Antioxidant activity and phenolic compounds identification of *Micromeria inodora* (Desf.) Benth. from Western Algeria

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Abstract

Micromeria inodora (Desf.) Benth. is most studied for the antimicrobial activity of their essential oils; nevertheless there are no reports on the phenolic compounds of this species and their antioxidant activity. In this study, aqueous and organic extracts were assayed for their antioxidant activity. The polyphenol and total flavonoid content, varied between 175.50 to 332.62 mg GAE/g extract and 65.38 to 86.30 mg catechin equivalent/g extract, respectively. The results of antioxidant activity have shown that all extracts reported an important activity and the ethyl acetate extract showed the stronger effect, higher than some standards molecules. Its IC_{50} value of scavenging DPPH is 1.5 ± 0.001µg/mL; β-Carotene Bleaching assay (BCB) IC_{50} = 28.1± 0.001µg/mL and the Ferric Reducing Antioxidant Power (FRAP) IC_{50} = 6.96± 0.05 µg/mL. RP-HPLCPDA analysis of phenolic compounds revealed the presence of phenolic acids and flavonoids as: gallic acid, quercetin, rutin, vanillin and naringenin.

Keywords: *Micromeria inodora*, polyphenol, flavonoid, antioxidant activity, RP-HPLC-PDA

1. Experimental

2.1. Plant material

The aerial part of *M. inodora* was collected in October during the flowering period from Ghazaouet-Tlemcen in western Algeria (Latitude: 35°05′49″N; Longitude: 1°50′02″O; Altitude: 103m). Botanical identification of the plant was authenticated in the laboratory of Ecology and Ecosystems Management in Department of Biology at University of Tlemcen-Algeria. In the herbarium of the University of Tlemcen, the code of this species is MI-0912-KA17 (Benomari et al. 2016). In laboratory, collected samples were cleaned with tap water and dried at room temperature and in darkness.

2.2. Preparation of plant extracts

Extracts were obtained by maceration at room temperature for 24 hours, 40g of dried plant in 600ml of solvent; distilled water for the aqueous extract (Aq), acetone- or methanol-water mixtures (70/30, v/v) to prepare hydroacetonic (EA), and hydromethanolic (EM) extracts. The Aq extract has undergone fractionation by liquid-liquid extraction using ethyl acetate and n-butanol.

1.3. Total polyphenol and flavonoid contents

Total polyphenols and flavonoids contents of the extracts were determined according to previous protocol (Adjdir at al., 2019).

1.4. RP-HPLC-PDA analysis of phenolic compounds

Separation and identification of phenolic compounds in the aqueous extract of *M. inodora* and its fractions; ethyl acetate, and n-butanol extracts were carried out by RP-HPLC-PDA technique (El-Haci et al., 2019).

1.5. DPPH free radical scavenging activity

The DPPH free radical scavenging essay was evaluated according to previous protocols (El Haci et al. 2009; Adjdir et al., 2019). BHT (Butylated hydroxytoluene), BHA (Butylated hydroxyanisole), ascorbic acid, gallic acid, catechin, and tannic acid were used as positive control. The radical scavenging activity of the tested samples was expressed as percentage inhibition of DPPH.

1.6. β-Carotene Bleaching assay (BCB)

The BCB assay was evaluated according to previous protocols (Adjdir et al. 2019). BHT, BHA, ascorbic acid, gallic acid, catechin, and tannic acid were used as positive control. The BCB activity is usually expressed as percentage of inhibition.

1.7. Ferric Reducing Antioxidant Power (FRAP)

The FRAP was evaluated using potassium ferricyanide reducing method according to previous protocols (Karagözler et al., 2008; Adjdir et al., 2019). The IC_{50} value corresponds to the extract concentration providing 0.5 of absorbance.

1.8. Total antioxidant capacity (TAC)

The Total antioxidant capacity was evaluated according to previous protocols (Adjdir et al., 2019).

2. Results

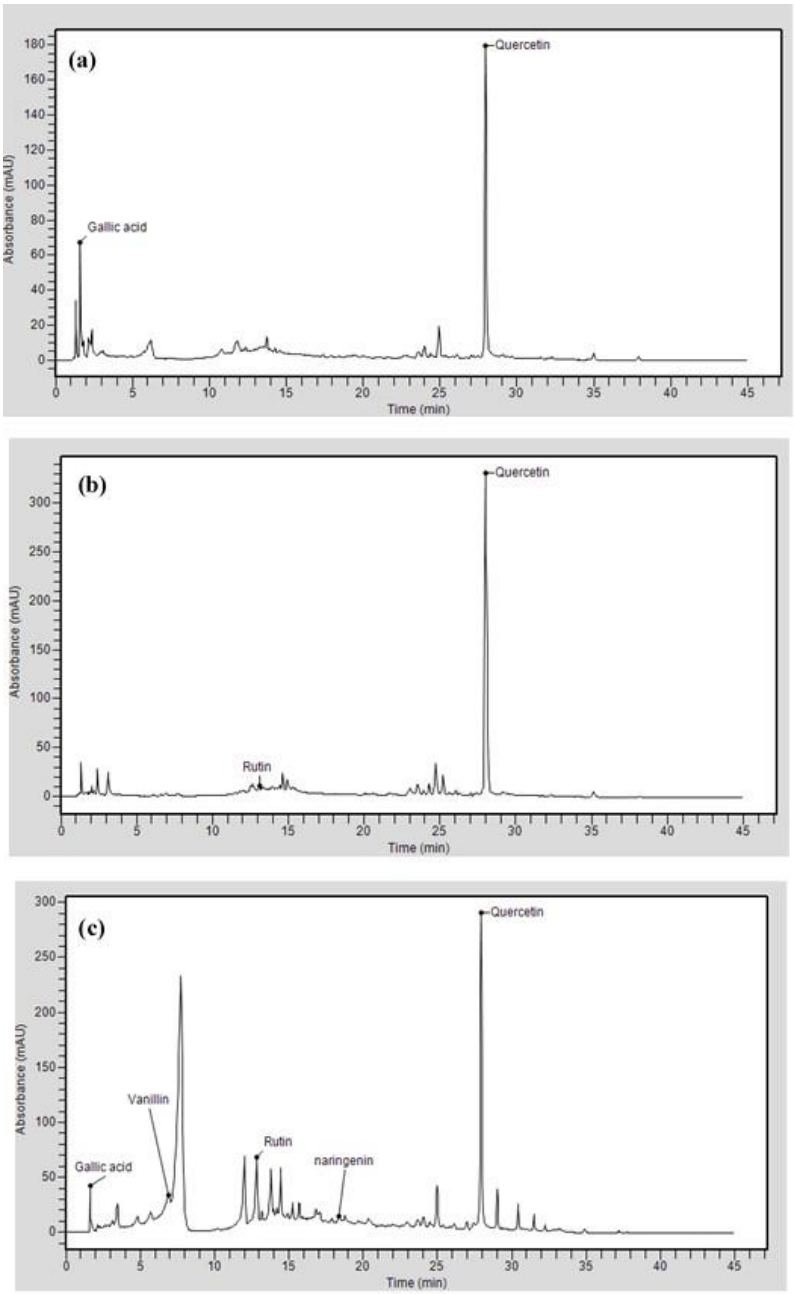

Figure S1: High-performance liquid chromatography profile of *M. inodora* extracts (at 280 nm) **(a)**: aqueous extract; **(b) :** n-butanol extract; **(c)** : ethyl acetate extract

	Concentration µg/mL					
	0.5	**6.25**	**12.5**	**25**	**75**	**250**
Aq	-	40.87 ±0.006	81.32 ±0.03	90.13 ±0.006	91.28 ±0.003	82.55 ±0.001
EA	-	15.56 ±0.01	33.60 ±0.02	66.44 ±0.01	82.71 ±0.004	89.21 ±0.009
EM	-	37.23 ±0.006	57.25 ±0.009	89.72 ±0.01	89.84 ±0.007	90.99 ±0.003
Acet	47.25	65.02	92.59	94.32	96.87	-

Table S1: Scavenging activity (%) of *M. inodora* extracts on DPPH radical. Each value is expressed as mean ±standard deviation (SD).

	±0.003	±0.008	±0.003	±0.004	±0.004	
	31.25	61.46	82.55	91.10	97.12	
n-but	±0.01	±0.004	±0.008	±0.000	±0.002	-
			Concentration µg/mL			

Table S2: β-carotene bleaching activity of *M. inodora* extracts. Each value is expressed as mean ±standard deviation (SD).

	31.2	62.5	187.5	625
Aq	3.77± 0.01	17.84 ±0.003	47.12±0.01	-
EA	48.19 ±0.01	78.45±0.01	-	-
EM	1.30± 0.01	-	42.01±0.02	63.73± 0.002
Acet	52.42± 0.02	62.96±0.01	77.08± 0.01	78.09± 0.01
n-but	14.62± 0.01	32.08± 0.007	65.96±0.005	99.52± 0.01

Table S3: Total antioxidant capacity of *M. inodora* extracts

	TAC µg GAE/DWE
Aq	76.9±0.02
EA	65.54±0.01
EM	72.61±0.001
Acet	102.83±0.02
n-but	78.76±0.03

GAE: Gallic Acid Equivalent; DWE: Dried Weight Extract.

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