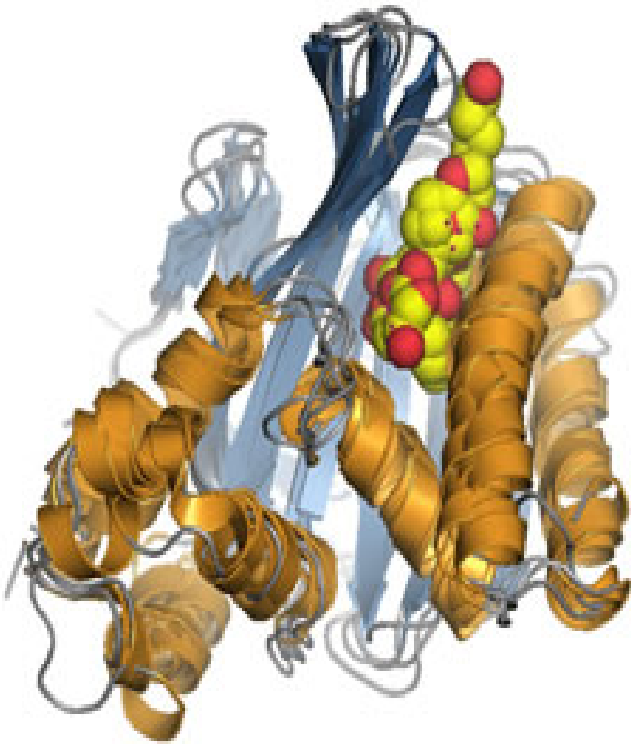
PhytoChem & BioSub Journal

Peer-reviewed research journal on Phytochemistry & Bioactives Substances

ISSN 2170 - 1768



PCBS Journal

Volume 8 N° 1, 2 & 3 2014

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ISSN 2170 - 1768

PCBS Journal

PCBS Journal

Volume 8 N° 1 2014

Edition LPSO

Phytochemistry & Organic Synthesis Laboratory

http://www.pcbsj.webs.com , Email: phytochem07@yahoo.fr

PhytoChem & BioSub Journal Vol. 8(1) 2014
ISSN 2170-1768
CAS-CODEN:PBJHB3

PCBS Journal
ISSN 2170-1768

PhytoChem & BioSub Journal

2014
Vol. 8 No. 1

Development of extraction and analysis of polyphenols of *Mesembryanthemum edule L.*

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DOI:10.163.pcbsj/2014.8.1.2

Abstract. *Mesembryanthemum edule (Aizoaceae family) is an edible halophyte widely used as a food ingredient and in traditional medicine. This study was intended to characterize the phenolic compounds of M. edule fresh and stone stems. The approach consisted to establish the phenolic composition through LC-DAD-ESI/MS consisted of an Acquity UPLC (Waters, Milford, MA) coupled with DAD and a Brucker Daltonics Ion trap mass spectrophotometer. The UPLC analysis revealed that the main phenolic compounds were catechin, epicatechin and procyanidin (4.9% yields) in the fresh stems, while catechin,epicatechin, procyanidin and propelargonidins (5.06% yields) were the most abundant phenolics in the stone stems.*

***Key Words*:** *Aizoaceae, Mesembryanthemum edule, Phenolic compounds, LC-DAD-ESI/MS*

1. Introduction

Proanthocyanidins or condensed tannins are commonly composed of flavan-3-ol units of (epi)catechin, (epi)gallocatechin and (epi)afzelechin with C4-C8 or C4-C6 interflavan linkages (B-type) and with an additional C2-O7 linkage (A-type).Proanthocyanidins exclusively composed of (epi)catechin units are called procyanidins, whereas propelargonidins and prodelphinidins contain (epi)afzelechin and (epi)gallocatechin, respectively, and are usually mixed with procyanidins[1,2]. Precise determination of these compounds remains difficult because of the great complexity of the chromatograms, due to the multiplicity of condensed tannins structures that are distributed in plant materials. Previously several methodfor their detection have been reported like reverse-phase HPLC methods coupled to UV, UV–Visible or electrochemical detection[3].
In this context and in connection with our research program on valorization of the Algerian floristic biodiversity particularly the aromatic and medicinal plants, we have undertaken a study on the condensed tannins of *Mesembryanthemum edule L.* called "*Lessan el Hamr*",

belong to the family of Aizoaceae[4].*Mesembryanthemum edule L*. is a multipurpose and edible halophyte that is used traditionally for the treatment of various diseases, sinusitis, diarrhea, infantile eczema and tuberculosis[5,6,7].The leaf juice of *M. edule* is widely consumed as a traditional remedy against a wide range of fungal and bacterial infections[6], and it can be taken orally for treating sore throat and mouth infections [8].*M. edule* contains high levels of polyphenolic compounds, notably procyanidins and propelargonidins[9].
The aim of this study was intended to isolate and identify the phenolic compounds of *M. edule*fresh and stone stems. The approach consist to determine contents in total phenolic and to establish the phenolic composition through UPLC-MS analysis.

2. Material and methods

2.1. Plant sampling

M. edule plants were collected in the littoral of Oran (west Algeria) characterized by an arid climate. The plants were separated into leaves, stems and roots. Stems samples were separated into bark, stone and fresh, rinsed with distilled water, ground to powder in Dangoumau and lyophilized.

2.2. Extraction and fractionation

The powder (5 g fresh and stone) was first extracted three times by 50 mL hexane,to remove lipids, carotenoids and chlorophylls. The resultingresidue was extracted by 50 mL methanol/TFA (three times) to dissolveorganic acids and phenolic compounds. Each extraction wascarried out by blending the powder with solvent for 5 min usingmagnetic stirring, and the mixture was filtered through a WhatmanNo. 4 filter paper. Methanol filtrates were combined andcleaned up on 5-g C18 Sep-Pak cartridges (Waters, Milford, MA).
The C18 solid-phase-extraction column was used to remove freesugars from procyanidin-rich extracts prior to LC-MS analysis. The C18 column was preconditioned with20 mL of methanol followed by 40 mL of acidified water (0.0.5% TFA). About 2 mL of filtered methanolic extracts were dilutedin 80 mL of acidified water (0.05% TFA) and loaded into theC18 cartridge, in order to remove sugars and other polarcompounds. For each organ, phenolics that had been adsorbedonto the resin were successively eluted with 10 mL of MeOH/TFA 0.05%.

2.3. Thiolysis

Fractions to be analyzed were dissolved in methanol,mixed with an equal volume of thiolytic reagent (5% solution ofphenylmethanethiol in methanol containing 0.2 M HCl), and heatedfor 2 min at 90 °C [10]. The released units were analyzed by UPLC. Quantification of each terminal and extension unit was based on peak areas around279-281 nm.

2.4. UPLC-DAD-ESI/MSanalysis

An Acquity UPLC system (Waters, Milford, MA), equipped with a photodiode array detection (DAD) detector was used for phenolic compounds analysis and quantification under UPLC conditions.Instrument control and data acquisition were performed using Bruker Compass DataAnalysis 4.0.
Chromatographic conditions UPLC–DAD quantification was performed on a reversed-phase column Acquity UPLC BEH C18. Injected volume was 5µL. The diodearrayUV–vis detector (DAD) was used for the detection and the wavelength for quantification was set around279-281 nm.The compound identification was performedby UPLC–MS experiment in positive mode.

Table 1. LC–MS/MS data for phenolic compounds in *M. edule* organs.

Organ	Peak	Proposed compound	RT (min)	m/z Value [M-H$^+$]
Fresh stems	1	Catechin	2.7	291
	2	Epicatechin	3.2	291
	3	Procyanidin (acid unit extension)	3.8	381
	4	Procyanidin (ester unit extension)	4.2	395
	5	Procyanidin (ester unit extension)	4.6	395
Stone stems	1	Catechin	2.9	291
	2	Epicatechin	3.3	291
	3	Procyanidin (ester unit extension)	3.8	395
	4	Procyanidin (acid unit extension)	3.9	381
	5	Procyanidin (ester unit extension)	4.3	395
	6	Propelargonidin (afzelechin unit extension)	4.8	379
	7	Propelargonidin (afzelechin unit extension)	5.1	379

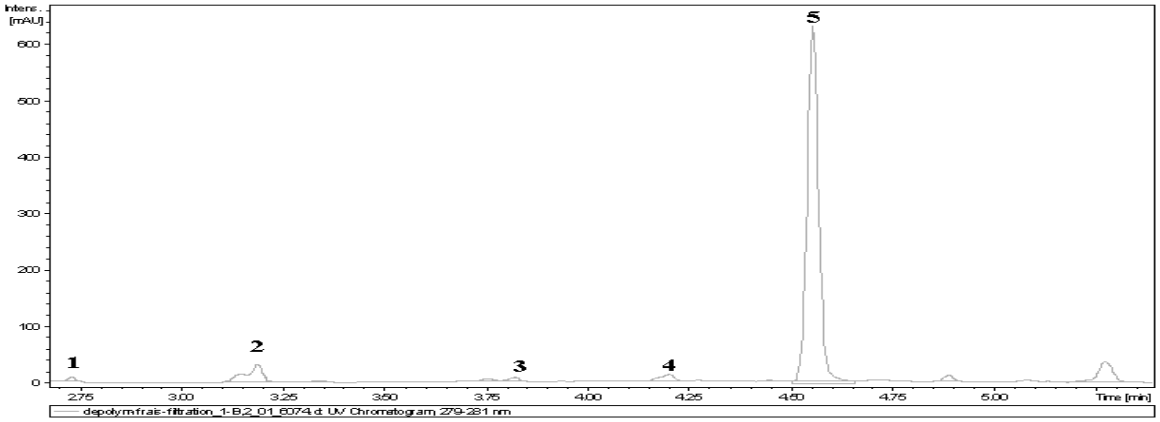

Fig.1. Fresh stems extract UPLC–DAD–ESI-MS chromatograms corresponding to the 279-281 nm trace in the positive mode

Phenolic compounds found in *M. edule* organs are given in Table 1 with their retention times and MS characteristics.

LC–MS analysis showed that fresh stems extract contain principally five allied compounds (Fig. 1), having a UV spectrum with a λ max around 279-281 nm. These molecules eluted at 2.7 (1), 3.2 (2), 3.8 (3), 4.2 (4) and 4.6 (5) min, with corresponding m/z at 291, 291, 381, 395 and 395, respectively (Table 1 and Fig.2). Considering the elution, profile and comparing our MS analyses to those of authentic standards and those reported in the literature, compounds 1 and 2 were respectively identified as catechin, epicatechin. Then, compounds 3, 4 and 5 were identified asprocyanidins derived from the extension unit acid and ester.

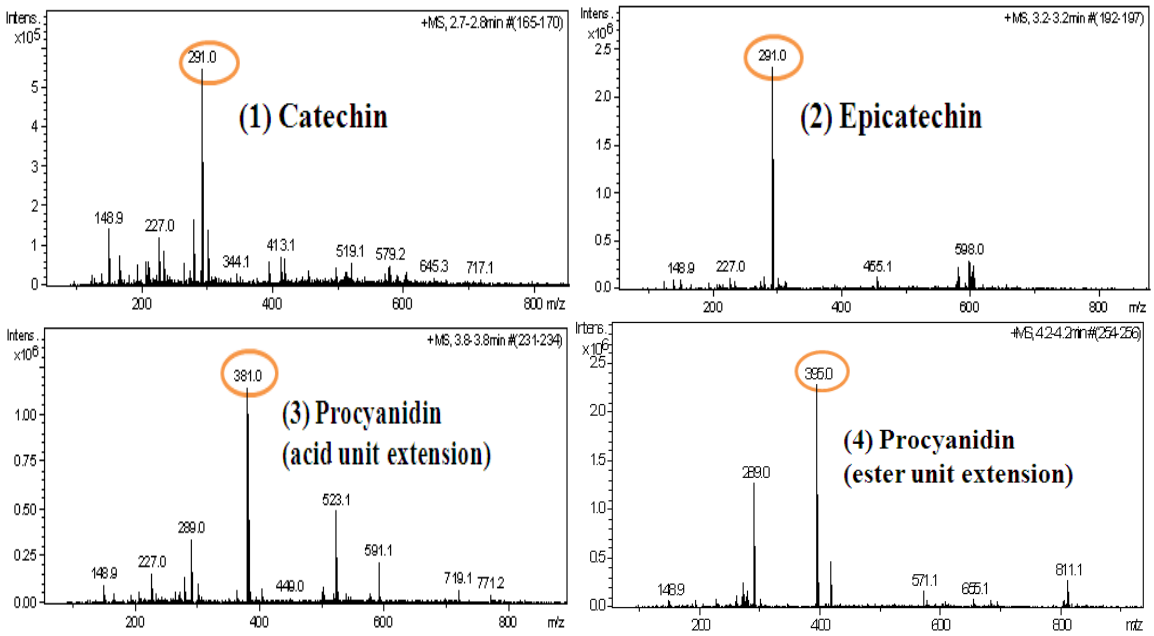

Fig.2. Mass spectrometric characterization of fresh stems extracts: MS spectrum (1), (2), (3), and (4).

MS spectra of stone stemsextract showed seven compounds ((Fig.3) having a UV spectrum with a λ_{max} around 279-281 nm, eluted at 2.9 (1), 3.3 (2), 3.8 (3), 3.9 (4), 4.3 (5), 4.8 (6) and5.1 (7) min, with corresponding m/z at 291, 291, 395, 381, 395, 379 and 379, respectively (Table 1 and Fig.4). The compounds 1, 2, 3, 4 and 5 were respectively identified as catechin, epicatechin and procyanidins derived from the extension unit ester and acid. Then, compounds 6 and 7 indicated the presence of an afzelechin unit, which is characteristic of propelargonidins (Fig.5).

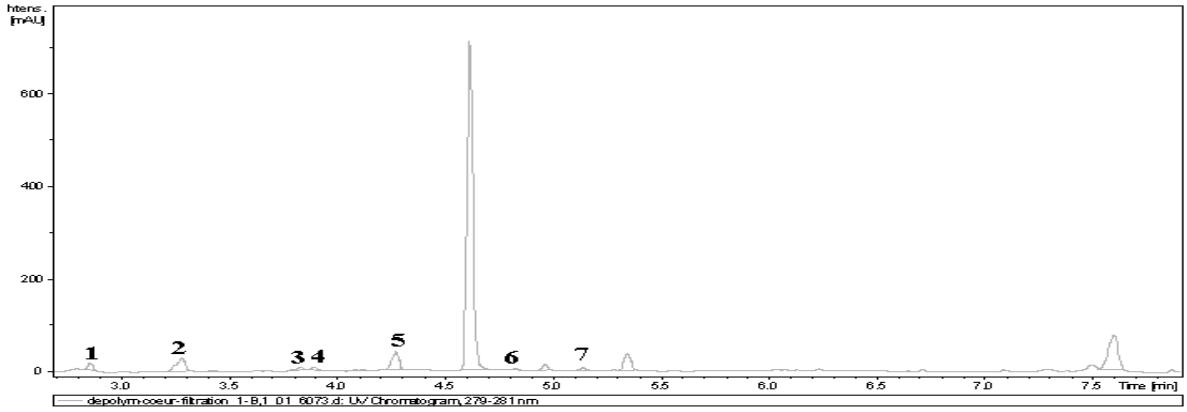

Fig.3. Stone stems extract UPLC–DAD–ESI-MS chromatograms corresponding to the 279-281 nm trace in the positive mode

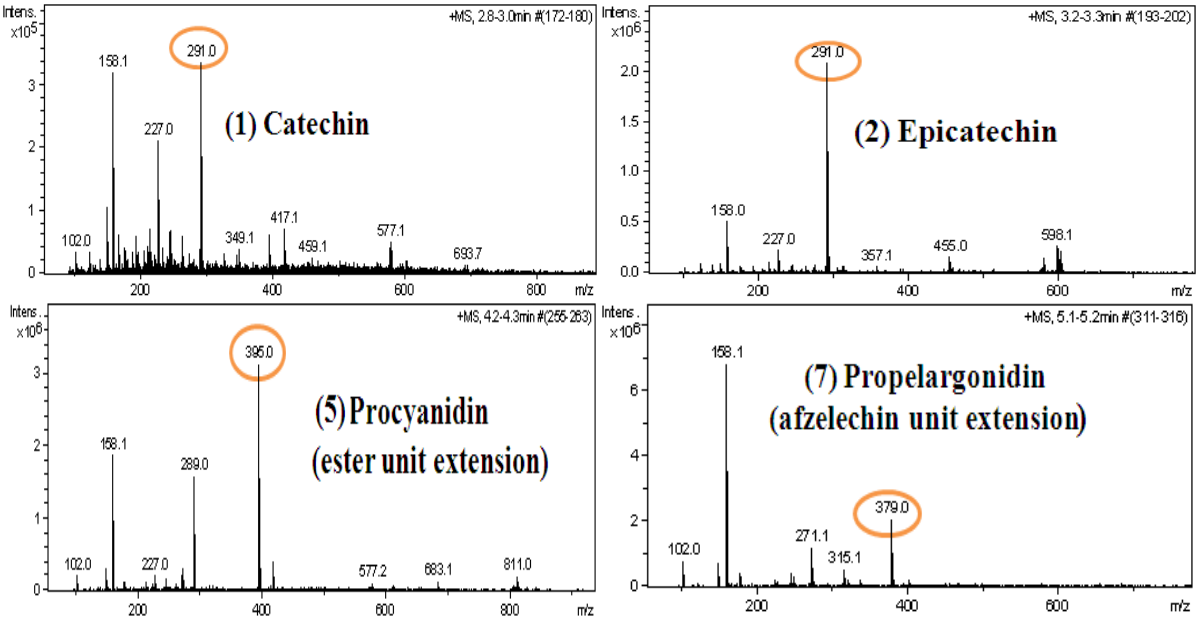

Fig.4. Mass spectrometric characterization of stone stems extracts: MS spectrum (1), (2), (5) and (7).

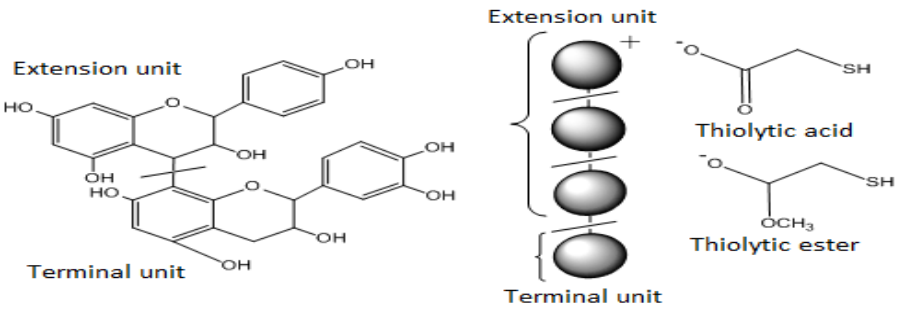

Fig.5. Example of propelargonidin and description of their extension and terminal unit

3. Conclusion

In conclusion, a rapid and reliable UPLC–DAD-ESI-MS method for determination of the major activeproanthocyanidins present in herbal medicine *M. edule* hasbeen developed. The extracted amount of fresh stemsis 0.049mg/g with 4.9% yields and stone stems is 0.0506mg/g with 5.06% yields. Thus, it is worthwhile to consider the utilization of this edible crop for the production of functional food ingredients or phytochemicals for medicinal exploration.

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ISSN 2170 - 1768

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