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Isolation and identification of new alkaloids from purslane (*portulacaoleracea l.*) leaves using HPLC/ESI-MS

Abstract

Purslane (*Portulacaoleracea L.*) leaves are well-known traditional Kurdish favorite foods. In the present study, from Purslane leaves ten alkaloids were extracted, isolated and Identified for the first time, By using HPLC/ESI-MS. The identified alkaloids were; Nandigerine(1), Reticuline(2), (R)-3,4-Dehydromagnocurarine(3), itingensine(4), (S)-Tembetarine(5), Homolycorine(6), Angustureine (7), Cyclobullatine-A (8), 10-(5-p-coumaroyl-6-methylpiperidin-2-yl) dodecan-2-one(9) and 10-(5-p-coumaroyl-6-methylpiperidin-2-yl)tetradecan-2-one(10).

Keywords: purslane, leaves of purslane, alkaloids, HPLC/ESI-MS

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Introduction

Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants and animals. Many alkaloids often have pharmacological effects and are used as medications, as recreational drugs or in Entheogenic rituals. Over 10000 alkaloids have been isolated from nature so far, which are distributed widely in plants.[1] Many studies demonstrate that alkaloids had many kinds of biological activities, such as anti-microbial, anti-oxidants, anti-cancer, anti-inflammatory, and anti-virus activities.[2,4]

The Purslane (*Portulacaoleracea L.)* is listed in the World Health Organization (WHO) as one of the most used medicinal plants under the family *Portulacaceae*.[5–6] The genus Portulacao comprising about 70species is characterized by conspicuously fleshy sessile leaves.[7] Many varieties of Purslane under many names grow in a wide range of climates and regions, it can be found in Europe, Africa, North America, Australia and Asia.

Purslane has been ranked as eight Most common plants in the world and is widespread as a weed, fast growing, self-compatible and has amazing ability to produce seeds even on death's doorstep.[8,9] There are many types of Purslane available in Kurdistan and mostly still morphologically under investigation. Different varieties, harvesting times and environmental conditions can contribute to purslane's nutritional composition and benefits.[10] The present study focused on isolation and identification of alkaloids from Purslane leaves.[1] Solvent extraction will be used to extract the alkaloids,[11] and then the extract will be ejected into HPLC/ESI-MS.

Materials and methods

Plant material

Purslane Leaves were collected in Rania - Kurdistan Region in northern of Iraq, in April 2015. The specimen taxonomically was identified in the Biology Department at University of Raparin. Fresh leaves were dried in open air without exposure to direct sunlight. The dried sample was powdered and weighed.

Chemicals and reagents

All chemicals and solvents used were in HPLC grade form Sigma-Aldrich (UK).

Extraction and isolation of alkaloids: Dried powdered Purslane leaves (1000g) immersed in glacial acetic acid (1000ml) at room temperature for overnight. The extract was filtered to remove any residue from the solution. Then, the extract was diluted with de-ionized water. The extract fractionated with 1000 ml chloroform in a separatory funnel (2×1000ml). The chloroform extracts were combined and washed with 10% aqueous sodium bicarbonate. The chloroform extract was dried over anhydrous Sodium Sulfate then the solvents were evaporated on a rotary evaporator with water bath, temperature set up at 45°C.

Instrumentation HPLC/ESI-MS: The chloroform extract (100 mg) dissolved in 10ml of methanol and filtered through micro-filter (40µl), then ejected into HPLC/ESI-MS. The samples were analyzed using HPLC/ESI-MS (Aligent 1100 series). The system was controlled with Chemstation for LC 3D Rev A.09.03. The chromatographic separations were performed on the column (Spherisil ODS2 C18, 25x4.6m, 5um, Waters) to separate alkaloids. The column temperature was 25°C. The solvent system was used a gradient of methanol (A) and water (B), running gradient was 90:10%. The flow rate was 1ml/min of solvents for 20minutes and the injection volume of samples and standards was 20µl. The detection of the system was combined with diode array detector (DAD). The chromatography analysis was developed to obtain a short retention time of analysis with good resolution. All the chromatograms were recorded at 254nm for aromatic system, 280nm for cinnamic acids and 330nm for poly phenolics. For identification the individual alkaloids, the MS analysis was carried out with an ESI interface operating the positive ion mode [M^+H^+]. The MS operating conditions were as follows: Ion spray voltage, 1.6eV; curtain gas (N_2), 20 Psi;nebulizing gas and heating gas (N_2), 50 Psi; heating gas temperature, 550°C; SQ detector; spectra range, m/z 200-900 (scan time, 4.8sec).The system was controlled with Xcalibur software, version 1.2.

Results and discussion

The Purslane leaves extract was dissolved in methanol then ejected into HPLC/ESI-MS. Overall 10 alkaloids were analyzed, the HPLC chromatogram shown in Figure 1. The HPLC fitted with ESI-MS system to provide molecular mass and further structural information of all peaks in the HPLC chromatogram. In the Purslane leaves, the presence of the following alkaloids were confirmed; Nandigerine (peak no.1), Reticuline (2) Nandigerine (3) Gitingensine (4) (*S*)-Tembetarine (5) Homolycorine (6) Angustureine (7) Cyclobullatine-A (8)10-(5-p-coumaroyl-6-methylpiperidin-2-yl) dodecan-2-one(9) 10-(5-p-coumaroyl-6-methylpiperidin-2-yl)tetradecan-2-one (10) (Table 1).

From the MS spectrum and by comparing with previous reports the identification of the alkaloids were confirmed.[12,13] In the mass spectrum (Figure 2), showed a protonated ion at m/z 312, which is match to Nandigerine (1) in the chromatogram, (the chemical structure are corresponded with numbers in Figure 3. In the MS/MS spectrum, $[M+H^+]$ ion of Nandigerine observed two fragment ions at 280 and 295m/z, protonated Nandigerine previously was reported with the same condition.[14] Also, the fragmentation behaviors of protonated Reticuline (2), (R)-3,4-Dehydromagnocurarine (3) and Gitingensine (4) were found and published with the same condition.[15–17]

The last 6 alkaloids were reported or tentatively characterized in other species.[18–23] But as far as authors concerned this first time of reporting those alkaloids in Purslane leaves. Alkaloids in peaks 5,6,7,8,9 and 10 assigned as (*S*)-Tembetarine, Homolycorine, Angustureine, Cyclobullatine-A, 10-(5-p-coumaroyl-6-methylpiperidin-2-yl) dodecan-2-one and 10-(5-p-coumaroyl-6-methylpiperidin-2-yl) tetradecan-2-one respectively. The alkaloids structures were tentatively identified based on their MS fragmentation behavior, as shown in Table 1.

Table 1 Retention time and ESI/MS fragmentation of Alkaloids identified in Purslane leaves

Peak number	Retention time (Minutes)	Proposed compounds	$[M+H]^+$ (m/z)	Fragment ion (m/z)
1	0.05	Nandigerine	312	295,280
2	0.117	Reticuline	330	299,213
3	3.377	(R)-3,4-Dehydromagnocurarine	312	297,285,
4	4.553	Gitingensine	329	236,221
5	7.066	(S)-Tembetarine	344	299,250
6	8.531	Homolycorine	316	302,298
7	10.764	Angustureine	217	217,202
8	12.11	Cyclobullatine-A	414	338,324
9	14.184	10-(5-p-coumaroyl-6-methylpiperidin-2-yl) dodecan-2-one	441	298,280
10	19.187	10-(5-p-coumaroyl-6-methylpiperidin-2-yl) tetradecan-2-one	472	326,308

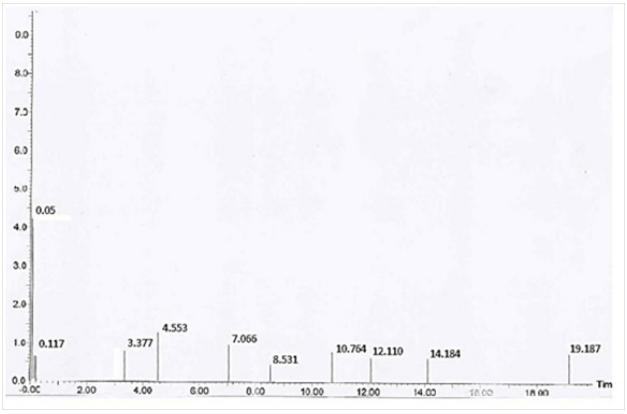

Figure 1 HPLC Chromatogram of alkaloids extract from Purslane leaves, each peak refer to a number of an alkaloids listed in Table 1: Nandigerine (1), Reticuline (2), Nandigerine (3), Gitingensine (4), (S)-Tembetarine (5), Homolycorine (6), Angustureine (7), Cyclobullatine-A (8), 10-(5-p-coumaroyl-6-methylpiperidin-2-yl) dodecan-2-one (9) and 10-(5-p-coumaroyl-6-methylpiperidin-2-yl)tetradecan-2-one (10).

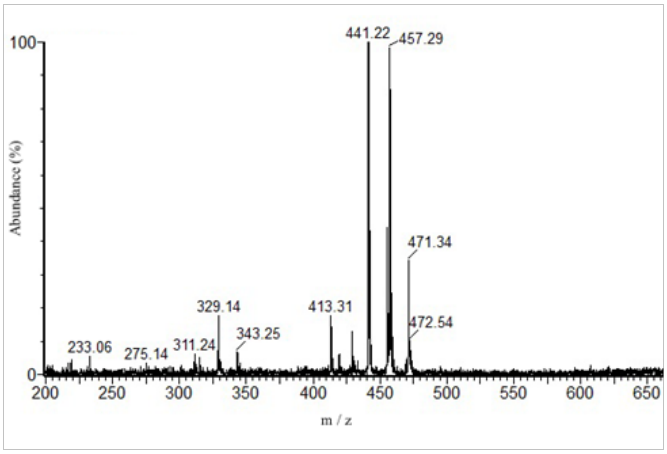

Figure 2 HPLC/ESI-MS spectrum of alkaloid extracts from Purslane leaves.

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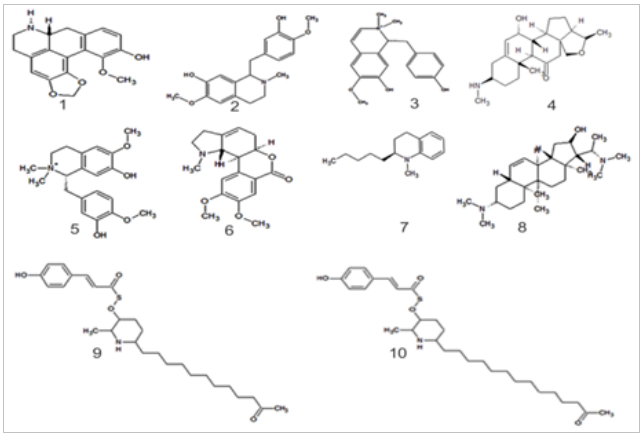

Figure 3 Chemical structures of alkaloids from Purslane leaves, listed in Table 1.

Conclusion

Leaves of Purslane (*Portulacaoleracea L.)* were extracted with glacial Acetic Acid and fractionated with Chloroform to extract alkaloids. Then, the extract ejected into HPLC/ESI-MS to analysis and confirm the chemical structure of alkaloids. In Purslane leaves, the following alkaloids were identified; which are; Nandigerine, Reticuline, (*R*)-3,4-Dehydromagnocurarine, Gitingensine, (*S*)-Tembetarine, Homolycorine, Angustureine, Cyclobullatine-A, 10-(5-p-coumaroyl-6-methylpiperidin-2-yl) dodecan-2-one and 10-(5-p-coumaroyl-6-methylpiperidin-2-yl)tetradecan-2-one.

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Conflict of interest

The author declares no conflict of interest.

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