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Characterization of phenolic compounds from *Globularia alypum* L. (Globulariaceae) leaves by LC-QTOF-MS²

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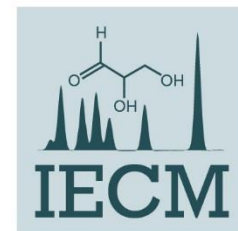
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Abstract:

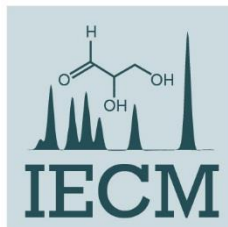
Globularia alypum L. (Globulariaceae) is a plant growing in the Mediterranean basin and is known and used in the folk medicine for its several pharmacological properties against typhoid, fever, gout, diabetes, and rheumatism. The methanol extract of *Globularia alypum* has been characterized for its phytochemical composition using the liquid chromatography hyphenated with an ESI-QTOF-MS. Thus, in this work the used technique leads to the tentative characterization of a total of 60 phytochemical compounds. The major compounds identified belong to the iridoids and phenolic acids derivatives. More than 20 iridoids and secoiridoids were identified, including several compounds not previously documented in *Globularia alypum*, such as gentiopicroside, acetylbarlerin isomers, serratoside A, specioside, shanziside, and decumbeside D isomers. Besides, the presence of 10 flavonoids, together with other nine polar compounds, has been confirmed. The methanol extract of *Globularia alypum* could thus be considered as a promising source of bioactive ingredients promoting the further use of this plant in the folk medicine.

Keywords *Globularia alypum*; Globulariaceae; Hydroxycinnamic acids; Flavonoids; LC-MS².



Introduction

- *Globularia alypum* L. (the globe daisy), is a wild plant belonging to the mint family (Plantaginaceae or Globulariaceae). It is a perennial shrub found throughout the Mediterranean area, Europe, and northeastern Africa.
- *Globularia alypum* leaves have been traditionally used as a hypoglycemic, laxative, diuretic, cholagogue, stomachic, tonic, purgative, sudorific agent and even as an aphrodisiac.
- Also used to treat hemorrhoids and cardiovascular diseases. To reduce histamine and serotonin contraction *in vitro* and showed active against lymphocytic, has antiviral activity.
- Its decoction used in the treatment of boils and intermittent fever. Also treatment of rheumatism, arthritis, besides having anti-tumor effects and phytotoxic potentials.
- Main biological activities described in *Globularia alypum* can be attributed to the bioactive compounds found in this plant such as phenolic compounds and iridoid glycosides. The wide use of this plant for the treatment of many diseases in addition to the fact that no phytochemical study has been reported on the *Globularia alypum* grown in Algeria prompted us to investigate the chemical composition of this plant matrix.



Materials and methods

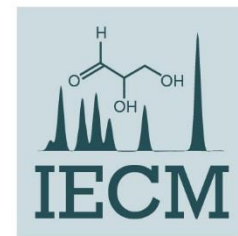
- Ground air-dried leaves of *Globularia alypum* were macerated with methanol at room temperature for 24 h.
- The supernatant was filtered and concentrated to dryness under reduced pressure in a rotary evaporator, and extracts were recovered with methanol and filtered.
- Afterwards, the *Globularia alypum* extract was injected into the LC-ESI-QTOF-MS system.

Materials and Methods

- The leaves of *G. alypum* were harvested in 2009, in remote areas in the suburbs of Souk Elbatel (Bajaia, Algeria).
- Fresh leaves were air-dried in shade at room temperature. After drying, ground to a fine powder (diameter < 250 μm) using an electric mill, thereafter, powder was exhaustively extracted by maceration with methanol, at room temperature for 24 h.
- Analyses were made using an Agilent 1200 Series RRLC system (Agilent Technologies). Zorbax Eclipse Plus C18 (150mm \times 4.6 mm, 1.8 μm) column has been used.
- The UHPLC was coupled to a microTOF-Q mass spectrometer (Bruker Daltonics).

Results and discussion

- A large number of metabolites present in *Globularia alypum* were identified by interpretation of their MS and MS/MS spectra obtained by QTOF-MS combined with the data provided in the literature.
- The analysis of the methanolic extract by LC-ESI-QTOF-MS revealed that iridoids, secoiridoids, hydroxycinnamic acids, and derivatives were the major classes of secondary metabolites in *Globularia alypum*.



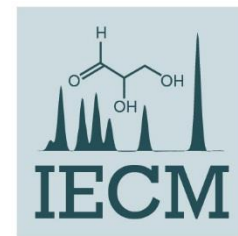
Results and discussion

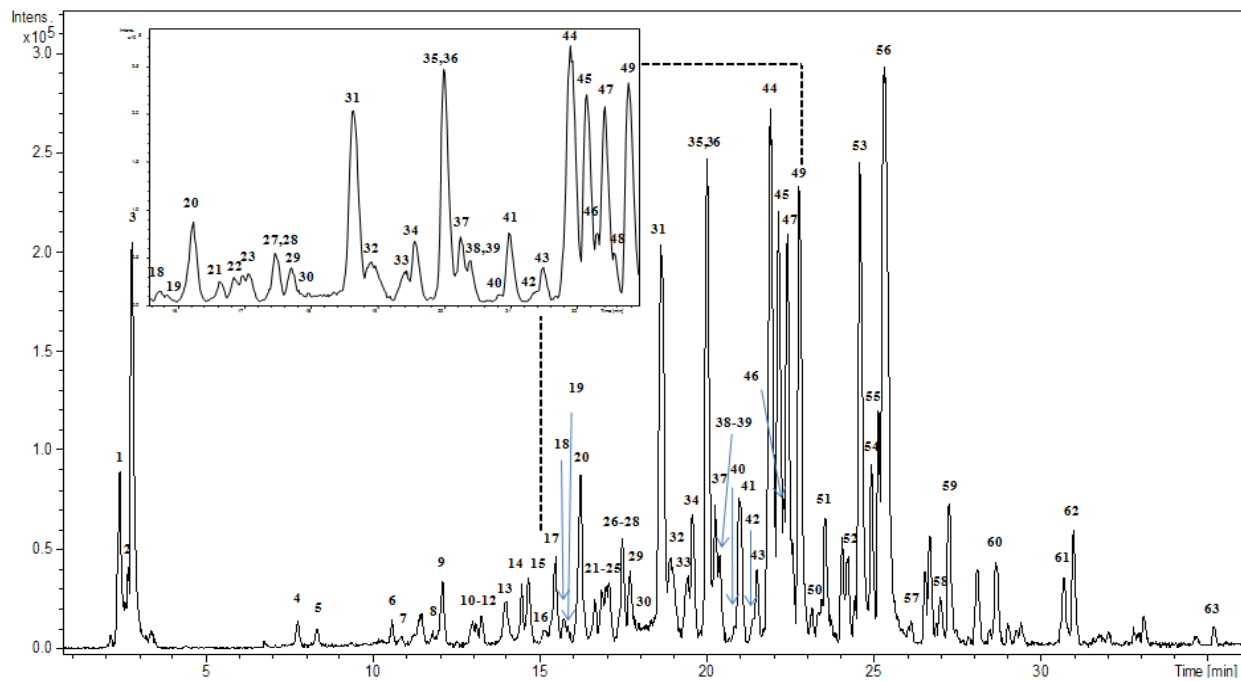
- **Hydroxycinnamic acids and derivatives**

Hydroxycinnamic acids and their derivatives represent another important group of metabolites characterized in this study, many of which were previously described in *Globularia alypum* or in the family Globulariaceae.

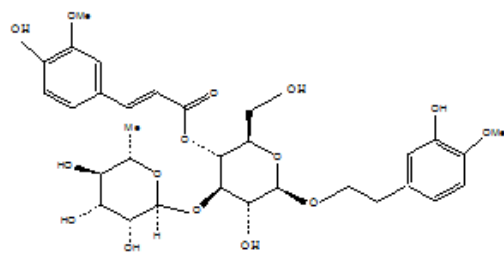
- **Flavonoids**

In the present work, five flavone glycosides previously described in *Globularia* were detected. Based on the MS² data and on the bibliography.

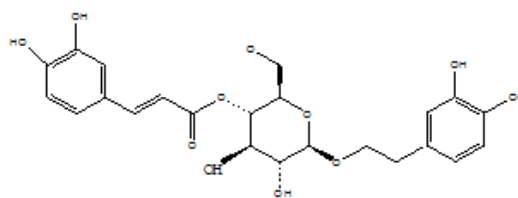




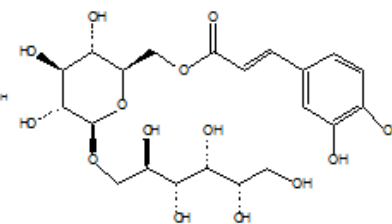
Base peak chromatogram (BPC) of *Globularia alypum* by LC-ESI-QTOF-MS in the negative ion mode. Peak labeling represents the compounds identified



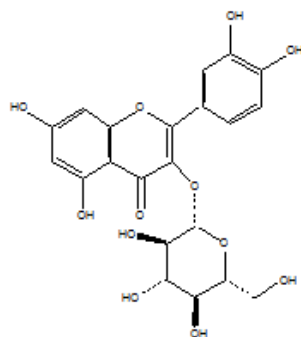
(a) (58) Martynoside



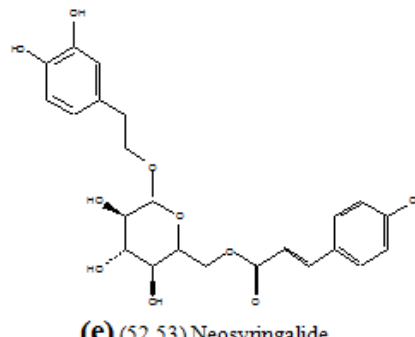
(b) (40, 47) Calceolanoside A



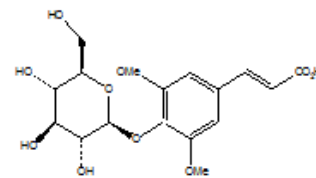
(c) (14) Hebitol II



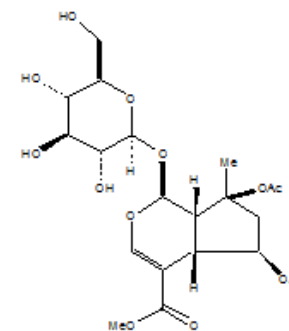
(d) (36) Quercetin glucoside



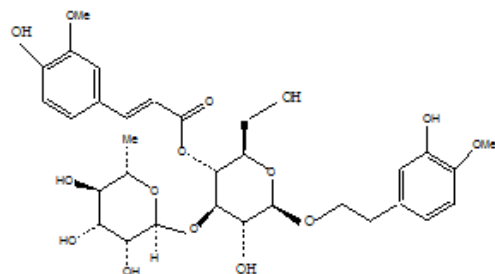
(e) (52,53) Neosyringalide



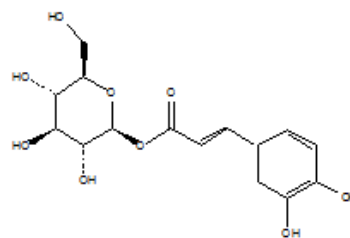
(f) (26) Sinapic acid-O-glucoside



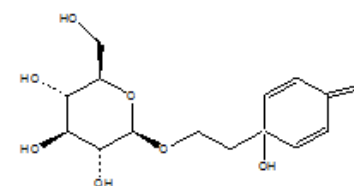
(g) (18,20) Acetylbarlerin



(h) (44) Verbascoside



(i) (15,17) Caffeoylglucose



(j) (9) Comoside

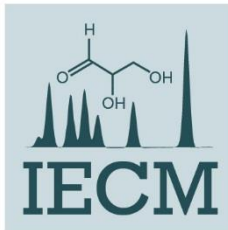
Chemical structures of several proposed compounds in *Globularia alypum* leaves

Table1. Flavonoids

^a Peak	<i>t_R</i> (min)	m/z experimental	Molecular formula	m/z calculated	Error (ppm)	mSigma value	MS/MS Fragments (% relative abundance)	Proposed compound
31	18.62	625.1418	C ₂₇ H ₂₉ O ₁₇	625.1410	-1.2	13.9	301.0358 (2), 461.1429 (1)	Hydroxyluteolin 7-O-laminaribioside
32	18.89	305.0692	C ₁₅ H ₁₃ O ₇	305.0667	-8.4	19.4	96.9592 (54), 225.1137 (100)	Gallocatechin/ Epigallocatechin
35	19.91	611.1597	C ₂₇ H ₃₁ O ₁₆	611.1618	2.1	3.4	151.0001 (2), 475.1085 (8)	Eriodictiol 7-O-sophoroside
36	19.98	463.0871	C ₂₁ H ₁₉ O ₁₂	463.0882	2.3	4.6	301.0366 (53)	Quercetin glucoside
37	20.23	609.1452	C ₂₇ H ₂₉ O ₁₆	609.1461	1.6	10.3	285.0394 (2)	Luteolin sophoroside (isomer 1)
39	20.38	609.1444	C ₂₇ H ₂₉ O ₁₆	609.1461	2.8	23.2	285.0398 (31)	Luteolin sophoroside (isomer 2)
45	22.12	447.0924	C ₂₁ H ₁₉ O ₁₁	447.0933	1.9	12.9	151.0037 (5), 285.0414 (49)	Cynaroside
48	22.52	533.1661	C ₂₆ H ₂₉ O ₁₂	533.1664	0.6	13.6	161.0233 (11), 179.0363 (8), 323.0777 (100)	Amurensin
49	22.74	477.1051	C ₂₂ H ₂₁ O ₁₂	477.1038	-2.6	3.5	299.0211 (5), 315.0509 (34), 462.0801 (3)	Nepitrin
54	24.89	517.1717	C ₂₆ H ₂₉ O ₁₁	517.1715	-0.4	11.3	145.0299 (27), 209.0828 (22), 307.0826 (100)	Phellamurin

Conclusions

- The LC-ESI-QTOF-MS based metabolite-profiling approach in this work enabled the tentative identification of more than 60 metabolites in a *Globularia alypum* extract on the basis of their MS and MS/MS spectra in negative ion mode together with the relevant data from the literature.
- The analyses of the leaf extract revealed a larger number of compounds, most being iridoids and hydroxycinnamic acid derivatives substituted with glycosides and/or acyl group.
- The described HPLC-ESI-QTOF-MS method proved to be a valuable tool for simultaneous characterization of a wide range of bioactive compounds from *Globularia alypum* leaves. Furthermore, the data obtained may encourage further use of this plant as folk and alternative medicine in human therapy.



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