SHORT REPORT

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Phytoconstituents and *in vitro* Evaluation of Antioxidant Capacities of *Cotula Cinerea* (Morocco) Methanol Extracts

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Abstract: The purpose of this study was to determine the phytochemical content of *Cotula cinerea* to establish principal components which may consolidate its use as a medicinal plant in the southeast of Morocco. The amount of total phenolic compounds as determined by analytical HPLC in methanol extracts was 79.23 ± 2.5 mg/g dry matter. The major phenolic compounds identified by HPLC-ESI-MS were neochlorogenic acid, chlorogenic acid, cryptochlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid and luteolin-4′-*O*-glucoside. All compounds displayed very strong antioxidant capacities in the DPPH, FRAP and ORAC assays. The data indicates that methanol extracts of *C. cinerea* via their antioxidant capacities, may be effective disease prevention potions in traditional African medicine which is probably related to the significant content of echinoids and flavonoids.

Keywords: *Cotula cinerea*; chlorogenic acids; flavonoids; antioxidant capacity.

1. Plant Source

The aerial parts of *C. cinerea* were collected in Errachidia (Morocco), during the flowering period (April/June, 2009). The plant was previously identified and authenticated by Dr Ben Tatou at the Scientific Institute, Rabat, Morocco and a voucher specimen (FSTBIO 04) is deposited at the herbarium of the Faculty of Science and Techniques, Errachidia. The dried plant material was stored in the dark, at room temperature (25°C) before extraction.

2. Previous Studies

C. cinerea (Compositae, subfamily Asteroideae, Tribe Anthemideae, subtribe Cotulinae) is a fragrant, perennial herb with yellow flowers, which grows in the southeast of Morocco and Algeria. It is known locally as “guertoufa”. Traditionally, the leaves of this plant are still used for colic, cough, bronchopulmonary cooling, diarrhoea and digestive disorders [1], and an analgesic effect has also been previously described [2]. To date, no information with regard to the phytochemical content of *C. cinerea* has been published except its essential oil composition (trans-thujone as a major component)

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[3] and the purpose of this study was to determine the phytochemical content of *C. cinerea* to establish principal components which may consolidate its use as a medicinal plant in the southeast of Morocco.

3. Present Study

The powdered aerial parts of *C. cinerea* (20 g) were extracted with hexane (1x3h) to remove lipid followed by methanol (3x3h) in a Soxhlet apparatus. The methanol extracts were concentrated to dryness, and the residues stored at 4 °C. Reverse-phase analytical HPLC, HPLC-ESI-MS and semi-preparative HPLC were conducted as described previously [4-6]. Full details of the analytical methods are given in the Supporting information file. Finally, antioxidant activity of the purified compounds was assessed by the 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric reducing ability of plasma (FRAP) and oxygen radical absorbance capacity (ORAC) assays as described previously [7].

Identification of phenolic compounds in solvent extracts of C. cinerea: The major phenolic compounds identified in the solvent extracts of *C. cinerea* were echinoid (chlorogenic acid isomers and dicaffeoyl quinic acid isomers) and a flavonoid as identified by HPLC-ESI-MS-MS (Table 1). An analytical HPLC chromatogram at 340 nm of a methanol extract is depicted in Fig. 1. along with the extracted negative-ion chromatograms $[M - H]^-$ at *m/z* 353, 515 and 447 representing the chlorogenic acid isomers, the dicaffeoylquinic acid isomers and luteolin-4′-*O*-glucoside respectively. Mass spectra are given in the Supporting information file. The concentrations in mg/g dry matter were neochlorogenic acid (7.9), chlorogenic acid (11.9), cryptochlorogenic acid (7.1), 3,4-dicaffeoylquinic acid (6.8), 3,5-dicaffeoylquinic acid (7.9), 4,5-dicaffeoylquinic acid (7.1) and luteolin-4′-*O*-glucoside (30.6). The structures are shown in Fig. 2. All compounds displayed very strong antioxidant capacities in the DPPH, FRAP and ORAC assays: e.g. far stronger (x 3.3 on average) in the ORAC assay, than the water soluble derivative of vitamin E, Trolox (Table 2)**.** These principal components may contribute to the effectivity of C. *cinerea* extracts in traditional African medicine.

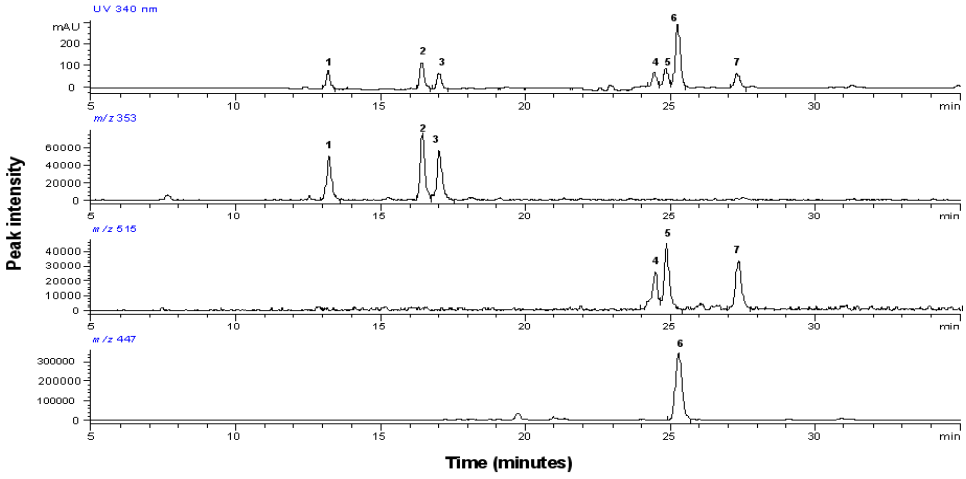

Figure 1. Reverse-phase HPLC (UV, 340 nm) and extracted ion chromatograms of a methanol extract of *C. cinerea* 1: neochlorogenic acid; 2: chlorogenic acid; 3: cryptochlorogenic acid; 4: 3,4-dicaffeoylquinic acid; 5: 3,5-dicaffeoylquinic acid; 6: luteolin-4′-*O*-glucoside; 7: 4,5-dicaffeoylquinic acid.

Table 1. HPLC-ESI-MS data of the phenolic compounds detected in the methanol extracts of *C. cinerea.*

No	Phenolic compound	$[M - H]^-$	HPLC-ESI-MS fragmentation (neg. ion *m/z*)
I	Neochlorogenic acid (M = 354)	353.1	191.1 = $[M - H]^-$-caffeic acid 179.1 = $[M - H]^-$-quinic acid
II	Chlorogenic acid (M = 354)	353.1	191.1 = $[M - H]^-$-caffeic acid 179.1 = $[M - H]^-$-quinic acid
III	Cryptochlorogenic acid (M = 354)	353.1	191.1 = $[M - H]^-$-caffeic acid 179.1 = $[M - H]^-$-quinic acid
IV	3,4-Dicaffeoylquinic acid (M = 516)	515.0	353.1 = $[M - H]^-$-caffeic acid 191.1 = $[M - H]^-$-2 x caffeic acid 179.1 = $[M - H]^-$-caffeic acid + quinic acid
V	3,5-Dicaffeoylquinic acid (M = 516)	515.1	353.1 = $[M - H]^-$-glucose 191.1 = $[M - H]^-$-2 x caffeic acid 179.1 = $[M - H]^-$-caffeic acid + quinic acid
VI	Luteolin-4′-*O*-glucoside (M = 448)	447.1	285.1 = $[M - H]^-$-glucose
VII	4,5-Dicaffeoylquinic acid (M = 516)	515.0	353.1 = $[M - H]^-$-glucose 191.1 = $[M - H]^-$-2 x caffeic acid 179.1 = $[M - H]^-$-caffeic acid + quinic acid

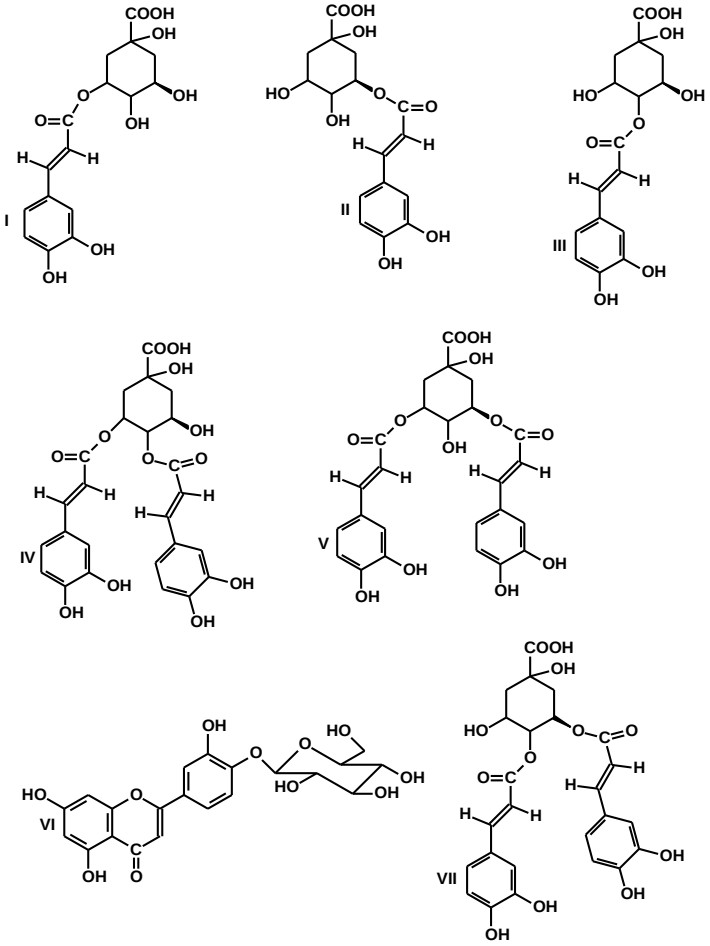

Figure 2. Structures of the phenolic compounds identified in *C. cinerea* methanol extracts. **I**, neochlorogenic acid; **II**, chlorogenic acid; **III**, cryptochlorogenic acid; **IV**, 4,5-dicaffeoylquinic acid; **V**, 3,5-dicaffeoylquinic acid; **VI**, luteolin-4′-*O*-glucoside; **VII**, 3,4-dicaffeoylquinic acid.

Table 2. Antioxidation assays: comparison of pure polyphenolic compounds isolated from the aerial parts of *C. cinerea* in comparison to reference compounds.

Phenolic compounds identified in *C. cinerea*	[a] DPPH IC_{50} (µM)	[b] FRAP EC_1 (µM)	[c] ORAC (units)
Chlorogenic acid	10.5	478	3.07
Neochlorogenic acid	11.0	527	2.42
3,4-Dicaffeoylquinic acid	30.25	329	3.33
3,5-Dicaffeoylquinic acid	23.84	407	3.62
4,5-Dicaffeoylquinic acid	31.49	337	3.76
Luteolin-4´-*O*-glucoside	30.25	422	3.46
Reference compounds			
Caffeic acid	38.82	473	4.23
Luteolin	25.28	560	4.33
Trolox	65.96	527	1.0

[a] IC_{50}: concentration of substance (µM) where 50% of the DPPH radical is scavenged.

[b] EC_1: concentration of substance (µM) giving an absorbance increase equivalent to 1 mM Fe(II) solution.

[c] 1 ORAC unit equals the inhibition of the declining fluorescence produced by 1 µM Trolox.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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