), with an increase of 20°C/min to 150°C (isothermal for 1 min), and with an increase of 20°C/min to 270°C (isothermal for 4 min). The compounds were identified and authenticated using their MS data by comparison with those of the NIST14 mass spectral library.



Fig. 1: (a and b) *Portulaca oleracea*



Fig. 2: (a and b) *Portulaca grandiflora*

The chemicals methanol, ascorbic acid, and DPPH were purchased from Sigma-Aldrich, USA [10].

RESULTS AND DISCUSSION

The DPPH radical scavenging analysis is considered to be a good *in vitro* model widely used to assess antioxidant efficacy of various types of a single compounds as well as of different plant extracts within a very short time. Furthermore, the DPPH method is considered as valid, accurate, and easy. DPPH is a stable and nitrogen-centered organic free radical and has strong absorption at 517 nm in alcoholic solution. The decentralized of the electron has the ability to increase violet color as evidence of the AOA. When DPPH solution is mixed with any type of antioxidants that have the ability to grant a hydrogen atom, this mixture will have the ability to reduce violet color as a sign of resistance to oxidative stress efficiently.

From the investigation, it is evident that the methanolic root extracts of both species possess effective AOA which may be due to the presence of respective phytochemicals. These phytochemicals may prevent the formation of new free-radical species and convert existing free radicals into less harmful molecules.

The antioxidant activities of plant extracts were researched to evaluate the promising directions of further pharmacological screening. Previous studies have reported that the aboveground part of *P. oleracea* is a rich source of natural antioxidants [11,12]. Aqueous juice of *P. oleracea* was screened for its AOA in adult male Waster albino rats. *P. oleracea* was caused a significant increase in glutathione reductase, glutathione peroxidase, and glutathione-S-transferase [13]. It was researched that mature plants of *P. oleracea* had higher total phenolic content and antioxidant activities than plants at the immature stages [14].

It was studied that *P. oleracea* polysaccharides had antioxidant effects on the oxidative injury in N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric cancer rats [15]. No information has been published regarding the AOA of the underground part of *P. oleracea* and *P. grandiflora*.

It was established that PGR and POR extracts revealed the AOA (Fig. 3), which was comparable to the activity of reference standard (ascorbic acid). DPPH assay was done in dynamic. More expressed activity was characteristic for PGR extract, which inhibited the formation of free radicals at 89.3%. POR extract inhibited the formation of the free radicals at 86.5%. Plants of genus *Portulaca* could be useful for the prevention of cardiovascular, neurodegenerative, and other chronic diseases caused by oxidative stress.

DPPH Assay

It is famous, that herb, stems, leaves of genus *Portulaca* are a rich source of biologically active compounds, such as antioxidants and omega-3

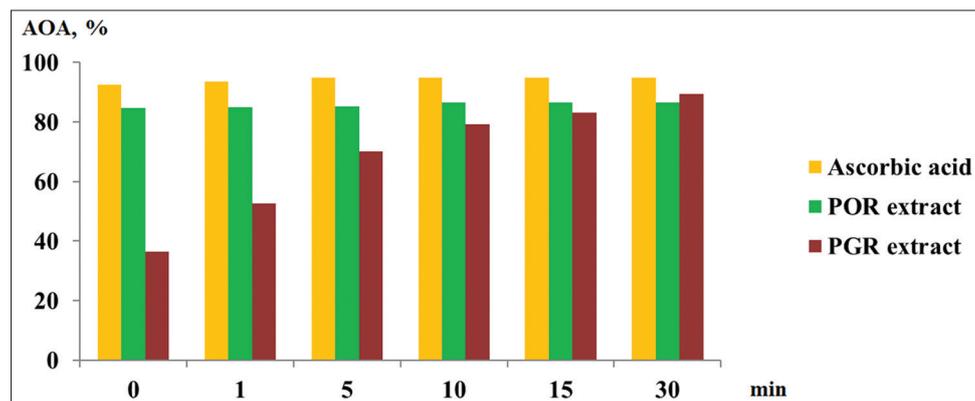


Fig. 3: Antioxidant activity data of plant extracts in dynamic (POR extract - *Portulaca oleracea* root extract; PGR extract - *Portulaca grandiflora* root extract; ascorbic acid is used as positive control. All the values were average of triplicates. The values were expressed in % \pm S.E.)

Table 1: Identified biological active compounds using GC-MS analysis of the POR extract

RT (min)	Molecular formula	Name
2.653	C ₆ H ₁₂ O ₃	3,3-Dimethoxy-2-butanone
2.722	C ₁₄ H ₂₈ O ₅	Butyl 2-(2-(2-butoxyethoxy) ethoxy) acetate
2.847	C ₈ H ₁₀	Ethylbenzene
2.93	C ₈ H ₁₀	Benzene, 1,3-dimethyl-
3.164		
3.458	C ₁₄ H ₁₉ N ₃ O ₃	Acetamide, 2-(1-piperidyl)-N-(2-methyl-5-nitrophenyl)-
3.637	C ₈ H ₁₀ O	1,5-Cyclooctadien-4-one
3.977	C ₅ H ₁₀ O ₅	DL-Arabinose
4.373	C ₁₄ H ₂₆ O ₂	2-Octen-1-ol, 3,7-dimethyl-, isobutyrate, (Z)-
5.021	C ₁₃ H ₁₀ F ₂ O	2,4-Difluorobenzene, 1-benzyloxy-
5.755	C ₆ H ₇ N ₃ O ₂	Imidazole, 2-amino-5-[(2-carboxy) vinyl]-
7.067	C ₁₀ H ₁₉ N	7-Azabicyclo[4.1.0]heptane, 2-methyl-5-(1-methylethyl)-
7.184	C ₁₀ H ₁₈ N ₂ O	N-[3-[NAziridyl] propylidene] tetrahydrofurfurylamine
8.528	C ₈ H ₇ N	m-Aminophenylacetylene
8.753	C ₉ H ₁₀ O ₂	Ethanone, 1-(2-hydroxy-5-methylphenyl)-
9.22	C ₈ H ₁₀ O ₃	Phenol, 2,6-dimethoxy-
9.901	C ₂₀ H ₂₈ O ₁₃	Vanillin lactoside
11.8	C ₈ H ₁₇ ClOSi	1-Chloromethyl-1-ethoxy-1-silacyclohexane
12.486	C ₉ H ₁₂ O ₂	2-Isopropoxyphenol
14.211	C ₁₂ H ₂₂ N ₂ O	1-Propyl-3,6-diazahomoadamantan-9-ol
14.408	C ₁₁ H ₁₈ N ₂ O ₂	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-
15.267	C ₁₇ H ₁₉ NO ₄	Papaveroline, 1,2,3,4-tetrahydro-3-O-methyl-
15.915	C ₁₇ H ₃₄ O ₂	Pentadecanoic acid, 13-methyl-, methyl ester
16.135	C ₁₅ H ₁₃ FO ₃	Benzaldehyde, 3-benzyloxy-2-fluoro-4-methoxy-
16.238	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid
16.544	C ₁₁ H ₁₄ O ₄	trans-Sinapyl alcohol
17.548	C ₁₇ H ₃₂ O	3-Heptadecyn-1-ol
17.784	C ₁₆ H ₂₀ O ₄	5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7ahexahydro-3,6-dimethyl-.alpha.-methylene-2-oxo-, methyl ester
17.886	C ₁₈ H ₃₂ O ₂	9,12-Octadecadienoic acid (Z, Z)-
17.936	C ₂₃ H ₃₈ O ₂	6,9,12,15-Docosatetraenoic acid, methyl ester
18.624	C ₂₀ H ₂₈ O ₆	1H-2,8a-Methanocyclopenta[a] cyclopropa[e] cyclodecen-11-one, 1a, 2,5,5a, 6,9,10,10a-octahydro-5,5a, 6-trihydroxy-1,4-bis (hydroxymethyl)-1,7,9-trimethyl-, [1S-(1.alpha., 1a.alpha., 2.alpha., 5.beta., 5a.beta., 6.beta., 8a.alpha., 9.alpha., 10a.alpha.)]-
18.936	C ₂₃ H ₃₂ O	2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1-en-1-carboxaldehyde
19.246	C ₂₀ H ₂₇ NO	.alpha.-N-Normethadol
20.977	C ₁₇ H ₂₄ O ₅	Pyrano[4,3-b] benzopyran-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-
21.115	C ₁₉ H ₃₈ O ₄	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester
22.423	C ₂₉ H ₅₀ O	.gamma.-Sitosterol
22.767	C ₂₁ H ₃₈ O ₄	9,12-Octadecadienoic acid (Z, Z)-, 2-hydroxy-1-(hydroxymethyl) ethyl ester
22.845	C ₂₀ H ₃₆ O ₂	Linoleic acid ethyl ester
13.832	C ₇ H ₁₄ O ₆	alpha.-Methyl mannopyranoside

GCMS: Gas chromatography-mass spectrometry, POR: *Portulaca oleracea* root

fatty acids. The phytochemical content of both extracts was screened by GC-MS method. The identification of phytochemical compounds was based on the retention time, molecular formula, and peak area. GC-MS analysis of POR extract revealed the presence of 39 biologically active compounds. The methanolic extract of *P. oleracea* was found alpha-N-normethadol, DL-arabinose, methyl esters of fatty acids pentadecanoic acid, n-hexadecanoic acid, 9,12-octadecadienoic acid, ethyl esters linoleic acid, vanillin lactoside, gamma-sitosterol, and alpha-methyl mannopyranoside.

The GC-MS data obtained for the POR extract of various bioactive compounds are presented in Table 1.

Thirty-two biologically active compounds were identified in PGR extract. The GC-MS analysis of the compounds carried out in PGR extract

is shown in Table 2. The methanolic extract of *P. grandiflora* was found oleic acid, valeric acid, methyl esters of fatty acids n-hexadecanoic acid, vanillin lactoside, and gamma-sitosterol.

CONCLUSION

It was established that PGR and POR extracts revealed the AOA.

PGR and POR GC-MS analysis was not published earlier.

The results of the GC-MS analysis of extracts of underground parts of *P. oleracea* and *P. grandiflora* revealed the presence above 30 biologically active compounds such as polyunsaturated fatty acids, terpenoids, and phytosterols. Many new compounds were identified in the current investigation, which needs to be extensively studied.

Table 2: Identified biological active compounds using GC-MS analysis of the PGR extract

RT (min)	Molecular formula	Name
2.662	C ₆ H ₁₂ O ₃	3,3-Dimethoxy-2-butanone
2.734	C ₆ H ₁₂ O ₃	1,3-Dioxolane-4-methanol, 2-ethyl-
2.849	C ₈ H ₁₀	Ethylbenzene
2.923	C ₈ H ₁₀	Benzene, 1,3-dimethyl-
3.172		
4.357	C ₁₀ H ₂₂	Decane
4.855	C ₇ H ₈ O	Benzyl alcohol
5.242	CH ₄ N ₂ O	Urea
5.369	C ₁₁ H ₂₁ ClO ₂	Valeric acid, 6-chlorohexyl ester
5.964	C ₈ H ₁₀ O	Phenylethyl alcohol
6.163	C ₁₇ H ₂₇ N ₅ O ₃	Pyrimidine-2,4,6-trione, 1-cyclohexyl-5-[(2-piperazin-1-yl-ethylamino) methylene]-
7.171	C ₁₂ H ₂₆	Dodecane
8.747	C ₉ H ₁₀ O ₂	Ethanone, 1-(2-hydroxy-5-methylphenyl)-
9.22	C ₈ H ₁₀ O ₃	Phenol, 2,6-dimethoxy-
9.887	C ₂₀ H ₂₈ O ₁₃	Vanillin lactoside
12.393	C ₁₀ H ₁₆ O ₃	1-(5-Methoxy-4,4-dimethyl-dihydro-furan-2-ylidene)-propan-2-one
12.48	C ₉ H ₁₂ O ₂	Phenol, 4-propoxy-
13.374	C ₇ H ₁₄ O ₆	Myo-inositol, 4-C-methyl-
14.211	C ₁₂ H ₂₂ N ₂ O	1-Propyl-3,6-diazahomoadamantan-9-ol
14.408	C ₁₁ H ₁₈ N ₂ O ₂	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butyryl]-
14.524	C ₁₃ H ₁₈ O ₃	2-Cyclohexen-1-one, 4-hydroxy-3,5,5-trimethyl-4-(3-oxo-1-butenyl)-
16.237	C ₁₆ H ₃₂ O ₂	n-Hexadecanoic acid
16.544	C ₁₁ H ₁₄ O ₄	trans-Sinapyl alcohol
17.945	C ₁₈ H ₃₄ O ₂	Oleic acid
20.875	C ₂₉ H ₃₄ N ₂ O ₃	Benzoic acid, 4-(4-butylcyclohexyl)-, 4-butoxy-2,3-dicyanophenyl ester
21.083	C ₂₁ H ₃₁ NO ₃	Phenanthrene-1-acetaldehyde, 7-acetoxy-2.beta.-cyano-2,4b-dimethyl-
21.735	C ₁₆ H ₁₈ N ₈ S ₂	Benzene, 1,3-bis (1-allyl-5-tetrazolythiomethyl)-
21.806	C ₂₈ H ₄₈ O	Cholest-14-en-3-ol, 4-methyl-, (3. beta., 4. alpha., 5. alpha.)-
21.957	C ₂₀ H ₂₈ O ₆	1H-2,8a-Methanocyclopenta[a] cyclopropa[e] cyclododec-11-one, 1a, 2,5,5a, 6,9,10,10a-octahydro-5,5a, 6-trihydroxy-1,4-bis (hydroxymethyl)-1,7,9-trimethyl-, [1S-(1. alpha., 1a. alpha., 2. alpha., 5. beta., 5a. beta., 6. beta., 8a. alpha., 9. alpha., 10a. alpha.)]-
23.265		Pyrano[4,3-b] benzopyran-1,9-dione, 5a-methoxy-9a-methyl-3-(1-propenyl) perhydro-Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester
20.977	C ₁₇ H ₂₄ O ₅	Gamma.-sitosterol
21.115	C ₁₉ H ₃₈ O ₄	Chlordiazepoxide
22.425	C ₂₉ H ₅₀ O	
23.633	C ₁₆ H ₁₄ ClN ₃ O	

GC-MS: Gas chromatography-mass spectrometry, PGR: *Portulaca grandiflora* root

Thus, *P. oleracea* and *P. grandiflora* are promising medicinal plants with beneficial biological active compounds and require further pharmacological and phytochemical investigations.

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AUTHORS' CONTRIBUTIONS

This work was done by the author named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the author. The review of literature, collection of plant material, preparation of extracts, data collection and analysis, and drafting of the manuscript were done by Ms. Kinichenko AO.

CONFLICTS OF INTEREST

The author has no conflicts of interest in this study.

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