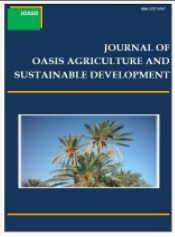

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Moringa oleifera leaves: LC-ESI-MS analysis of phenolic compounds and antioxidant activities

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Abstract

Research on bioactive compounds derived from medicinal plants is still topical and their applications are of interest to food industry, phytopharmacy and cosmetics. The present study deals with the phenolic compounds profile and antioxidant activities of *Moringa oleifera* Lam. leaves. Therefore, this work is a contribution to study the potential of *M. oleifera* as a new crop in Tunisia. Composition of phenolic compounds was realized using LC-ESI-MS analysis. Antioxidant activities were assessed using many complementary methods. Extracts from Moringa leaves contained interesting amounts of total phenolics (33–99 mg GAE/g extract) and flavonoids (7–18 mg QE/g extract), with appreciable antioxidant activities in Fe^{3+} reducing ($EC_{0.5}$: 0.5–1.1 mg/ml), DPPH• radical-scavenging (IC_{50}: 0.6–1.9 mg/ml), β-carotene/linoleic acid bleaching (IC_{50}: 66–120 µg/ml) and Fe^{2+} chelating (IC_{50}: 0.5–0.8 mg/ml) assays. Quinic acid, gallic acid, quercetin-3-*O*-galactoside and quercetin-3-*O*-rhamnoside were the major compounds measured in the different extracts (5.5–13.5 mg/g extract). *M. oleifera* grown in oasis of Chenini-Gabes, southeastern Tunisia, can be a source of potent antioxidants for various food, nutraceutical or cosmeceutical applications.

1. INTRODUCTION

Moringa oleifera Lam. belongs to the Moringaceae family, native to India. It is one of the most useful and versatile plants, known as the tree of life, given its interesting properties. In fact, each part of the Moringa tree has interesting potential in food, agronomic, cosmetic and medicinal applications (Singh et al., 2020).

The Moringa distribution is cosmopolitan, since it can be grown under stressful conditions. Moringa is a fast-growing, drought tolerant and high temperature resistant tree, which can adapt to the regions affected by climate change, such as the Mediterranean ecosystem type (Trigo et al., 2020). It is mainly occurred in southern Tunisia characterized by arid and semi-arid climates, where it becoming a new food and economic phytoresource (Bennour et al., 2020). In the same context, Moringa can adapt to the oasis conditions of the Gabes region whose climate seems to be adequate for its plantation.

Moringa leaves can be eaten fresh, in salads or as a seasoning, as they can also be cooked in soups and stews. Moreover, the leaves can be used in infusions for medicinal properties. The dried leaves are attractive for preparing nutritionally fortified foods, as they have a high protein content, similar to that of soybeans, as well as they are a good source of essential amino acid methionine and minerals, such as phosphorus, calcium, potassium and iron. On the other hand, leaves are also rich in bioactive metabolites, such as carotenoids, ascorbic acid, glucosinolates, phenolic acids (ellagic acid, gallic

acid, ferulic acid, chlorogenic acid, etc.) and flavonoids (quercetin, rutin, kaempferol, vanillin etc.) (Singh et al., 2020).

Given the potential of this plant, it is interesting to extract the bioactive compounds of the introduced species in Tunisia and also to confirm its nutraceutical properties. Thus, the present work focuses on the phenolic compounds of the oven-dried leaves from M. oleifera grown in the oasis of Chenini-Gabes, southeastern Tunisia. The phenolic compounds of different solvent extracts were measured by LC-ESI-MS technique. In addition, antioxidant properties of these extracts were studied.

2. MATERIEL ET METHODES

2.1. Plant material

Moringa oleifera Lam. (Moringaceae) leaves were collected from the oasis of Chenini-Gabes (southeastern Tunisia, characterized by an arid climate) on April 2020. The leaves were oven-dried at 50°C until a constant mass was obtained, then ground into fine powder and stored at ambient temperature in a dry place and in the dark until use.

2.2. Preparation of M. oleifera extracts

The leaves powder was extracted using: (i) ethanol 100%; (ii) ethanol/water (50:50, v:v) and (iii) distilled water. Five g powder were macerated in 100 ml of each solvent under stirring at 250 rpm for 12 h. After filtration, the extracts were lyophilized and kept in the dark at +4°C until further analysis.

2.3. Total phenolics and flavonoids contents

The total phenolics and flavonoids contents were measured as previously described by Dewanto et al. (2002). The total phenolics was expressed in terms of gallic acid equivalent (mg GAE/g extract). The flavonoids content was expressed in terms of quercetin equivalent (mg QE/g extract).

2.4. Liquid chromatography-electrospray ionization mass spectrometry (LC-ESI-MS) analysis

The M. oleifera extracts (20 mg/ml) were analyzed using a LC-MS-2020 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) equipped with an electrospray ionization source and operated in negative ionization mode as previously described by Jdir et al. (2017). The identification of phenolics was done by comparing the retention times and the mass spectra with those of authentic standards. The chemical standards (quinic acid, gallic acid, protocatechuic acid, chlorogenic acid, caffeic acid, syringic acid, p-coumaric acid, trans-ferulic acid, o-coumaric acid, trans-cinnamic acid, 4-o-caffeoylquinic acid, 1,3-di-o-caffeoylquinic acid, 3,4-di-o-caffeoylquinic acid, 4,5-di-o-caffeoylquinic acid, rosmarinic acid, salvianolic acid, catechin, epicatechin, acacetin, apigenin-7-O-glucoside, apigenin, cirsilineol, cirsiliol, hyperoside (quercetin-3-O-galactoside), luteolin-7-O-glucoside, luteolin, naringenin, naringin, quercitrin (quercetin-3-O-rhamonoside), quercetin, rutin and silymarin) of highest purity (≥99.0%) were purchased from Sigma Chemical Co. (St Louis, MO, USA).

2.5. Antioxidant activities

The ferric (Fe3+) reducing power (OD700), DPPH•-radical scavenging activity (%), β-carotene/linoleic acid bleaching activity (%) and metal (Fe2+) chelating capacity (%) of M. oleifera extracts were measured as previously described (Dinis et al. 1994; Yildirim et al. 2001; Zouari et al. 2011). Results of DPPH•-radical-scavenging, β-carotene/linoleic acid bleaching and metal (Fe2+) chelating assays are presented by IC_{50} values, defined as the extract concentration needed to scavenge 50% of DPPH•, to obtain 50% inhibition of β-carotene/linoleic acid peroxidation and to chelate 50% of Fe2+, respectively. The ferric (Fe3+) reducing power represented the absorbance measured at 700 nm and the extract concentration ($EC_{0.5}$) providing 0.5 of an absorbance at 700 nm was determined. Lower IC_{50} and $EC_{0.5}$ values reflected better antioxidant activities.

3. RESULTS AND DISCUSSION

3.1. Phenolic compounds profile

Table 1 shows the total phenolics and flavonoids contents, as well as phenolic compounds profile of the different extracts from *M. oleifera* leaves. The highest amounts of total phenolics (99 mg GAE/g extract) and flavonoids (18 mg QE/g extract) were measured for the ethanol/water (50:50) extract (Table 1). Comparable results were reported for a 70% methanol extract from air-dried leaves of *M. oleifera* cultivated in Menzel Habib area (Gabes, southeastern Tunisia) (Bennour et al., 2020). The LC-ESI-MS analysis resulted in the identification of 15 compounds divided into 8 flavonoids and 7 phenolic acids (compounds 1-6, and 10). The identification of phenolic compounds was done by comparing the obtained mass spectra with those of 32

Table 1. Phenolics profile and antioxidant activities of *M. oleifera* leaf extracts.

No[1]	Compounds[2]	Molecular formula	Molecular mass	[M-H]⁻ m/z	Retention time (min)	Extract content (µg/g extract)		
						Ethanol	Ethanol/ Water	Water
1	Quinic acid	$C_7H_{12}O_6$	192	191	2	1321	13506	447
2	Gallic acid	$C_7H_6O_5$	170	169	4.5		57	6808
3	Protocatechuic acid	$C_7H_6O_4$	154	153	6.5	1000	211	1383
4	Caffeic acid	$C_9H_8O_4$	180	179	14.8	58	21	117
5	*p*-Coumaric acid	$C_9H_8O_3$	164	163	21.2	150	83	277
6	*trans*-Ferulic acid	$C_{10}H_{10}O_4$	194	193	23.3	23		
7	Quercetin-3-*O*-galactoside	$C_{21}H_{20}O_{12}$	464	463	24.8	11605	6598	21
8	Quercetin-3-*O*-rhamnoside	$C_{21}H_{20}O_{11}$	448	447	26.8	5515	3712	11
9	Naringin	$C_{27}H_{32}O_{14}$	580	579	26.4	80	36	
10	*trans*-Cinnamic acid	$C_9H_8O_2$	148	147	32.1	333		
11	Quercetin	$C_{15}H_{10}O_7$	302	301	32.2	58	10	
12	Naringenin	$C_{15}H_{12}O_5$	272	271	34.2			64
13	Apigenin	$C_{15}H_{10}O_5$	270	269	34.9			650
14	Luteolin	$C_{15}H_{10}O_6$	286	285	35.2	23	5	
15	Cirsiliol	$C_{17}H_{14}O_7$	330	329	35.9	92	41	11
Total of identified compounds (mg/g extract)						20.3	24.3	9.8
Total phenolics (mg GAE/g extract)*						33	99	66
Flavonoids (mg QE/g extract)**						7	18	16
Reducing power ($EC_{0.5}$, mg/ml)						1.1	0.5	0.8
DPPH• scavenging activity (IC_{50}, mg/ml)						1.9	1.1	0.6
β-Carotene/linoleic acid bleaching activity (IC_{50}, µg/ml)						120	84	66
Fe2+ chelating capacity (IC_{50}, mg/ml)						0.7	0.8	0.5

[1]The numbering refers to elution order of compounds from an Aquasil C18 column.
[2]Identification was confirmed using 32 authentic commercial standards.
**Polyphenolics content as gallic acid equivalent (GAE); **Flavonoids content as quercetin equivalent (QE).*

standards. However, if the analyzed extracts contained compounds different from the used standards, they cannot be identified. Quinic acid, gallic acid, quercetin-3-*O*-galactoside and quercetin-3-*O*-rhamnoside were the major compounds measured in the different extracts (> 5 mg/g extract). The content of these compounds varied considerably with the solvent used, which suggest the important effect of the solvent nature on their extractions. In fact, the highest content of quinic acid was measured in ethanol/water extract, compared to ethanol and water extracts. However, quercetin-3-*O*-galactoside and quercetin-3-*O*-rhamnoside were more extracted in ethanol, compared to other solvents. As for gallic acid, the highest content was measured in the aqueous extract. Bennour et al. (2020) identified many phenolic compounds in the leaves of *M. oleifera* cultivated in Menzel Habib area (Gabes, southeastern Tunisia). These authors reported that the contents of all identified compound were less than 40 µg/g extract; while, the major identified compounds were 4-*O*-caffeoylquinic acid (7.7–13.7 mg/g extract), rutin (2.7–6.2 mg/g extract), quinic acid (1.8–3.3 mg/g extract) and quercitrin (1.7–3.8 mg/g extract). These differences between the results in the qualitative and quantitative phenolic composition of Moringa leaves could be explained by the edaphoclimatic factors and plant growth stage, as well as by the plant sample pretreatment and extraction technique (Bennour et al., 2020). Changes in climatic conditions increased biotic and abiotic stresses for the living organism, which affects the productivity of medicinal plants. Abiotic stresses caused changes in agro-ecological conditions and affected plant growth and thus the production and accumulation of secondary metabolites (Mahajan et al., 2020).

Phenolic acids and derivatives are interesting bioactive compounds widely distributed in the plant tissues. Recently, it has been reported that they could be applied as functional food ingredients with physiological benefits (Bodoira

et al., 2022; Liu et al., 2022). Quinic acid is a cyclitol that represents a major biochemical intermediate in the shikimate pathway, involved in the biosynthesis of many aromatic compounds in plants. Quinic acid has been reported to have potent antioxidant, anti-inflammatory and hepatoprotective properties (Pero et al., 2009). These authors reported that quinic acid as supplementary food enhanced the nicotinamide and tryptophan synthesis, which improved DNA repair. Furthermore, gallic acid (3,4,5-trihydroxybenzoic acid) and its derivatives were shown to possess several beneficial health effects, such as potent antioxidant, antimicrobial, anti-inflammatory and cardio-protective properties, among others (Al Zahrani et al., 2020). On the other hand, gallic acid or some of its derivatives showed potent inhibitory activity against tyrosinase, a key enzyme in melanin synthesis in mammals, which played an important role in the prevention of skin pigmentation and melanoma (Kubo et al., 2003). Quercetin is one of the major dietary flavonoids belonging to a group of flavonols. which occurs mainly as glycosides. Quercetin-3-O-galactoside (hyperoside) was shown to have potential protective effects on kidney-2 cells against oxidative damage (Chen et al., 2018), as well as against liver diseases (Hu et al., 2020). It was reported that quercetin-3-*O*-rhamnoside (quercitrin) showed cytotoxic activity and inhibited cell migration against the HeLa cells line, which suggest the potential for therapeutic application in cancer treatment (Herni et al., 2021).

3.2. Antioxidant activities

The ferric (Fe^{3+}) reducing power (OD_{700}), DPPH•-radical scavenging activity (%), β-carotene/linoleic acid bleaching activity (%) and metal (Fe^{2+}) chelating capacity (%) were measured (Fig. 1). The obtained results showed that *M. oleifera* extracts exhibited dose-dependent activities in the different antioxidant assays. Table 1 also shows the IC_{50} and $EC_{0.5}$ values for the different assays. The ethanol/water extract, which contained the highest quinic acid content, presented the highest Fe^{3+} reducing power (IC_{50}: 0.5 mg/ml) compared to the other extracts. However, the water extract, which contained the highest gallic acid content, showed the highest DPPH• radical-scavenging (IC_{50}: 0.6 mg/ml), β-carotene/linoleic acid bleaching (IC_{50}: 66 µg/ml) and metal (Fe^{2+})

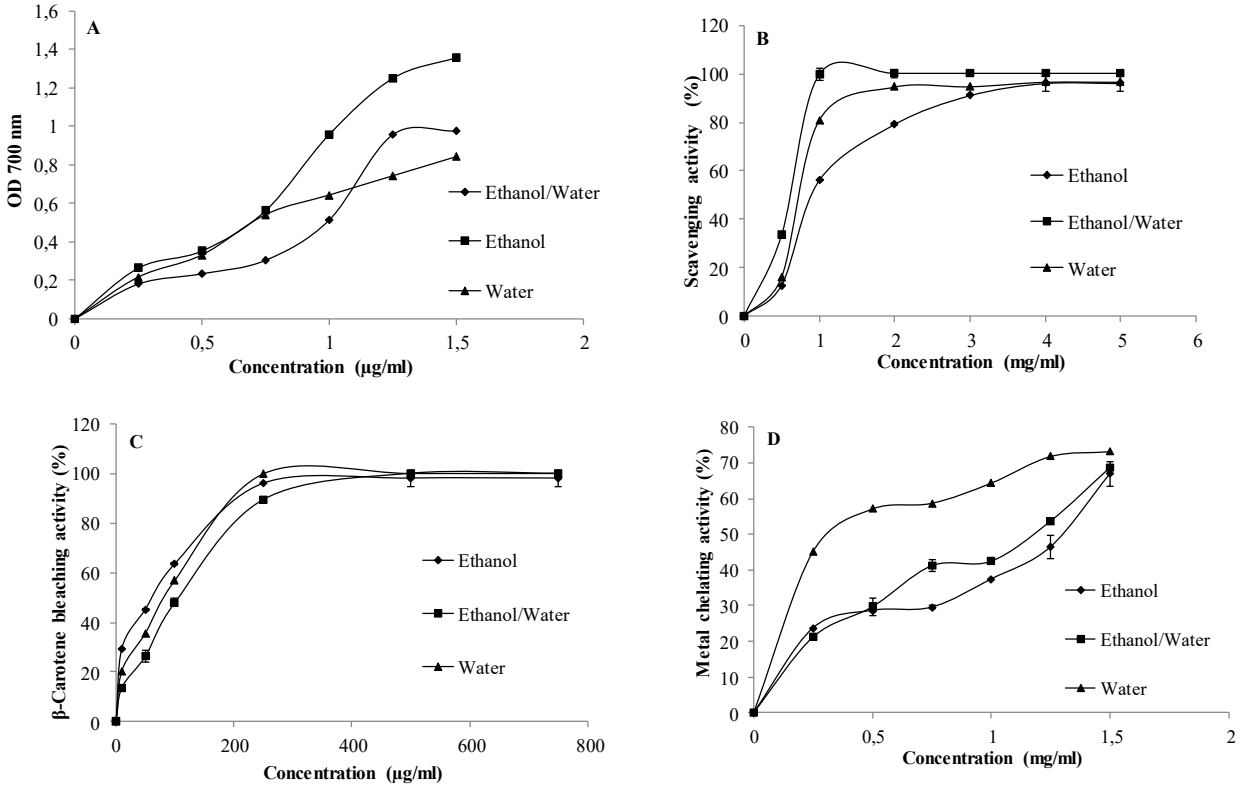

Fig. 1. Antioxidant activities of *M. oleifera* extracts. (A) Ferric (Fe^{3+}) reducing power (OD_{700}); (B) DPPH•-radical scavenging activity (%); (C) β-carotene/linoleic acid bleaching activity (%); (D) Metal (Fe^{2+}) chelating capacity (%).

chelating (IC_{50}: 0.5 mg/ml) activities (Table 1). Bennour et al., (2020) reported lower (Fe^{2+}) chelating activity (IC_{50}: 1.5–2.8 mg/ml); while higher DPPH• scavenging activities (113–140 µg/ml) for different extracts from *M. oleifera* grown in Tunisia. These authors reported that drying and extraction methods had a significant influence on chemical composition and thereby the biological activities of plant extracts.

A survey of the literature shows that most of major compounds identified in *M. oleifera* extracts had potent antioxidant potential in DPPH• radical-scavenging activity. In fact, it was reported that IC_{50} values (µg/ml) of gallic acid, quercetin-3-*O*-galactoside and quercetin-3-*O*-rhamnoside were 0.7 (Mishra et al., 2012), 5.2 (Zhao et al., 2013) and 12.5 (Khanduja and Bhardwaj, 2003), respectively. The present study suggests the appreciable antioxidant activity of the Moringa leaves grown in the oasis of Chenini-Gabes, southeastern Tunisia**.**

4. CONCLUSION

The *M. oleifera* grown in oasis of Chenini-Gabes was a valuable source of phenolic compounds, among which quinic acid, gallic acid, quercetin-3-*O*-galactoside and quercetin-3-*O*-rhamnoside were the major compounds. Moringa leaves can be a source of potent antioxidants that can be exploited for food and nutraceutical applications. Given the potential of this plant, it is interesting to cultivate Moringa in arid and semi-arid regions, such as the oasis environment, which could be a good alternative for diversifying and enriching agricultural products.

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