

INVESTIGATION OF THE ACTIVE CONSTITUENTS OF *PORTULACA OLERACEAE* L. (PORTULACACEAE) GROWING IN JORDAN

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ABSTRACT

The phytochemical analysis of the fresh aerial parts of *Portulaca oleracea* (Portulacaceae), growing in Jordan, using conventional chromatographic procedures resulted in the isolation of β -sitosterol, β -sitosterol-glucoside, N,N'- dicyclohexylurea, and allantoin. The last three compounds were isolated for the first time from this plant. The structure elucidation of these compounds was attained by the use of spectral data (UV, IR, MS, ¹H-, ¹³C- and 2D-NMR), X-ray crystallography and by comparison with authentic samples.

INTRODUCTION

Portulaca oleracea L., a member of family Portulacaceae, is a warm climate, annual, green herb, with branched and succulent stems which are decumbent near the base and ascending near the top to a height of 15-30 cm. The plant is fleshy, stout and succulent (water content of over 90%), with obovate to spatulate, obtuse opposite leaves tapering towards the base. The flowers are small, yellow, and sessile in clusters of 3-5 on the forks and tips of the branches, opening in the morning only. The fruit is oblong and transversely dehiscent. The seeds are orbicular and 0.5 mm in diameter (Hussein, 1985; Mitich, 1997; Mossa *et al.*, 1987 and Feinbrun-Dothan & Darin, 1991). It has a cosmopolitan distribution in Africa, China, India, Australia, Middle East, Europe and United States (Chan *et al.*, 2000; Oran & Al-Eisawi, 1998 and Mitich, 1997).

Several reports in the literature claim that *P. oleracea* contains several biologically active compounds and it is a source of many nutrients. Some of the reported biologically active compounds include organic acids (free oxalic acids in traces only, cinnamic acids, caffeic acid, malic acids and citric acids), alkaloids, coumarins, flavonoids, cardiac glycosides, anthraquinone glycosides, alanine, catechol, saponins and tannins. *P. oleracea* reported to contain also other chemical constituents, including urea, calcium, iron, phosphorous, manganese, copper and fatty acids, especially omega-3-acids whose concentration in *P. oleracea* is the highest found in leafy vegetables (Ezekwe *et al.*, 1999; Garti *et al.*, 1999; Hussein, 1985; Mohamed & Hussein, 1994 and Simopoulos *et al.*, 1992). Furthermore, the occurrence of glutathione; glutamic acid; and aspartic acid has been published by Simopoulos *et al.* (1992). The seeds contain 17.4% of a fixed oil containing β -sitosterol (Rizk, 1986). Moreover, the whole plant contains large amounts of *l*-norepinephrine (0.25% in fresh herb), soluble carbohydrates, fructose/fructane, vitamins, A, B₁, B₂ and it is rich in ascorbic acid (Garti *et al.*, 1999a). The leaves contain 0.42% mucilage, which is composed of an acidic and a neutral fraction. The acidic fraction consists of galacturonic acid residues joined by α -(1 \rightarrow 4) linkages. The neutral fraction is composed of 41% of arabinose and

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43% of galactose residues, besides traces of rhamnose residue (Mohamed & Hussein, 1994 and Rizk, 1986).

Imperato (1975) studied the acylated betacyanins of *P. oleracea* that grow in Japan and isolated two red-violet pigments (Olecacin I and II). He found that Olecacin I converted upon treatment with aqueous citric acid to Olecacin II, the diastereoisomer of Olecacin I, and both appear as mixture. Upon alkali treatment, this mixture yielded ferulic acid and two new diastereoisomeric pigments (DO1 and DO2), which were separated by chromatography on polyamide. DO1 was identified as betanidin 5-0-cellobioside and DO2 was isobetanidin 5-0-cellobioside. Hydrolyzes of the mixture of DO1 and DO2 with 10% acetic acid (3-5 hr. under reflux) gave 2 sugars, glucose and cellobioside. Sakai *et al.* (1996) isolated from the methanolic extract of *P. oleracea*, collected in Japan, a monoterpene glucoside, Portuloside A. The structure of Portuloside A was established by spectroscopic methods and found to be (3S)-3-(3,7-dimethylocta-1,7-dien-6-yl)- β -D-glucopyranoside, which was confirmed by synthesizing it from linalool. Portuloside A exhibited no antimicrobial activity.

The fatty acid and β -carotene contents of the Australian varieties of *P. oleracea* were determined by gas chromatography (GC) and high performance liquid chromatography (HPLC). The fatty acid content ranged from 1.5 to 2.5 mg/g of fresh mass in leaves, 0.6 to 0.9 mg/g in stems and 80 to 170 mg/g in seeds. The β -carotene content ranged from 22-to-30 mg/g fresh mass in leaves. Longer-chain omega-3 fatty acids were not detected (Liu *et al.*, 2000). α -Linolenic acid (C_{18:3} ω ₃) accounted for around 60% and 40% of the total fatty acid content in leaves and seeds, respectively. Based on these studies the uniqueness of purslane as the "richest vegetable source" of omega-3 fatty acids and protein compared to other vegetables has been concluded (Ezekwe *et al.*, 1999). A water-soluble anionic, low molecular weight polysaccharide (gum) with surface, interfacial, and emulsification properties was extracted from leaves of *P. oleracea* and named P. oleracea gum (POG). POG is regarded as a good example of a new gum that can be considered as a food emulsifier (Garti *et al.*, 1999a, b).

There are two species of the genus *Portulaca*, namely *P. oleracea* and *P. afra* found in Jordan. *P. oleracea*, an edible plant, is widely distributed, especially in Deir 'Alla, northern Ghor, Zarka and in Sail Tawaheen Adwan. The second species is also found in the same region as *P. oleracea* (Al-Eisawi, 1982). People in Jordan use *P. oleracea* as food either raw in salad or cooked as a vegetable dish. As nothing was found concerning the identification of chemical constituents present in *Portulaca oleracea*, the aim of this study was to isolate and identify the major active compounds, which to our knowledge, has not been previously studied in Jordan based on the literature survey. However, Rashed (2002) studied the wound healing activity of *P. oleracea*, growing in Jordan, on white Swiss mice skin excision wounds and found that the fresh homogenized crude extract of *P. oleracea* has accelerated the wound healing process.

EXPERIMENTAL

General Experimental Procedure:

1 Chromatographic materials

Thin Layer Chromatography Analytical pre-coated silica gel TLC sheets for chromatography, 20 × 20 cm, 25 sheets, ALUGRAM[®] SIL G/ UV₂₅₄, Germany. Silica gel 60 particle size 0.06-0.20 mm, 70-230 mesh (Scharlau, Chemie, Spain) was used for column chromatography.

2 Chemicals

All chemicals used were analytical grade.

3 Instruments

Melting points were determined on STUART (SMP1) scientific apparatus, U.K. Spectroline® model CX-20, ultraviolet, fluorescence analysis cabinet (long wave UV 365 nm and short wave UV 254 nm), U.S.A. UV spectra were measured using UV- visible spectrophotometer DMS 80, Carry, Varian, Australia. IR spectra were recorded using Infrared spectrophotometer, Impact 400, Nicolet, U.S.A. MS spectra were obtained using mass spectroscopy; MS model VG 7070 E, from VG analytical using EI. ¹H-, ¹³C- and 2D-NMR spectra were obtained using Nuclear magnetic resonance spectrometer, Bruker, Avance, DPX 360 and 500 MHz, University of Hamburg, Germany and King's College, London. Spectra were recorded using tetramethylsilane as internal standard. HMQC and DEPT spectra were generated.

Plant Material

Aerial parts of *P. oleracea* L., growing in Jordan, were purchased from the local market in Amman in July 2000. The plant was identified botanically using descriptive references (Hussein, 1985; Chaudhary and Zawawi, 1983; Mossa *et al.*, 1987) and authenticated in comparison with the herbarium specimen of the Faculty of Science, University of Jordan. A voucher specimen has been deposited in the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan (Herbarium No. 1 PORT-FMJ).

Extract Preparation

Twenty kilograms of fresh aerial parts of *P. oleracea* L. grown in Jordan were cut into small pieces and macerated in 96% EtOH at room temperature for four weeks. This process was repeated twice. Then the solvent was evaporated under reduced pressure in a rotary evaporator at temperature 40°C to yield 278 g of crude extract. The obtained crude extract was fractionated against petroleum ether, chloroform, ethylacetate, butanol and methanol. Methanol fraction was subjected to repeated column chromatography and eluted using chloroform and methanol as mobile phases to obtain different compounds.

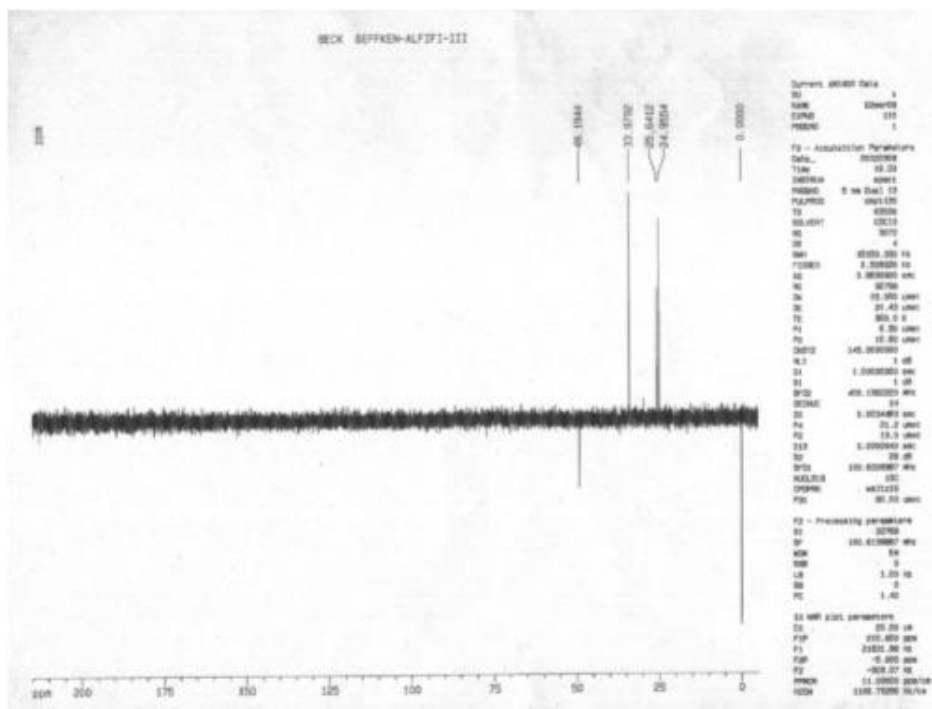
RESULTS AND DISCUSSION

The present study dealt with the investigation of the alcoholic extract of the aerial parts of *P. oleracea* growing in Jordan. Allantoin, N,N'-dicyclohexylurea, β -sitosterol and β -sitosterylglucoside were isolated. The chemical identity and the structural elucidation of these compounds were obtained based on their spectroscopical analysis.

Compound POM-5: Colorless crystals obtained from MeOH fraction were identified as allantoin based on the spectroscopical data. The ¹H-NMR spectrum of POM-5 (Table 1) indicated the presence of six protons in the region δ 5.30-10.50 ppm. The signals for H-3 and H-6 appeared as doublets at δ 6.9 ppm ($J = 8.1$ Hz) and at δ 5.3 ppm ($J = 8.1$ Hz), respectively. The remaining protons, appeared as singlets at δ 10.5, 8.1 and 5.8 ppm, were assigned to H-1, H-4 and H-8, respectively. The stereochemistry of the chiral center at C₄ indicated the appearance of H-4 as singlet, while H-3 and H-6 as doublets. The ¹³C-NMR spectrum showed only four carbons present in this substance and indicated the presence of three carbonyl groups (Table 2). The two carbonyl carbons C₂ and C₇ having similar environment appeared very close to each other at δ 157.1 and δ 157.6 ppm, respectively, while C₅ less deshielded found at δ 174.0 ppm. The remaining carbon C₄ appeared at δ 62.7 ppm. The IR stretching bands [NH₂-(3439, 3343, 1603 cm⁻¹), NH-(3226, 3062, 1532 cm⁻¹) and CO-(1781, 1716, 1655 cm⁻¹)], MS fragmentation pattern [m/z 158 [M⁺ 9%],

corresponding to the molecular formula ($C_4H_6N_4O_3$) and further peaks at m/z 141 $[M-NH_3]^+$ (12%) and m/z 130 $[M-CO]^+$ (100%), 115 $[M-CONH]^+$ (39%), 114 $[M-CONH_2]^+$ (19%), 60 $[M-N_2H_4CO]^+$ (20%), UV spectrum (MeOH) λ_{max} 226 and 230 nm and m.p (230-232°C) POM-5 supported the suggested structure of POM-5 as allantoin. Its structure which was further confirmed by X-ray crystallography. Single-crystal diffraction using three-dimensional photographic data was identical to those reported for allantoin (Mootz, 1965). The occurrence of allantoin was not reported in *P. oleracea* before this study.

Compounds POMW-I, POMW-II and POMW-III: These three substances obtained from MeOH/H₂O fraction were identified as N,N'-dicyclohexylurea (m.p 229-232°C) based on spectral data (D'agostino *et al.*, 1987; Tasi *et al.*, 1997). These UV active compounds ((CHCl₃) λ_{max} 215, 240 and 323 nm; 256 nm) with $R_f = 0.45$ (solvent system CHCl₃: MeOH (8:2)) gave positive reaction with MeOH: H₂SO₄ (1:1) upon spraying of the TLC. The IR stretching bands at 3326 cm⁻¹ and at 1626 cm⁻¹ indicated the presence of an NH and NHCO groups respectively. In EIMS spectrum the molecular ion appeared at m/z 224 (M⁺ 21%) corresponding to the molecular formula (C₁₃H₂₄N₂O) and further peaks at m/z 143 $[M-C_6H_{10}]^+$ (20%), 99 $[M-C_7H_{12}O]^+$ (34%), 70 $[M-C_8H_{14}N_2O]^+$ (16%), 56 $[M-C_9H_{16}N_2O]^+$ (100%) and 28 [CO] (92%) supported the suggested structure. The ¹H- and ¹³C assignments are reported in tables 1 and 2. The ¹³C-NMR signals at δ 24.9 and 33.9 ppm were attributed to methylene (CH₂) groups in cyclohexane (C_{3,5,3',5'}) and (C_{2,6,2',6'}), respectively. Signal for CH groups (C_{1,1'}) appeared at δ 49.1 ppm, while the signal of the carbonyl group (C₇) appeared at δ 157.7 ppm. The remaining signal at δ 25.6 ppm corresponded to CH₂ groups at C_{4,4'}. The assignment of ¹H- and ¹³C-NMR signals has been further verified using DEPT (135) and 2D-NMR (HMQC) (Fig. 1 and 2). This is the first report of N, N'-dicyclohexylurea being isolated from the fresh aerial parts of *P. oleracea* and the second time from natural sources. Tasi *et al.* (1997) isolated this compound for the first time from natural sources, namely from the root wood of Formosan *Toddalia asiatica* (Rutaceae).



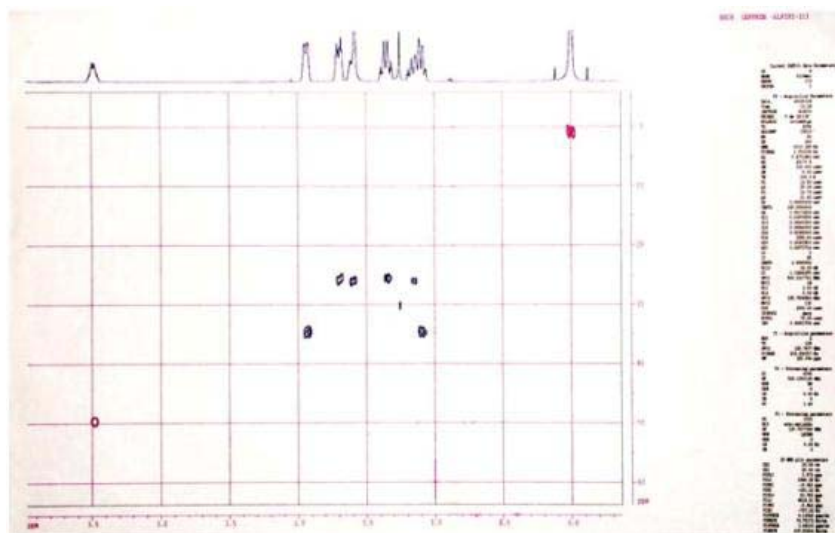
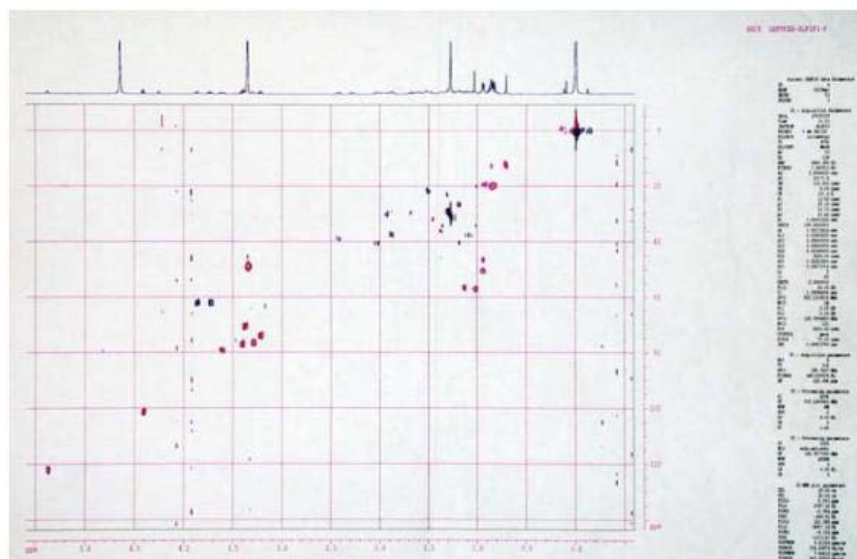


Fig. 2: 2D- NMR (HMQC) of N,N'- dicyclohexylurea.

Fig. 3: 2D- NMR (HMQC) of β - sitosterol glucoside.

Compounds POB-1 and OB-3: TLC analysis of these two white substances using vanillin/H₂SO₄ as spraying reagent, suggested that the isolated compounds were terpenoids. Spectral analysis revealed the identity of the two compounds to be β -Sitosterol glucoside (m.p. 272-276°C), which was confirmed by comparing both, the obtained and published data. The obtained IR, MS, ¹H-NMR and ¹³C-NMR data were in full agreement with the published data of β -sitosterol glucoside (Aquino *et al.*, 1998; Hamed, 1996; Kusano *et al.*, 1973; Misra and Tiwari, 1973). The NMR data were further confirmed by 2D-NMR (HMQC) (Fig. 3).

Compound POB-2, obtained from the butanol fraction was identified as β -Sitosterol. The identification of this compound was based on the comparison of the spectral data (MS m/z 414; $C_{29}H_{50}O$), melting point (134-136°C) and mixed melting point (135-137°C) of both, the isolated compound and the reference substance with the published data (Tasi *et al.*, 1997).

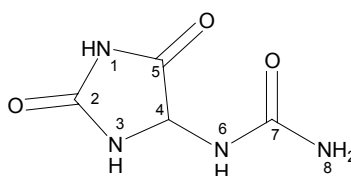
In the present study 1H -NMR and ^{13}C -NMR shifts were calculated for all the isolated compounds using Internet Chemical Software, Ultraversion 6, Drawing Modeling and Information Program (Cambridge Soft Corporation) for further conformation of the structures of the isolated compounds.

Table 1
 1H -NMR spectral data of allantoin (δ values in DMSO) and
N,N'-dicyclohexylurea (δ values in $CHCl_3$)

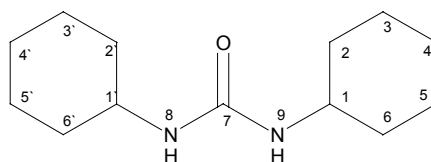
| Compound | ppm | Integration | Multiplicity | J (Hz) | Inference |
|---------------------------|------|-------------|---------------|--------|--------------------------|
| Allantoin | 6.9 | 1H | Doublet | 8.1 | H-3 |
| | 10.5 | 1H | Singlet | - | H-1 |
| | 8.1 | 1H | Singlet | - | H-4 |
| | 5.3 | 1H | Doublet | 8.1 | H-6 |
| | 5.8 | 2H | Singlet | - | H-8 |
| N,N'- dicyclohexylurea | 3.5 | 2H | Multiplet | - | H-1, 1' |
| | 1.9 | 4H | Multiplet | - | H-2, 6, 2', 6' |
| | 1.7 | 4H | Multiplet | - | H-2, 6, 2', 6' |
| | 1.6 | 2H | Multiplet | - | H-4, 4' |
| | 1.3 | 4H | Multiplet | - | H-3, 5, 3', 5' |
| | 1.1 | 6H | Multiplet | - | H-3, 4, 5, 3', 4', 5' |
| | 4.0 | 2H | Broad-doublet | 7.2 | H-8, 9 |

Table 2
 ^{13}C -NMR spectral data of allantoin (δ values in DMSO) and
 N,N'-dicyclohexylurea (δ values in CHCl_3)

| Compound | ppm | Integration | Inference |
|-----------------------|-------|-----------------|------------------------|
| Allantoin | 157.1 | CO | C_2 |
| | 174.0 | CO | C_5 |
| | 62.7 | CH | C_4 |
| | 157.6 | CO | C_7 |
| N,N'-dicyclohexylurea | 24.9 | 4 CH_2 | $\text{C}_{3,5,3',5'}$ |
| | 25.6 | 2 CH_2 | $\text{C}_{4,4'}$ |
| | 33.9 | 4 CH_2 | $\text{C}_{2,6,2',6'}$ |
| | 49.1 | 2 CH | $\text{C}_{1,1'}$ |
| | 157.7 | CO | C_7 |



Allantoin



N,N'-dicyclohexylurea

ACKNOWLEDGEMENTS

The authors are grateful to Dr. A. Kennedy, University of Strathclyde, Dr. Mire Zloh, University of London, Dr. Amala Raman, King's College, Prof. Dr. D. Geffken, University of Hamburg and Dr. Yaser Al-Haj, Faculty of Science, Yarmouk University for their help in running X-ray Crystallography, Mass Spectra, ¹H- and ¹³C-NMR.

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