

Antioxidant Activity of *Moringa oleifera* Tissue Extracts

Andréa F. S. Santos,² Adriana C. C. Argolo,¹ Patrícia M. G. Paiva¹ and Luana C. B. B. Coelho^{1*}

¹Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco (UFPE), Av. Prof. Moraes Rego s/n, 50670-901, Recife, PE, Brazil

²IBB-Institute for Biotechnology and Bioengineering, Centre for Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057, Braga, Portugal

***Moringa oleifera* is an important source of antioxidants, tools in nutritional biochemistry that could be beneficial for human health; the leaves and flowers are used by the population with great nutritional importance. This work investigates the antioxidant activity of *M. oleifera* ethanolic (E1) and saline (E2) extracts from flowers (a), inflorescence rachis (b), seeds (c), leaf tissue (d), leaf rachis (e) and fundamental tissues of stem (f). The radical scavenging capacity (RSC) of extracts was determined using dot-blot on thin layer chromatography stained with a 0.4 mM 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) solution; spectrophotometric assays were recorded (515 nm). Antioxidant components were detected in all E1 and E2 from a, b and d. The best RSC was obtained with E1d; the antioxidants present in E2 reacted very slowly with DPPH. The chromatogram revealed by diphenylborinate-2-ethylamine methanolic solution showed that the ethanolic extract from the flowers, inflorescence rachis, fundamental tissue of stem and leaf tissue contained at least three flavonoids; the saline extract from the flowers and leaf tissue revealed at least two flavonoids. In conclusion, *M. oleifera* ethanolic and saline extracts contain antioxidants that support the use of the plant tissues as food sources. Copyright © 2012 John Wiley & Sons, Ltd.**

Keywords: antioxidant activity; *Moringa oleifera*; radical scavenging capacity; DPPH.

INTRODUCTION

Moringa oleifera (Lam), Moringaceae family is a plant rich in antioxidant compounds (Santos *et al.*, 2005; Iqbal and Bhangar, 2006; Arabshahi-D *et al.*, 2007; Chumark *et al.*, 2008; Amaglo *et al.*, 2010). Antioxidants are of great importance in preventing stress that may cause several degenerative diseases (Helen *et al.*, 2000). On the other hand, antioxidants are molecules capable of stabilizing or deactivating free radicals before they attack cells (Kaliara *et al.*, 2006). Food antioxidants are important for human nutrition, decreasing oxidative damage to lipids, proteins and nucleic acids induced by free radicals (Soler-Rivas *et al.*, 2000). The frequent consumption of fruits and vegetables is associated with a lowered risk of cancer, heart disease, hypertension and stroke; the effect has been attributed to the presence of phytochemicals and antioxidants present in foods including flavonoids and anthocyanins (Lako *et al.*, 2007).

M. oleifera has been used in human and animal nutrition. The young leaves, flowers and green pods are common vegetables in many countries, particularly in India, Pakistan, Philippines, Hawaii and many parts of Africa (Guevara *et al.*, 1999; Anwar *et al.*, 2007). Amaglo *et al.* (2010) showed that tissues of *M. oleifera* had a relatively complex flavonoid profile consisting of glucosides, rutinosides, malonylglucosides and traces of acetylglucosides of kaempferol, quercetin and

isorhamnetin. Leaves are consumed by South East Asian populations and are believed to have beneficial effects on improved vision and the prevention of eye diseases (Liu *et al.*, 2007). In Nigeria, *M. oleifera* leaves are eaten as vegetables without any reported side effects and are also eaten commonly by infants and children in South India; the high content of β -carotenes helps to prevent the development of vitamin A deficiency (Ghasi *et al.*, 2000). According to Makkar and Becker (1996), *M. oleifera* leaves had negligible antinutritional factors; tannins, trypsin inhibitors and lectins were not detected. Richter *et al.* (2003) showed that *M. oleifera* leaves could be used to substitute up to 10% of the dietary protein in Nile tilapia without a significant reduction on growth. Hypocholesterolemic (Ghasi *et al.*, 2000; Chumark *et al.*, 2008), hypotensive (Faizi *et al.*, 1995), antiatherosclerotic (Chumark *et al.*, 2008) and antioxidant activities with linoleic acid, α -tocopherol and sunflower oil (Arabshahi-D *et al.*, 2007) were also found in the leaf extract.

In this report the antioxidant activity from *M. oleifera* ethanolic and saline extracts was evaluated.

MATERIALS AND METHODS

Chemicals. Ethanol, methanol, ethyl acetate, formic acid, acetic acid and rutin were purchased from Merck (Darmstadt, Germany). Epicatechin, epicatechin gallate, epigallocatechin gallate and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were acquired from Sigma Chemical Co. (St Louis, MO, USA). All other reagents were of analytical grade.

* Correspondence to: Luana C. B. B. Coelho, Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Pernambuco (UFPE), Av. Prof. Moraes Rego s/n, 50670-901, Recife, PE, Brazil.
E-mail: lcbcoelho@gmail.com

***M. oleifera* extract preparations.** Tissues from *Moringa oleifera* were collected in Recife City, State of Pernambuco, and Northeast of Brazil. A sample of the collected material is archived as voucher specimen number 63184, IPA, at the herbarium 'Dárdano de Andrade Lima' (Empresa Pernambucana de Pesquisa Agropecuária, Recife City, Brazil). The ethanolic (E1) and saline (E2) extracts were prepared from dried and powdered flowers (a), inflorescence rachis (b), seeds (c), leaf tissue (d), leaf rachis (e) and fundamental tissues of stem (f). After filtration the extracts were evaporated to dryness, dissolved in methanol and evaporated at room temperature.

Determination of antioxidant activity using DPPH radical. The qualitative assay to evaluate the radical scavenging capacity (RSC) of extracts (E1 and E2, 5 mg/mL, 2 μ L) and standards (1 mg/mL, 2 μ L) epicatechin, epicatechin gallate and epigallocatechin gallate were determined (Soler-Rivas *et al.*, 2000) using dot-blots on thin layer chromatography (TLC) plates (Merck, Darmstadt, Germany; silica gel 60 F254 20·10 cm aluminium backed), stained with a 0.4 mM DPPH solution. The negative control was pure methanol used to prepare the extracts. Dissolved samples were diluted (1:2, 1:4, 1:8 and 1:16) in order to avoid the background color of the samples which could mask the reaction with DPPH, applied in triplicate and left to dry (2–3 min). The TLC sheet was immersed upside down in a 0.4 mM DPPH methanol solution for 2 s. The stained layer (purple) revealed yellow spots where drops were placed depending on the RSC of samples. The intensity of the yellow staining depended upon the amount and nature of the radical scavengers present in the sample.

Spectrophotometric analysis to evaluate radical-scavenging capacity. Quantitative assays were performed by spectrophotometer analysis of extracts recorded using the stable DPPH radical. The procedure followed the method of Brand-Williams *et al.* (1995) and Soler-Rivas *et al.* (2000) with some variations. For each extract (E1 and E2) different concentrations were tested. Reactions were monitored for 30 min by the disappearance of DPPH, the time during which this radical was stable. The analysis was used to determine IC₅₀ and IP of samples which are parameters used widely for determining the RSC from plant extracts. The IC₅₀ is the amount of antioxidant

necessary to decrease the initial concentration of DPPH (90 μ M) by 50%. IP was the amount of total DPPH (90 μ M) reacting with antioxidant at a steady state. An aliquot (20 μ L) of each extract (5 mg/mL, 2.5 mg/mL and 1.25 mg/mL) was mixed separately with 90 μ M methanol solution of DPPH to a final volume of 1 mL. An equal volume of pure methanol was added to the control tube. The disappearance of DPPH was monitored by the decrease in absorbance at 515 nm, which was recorded after 0, 1, 2, 3, 4 and 5 min, and subsequently every 5 min up to 30 min. The experiments were performed using a Perkin-Elmer Lambda-2 UV-Vis spectrophotometer (Toronto, Canada). The concentration of DPPH in the reaction mixture was calculated from a calibration curve according to the following linear regression equation ($r = 0.99962$): $A_{515 \text{ nm}} = 0.0518 + 0.00923 [\text{DPPH}]$, where [DPPH] is expressed in mg/mL.

The percentage of the remaining DPPH (%DPPH_{REM}) was calculated according to Brand-Williams *et al.* (1995), as follows: %DPPH_{REM} = $[\text{DPPH}]_T / [\text{DPPH}]_{T_0} \times 100$, where T is the time at which the absorbance was determined (1–30 min) and T₀ is the zero time. The IC₅₀ was calculated by plotting the %DPPH_{REM} at steady state (20 min) against various concentrations of each extract. The results were expressed as mg antioxidant/g DPPH \pm standard deviation. An aliquot (50 μ L) of each sample (IP determination) was then added to 2 mL of a 90 μ M methanol solution of DPPH radical and the absorbance was determined (515 nm) at the steady state. The IP was calculated according to the expression: $\text{IP} = [(A_{T_0} - A_{T_S}) / A_{T_0}] \times 100$, where A_{T₀} is the absorbance at zero time and A_{T_S} is the absorbance in the steady state. The results were expressed as % of inhibition.

Thin layer chromatography. In order to determine the number of active antioxidant compounds, a drop (15 μ L) of ethanol and saline extracts together with positive controls were placed individually on the base line of a TLC plate which was eluted with ethyl acetate/formic acid/acetic acid/H₂O (100/11/11/26, v/v/v/v). Spots were visualized under 365 nm UV light (UVP, Model UVGL-25, Upland, CA, USA) and, after treatment with a 0.4 mM methanol solution of DPPH, with 1% methanol solution of diphenylborinate-2-ethylamine specific to flavonoids using rutin as a positive control.

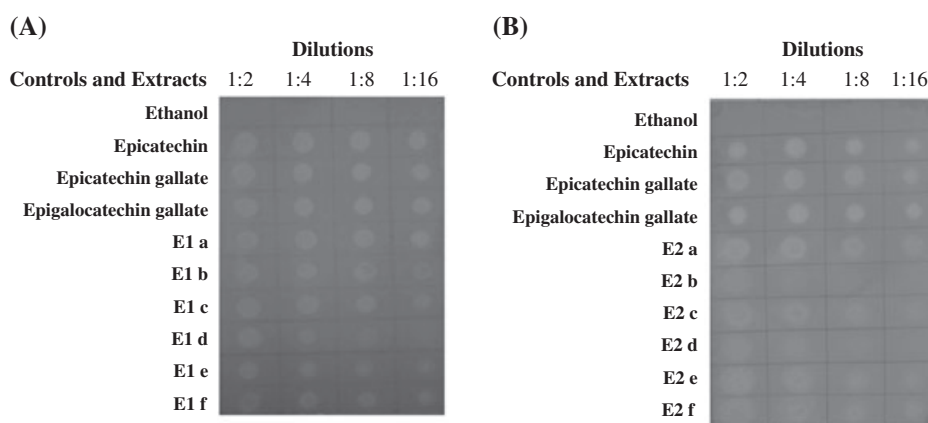


Figure 1. Qualitative assay using silica gel TLC plates stained with 0.4 mM methanolic solution of DPPH. Standard concentrations: 1.0, 0.5, 0.25 and 0.125 mg/mL; extract concentrations: 2.5, 1.25, 0.625 and 0.312 mg/mL. (A): Ethanolic, E1 and (B): saline, E2 extracts: Flowers (a), fundamental tissues of stem (b), leaf rachis (c), seeds (d), inflorescence rachis (e), and leaf tissue (f).

Chemical assays of *M. oleifera* extracts. The extracts were analysed using chemical tests with various reagents for classes of compounds (phenols; flavonones; flavonoids, flavanonols, flavanols, free xanthenes and heterosides; flavones, flavonols and xanthenes; free steroids; pentacyclic triterpenes; leucoanthocyanidins) normally associated with antioxidant activity following protocols described by Matos (1988).

Statistical analysis. Values of experimental results shown in the tables and figures were the mean of at least three determinations (\pm standard deviation). Linear regression equations were established using the Origin version 6.0 program (Microcal, Northampton, MA, USA).

RESULTS AND DISCUSSION

M. oleifera is a plant of multipurpose uses; some tissues are used by the population in the diet and the seeds are applied to water treated for human consumption. These plant properties stimulated the present study to evaluate *M. oleifera* tissues as sources of natural antioxidants.

There is an increasing interest in antioxidants due to the presumed capacity to prevent the deleterious effects of free radicals in the human body and a preference for antioxidants from natural instead of synthetic sources. Studies have shown that diets rich in vegetables and fruits can reduce the risk of many diseases and these effects have been associated with the presence of antioxidant compounds, which makes them important tools in nutritional biochemistry. Phenolic compounds are naturally occurring substances in fruits, vegetables, nuts, seeds, flowers and some herb beverages; they constitute an integral part of the human diet (Katalinić *et al.*, 2004). In view of the diversity among the number of antioxidant assays available, the results of a single assay can suggest the antioxidant property of plant extracts (Brand-Williams *et al.*, 1995). The relatively stable DPPH radical has been used widely to test the ability of compounds to act as free radical scavengers or hydrogen donors and thus to evaluate antioxidant activity.

Antioxidant radicals formed are stabilized through the formation of nonradical products (Argolo *et al.*, 2004). Compounds with radical scavenger capacity (RSC) are able to reduce DPPH as well as the type and concentration of antioxidant in samples, interfere with the time of appearance and final intensity of yellow staining (Soler-Rivas *et al.*, 2000). Antioxidant components were detected in all *M. oleifera* ethanolic and saline extracts from flowers, inflorescence rachis and leaf tissue (Fig. 1) by DPPH reduction after 30 min. The methanolic extract showed high (DPPH)

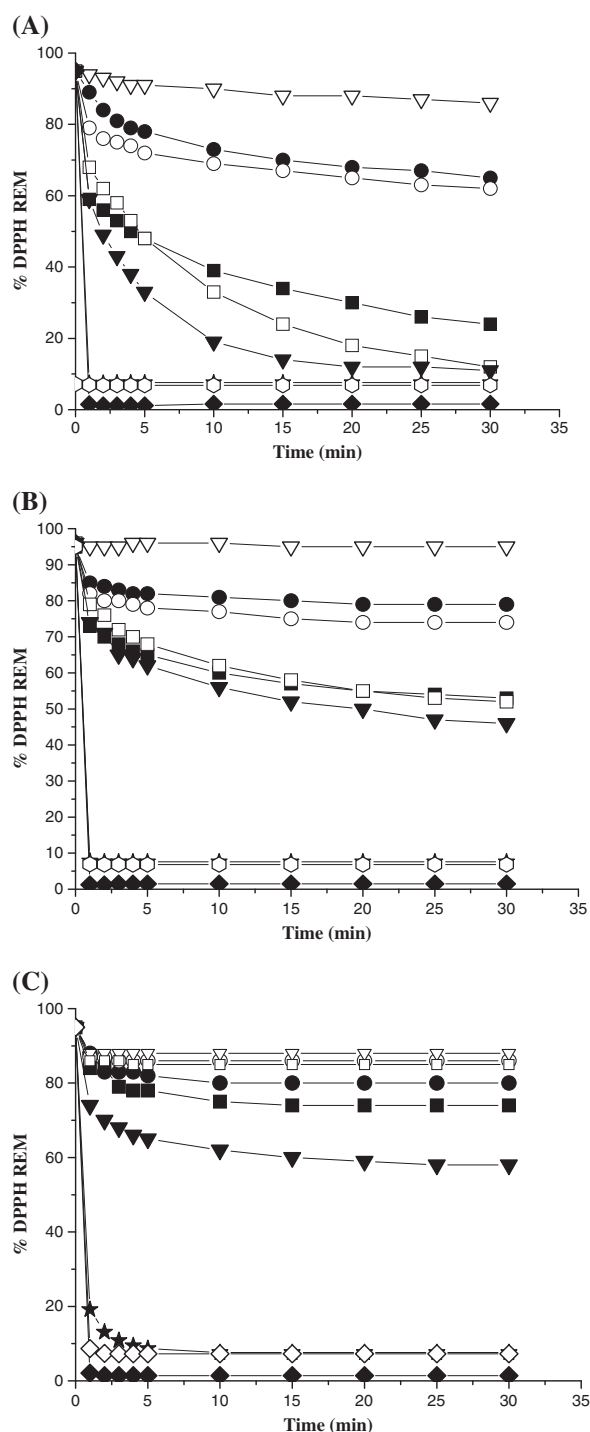


Figure 2. Kinetic behaviour of ethanolic extracts from *M. oleifera*. Reaction (515 nm) was performed with 5 mg/mL (A), 2.5 mg/mL (B) and 1.25 mg/mL (C) in a methanolic solution of DPPH. Samples: (○) flowers, (□) inflorescence rachis, (▽) seeds, (▼) leaf tissue, (■) leaf rachis, (●) fundamental tissues of stem, (★) epicatechin, (◆) epigallocatechin gallate, and (◇) epigallocatechin gallate.

Table 1. Percentage of total DPPH reacting with antioxidant ethanolic extracts and amount of antioxidant from extracts decreasing initial concentration of DPPH by 50%

Extract	IP (%)			IC ₅₀ (mg/g DPPH)
	5 mg/mL	2.5 mg/mL	1.25 mg/mL	
a	35.0	25.4	13.8	0.210
b	31.7	20.4	20.8	0.300
c	69.7	44.6	20.0	0.090
d	10.8	2.6	10.8	2.920
e	81.2	43.6	12.4	0.008
f	86.9	49.5	39.9	0.060

Flowers (a), fundamental tissues of stem (b), leaf rachis (c), seeds (d), inflorescence rachis (e) and leaf tissue (f). IP extracts (5 mg/mL, 2.5 mg/mL and 1.25 mg/mL) were calculated at the steady state (20 min). IC₅₀ was calculated at the steady state (20 min). Values represent the mean of three assays (\pm standard deviation).

Table 2. Classes of compounds detected in *M. oleifera* extracts evaluated in this study

Extract	Phenols	Flavonones	Flavonoids, flavanonols, flavanonois, free xanthonnes, heterosides	Flavones, flavonoids, xanthonnes	Free steroids	Pentacyclic triterpenes	Leucoanthocyanidins
E1 a	+	+	+	+	+	-	-
E1 b	-	-	-	+	-	-	-
E1 c	+	+	+	+	-	+	-
E1 d	-	+	-	+	-	-	-
E1 e	+	+	-	+	+	-	-
E1 f	+	+	+	+	-	+	-
E2 a	+	+	+	+	ND	ND	-
E2 e	+	+	-	+	ND	ND	-
E2 f	+	+	-	-	ND	ND	-

Ethanollic (E1) and saline (E2) extracts: Flowers (a), fundamental tissues of stem (b), leaf rachis (c), seeds (d), inflorescence rachis (e), and leaf tissue (f). +, detected; -, not detected; ND, not determined.

radical-scavenging activity similar to *Ferula orientalis*, Apiaceae (Kartal *et al.*, 2007).

Quantitative analysis revealed that the ethanolic extracts of leaf rachis, inflorescence rachis and leaf tissue reduced DPPH faster than the other extracts; the ethanolic extract of seeds reacted very slowly (Fig. 2). When the antioxidant activity of the *M. oleifera* leaf ethanol extract was assayed with linoleic acid, α -tocopherol and sunflower oil a strong reaction was revealed (Arabshahid *et al.*, 2007). This behavior was correlated with the nature and concentration of the antioxidant (Argolo *et al.*, 2004). The steady state was reached in less than 20 min for all ethanol samples (Fig. 2); the saline extracts reacted very slowly.

The median inhibitory concentration (IC_{50}) and inhibition percentage (IP) values are considered to be good measures of the antioxidant efficiencies of a compound. The seed ethanolic extracts revealed the highest IC_{50} , therefore, a high dose of extract is required; the IP values showed that the ethanolic extract of leaf rachis, inflorescence rachis and leaf tissue reacted with more than 60% of total DPPH up to steady state (Table 1). The ethanolic extract of flowers, fundamental tissues of stem and seeds reacted with less than 40%. Antioxidants present in the saline extracts reacted very slowly with the DPPH radical. A lichen extract of *Cetraria islandica* showed an IP value of 100% peroxidation inhibition of a linoleic acid emulsion (Güçin *et al.*, 2002) and the leaf extracts from *Bauhinia monandra* presented IP values in the range of 60% (Argolo *et al.*, 2004).

Table 2 shows a summary of the classes of compounds that were detected by chemical assays in *M. oleifera*. Flavonoids were identified in ethanolic and saline extracts; steroids and triterpenoids were only present in ethanol extracts. The ethanol extract from the leaves of *B. monandra* are used widely for treating human diabetes and also showed an antioxidant property (Argolo *et al.*, 2004).

The chromatogram revealed with a methanolic solution of diphenylborinate-2-ethylamine showed that the flowers, inflorescence and leaf rachis as well as the leaf tissue ethanolic extracts contained at least three flavonoids with the same R_f values: 0.18, 0.38 and 0.44, respectively (Fig. 3). In agreement with the findings of antioxidant activity from *M. oleifera*, Lako *et al.* (2007) reported that the leaves showed a high antioxidant capacity and are rich in total polyphenol content, quercetin, kaempferol and β -carotene. Phenolics and

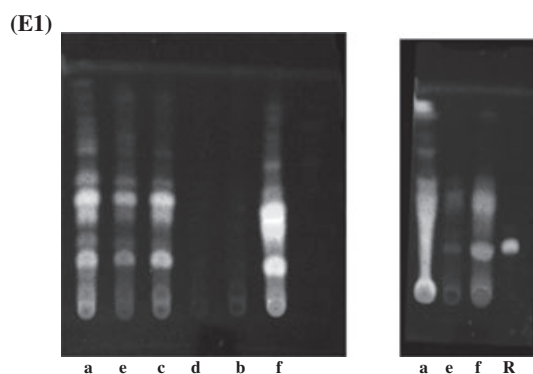


Figure 3. Chromatogram revealed with a methanolic solution of diphenylborinate-2-ethylamine specific to flavonoids. E1: Ethanolic and E2: Saline extracts: flowers (a), fundamental tissues of stem (b), leaf rachis (c), seeds (d), inflorescence rachis (e) and leaf tissue (f). R, Rutin.

polyphenolic compounds constitute the main class of natural antioxidants and are believed to be the major class responsible for the antioxidant activity of plant materials (Kanatt *et al.*, 2007).

The saline extracts of flower and leaf tissue showed at least two flavonoids with R_f values of 0.35, 0.63 and 0.16, 0.34, respectively (Fig. 3). When DPPH was used to reveal the chromatogram, the presence of antioxidant components was confirmed in the extracts.

CONCLUSION

In the present work it was found that *M. oleifera* ethanolic and saline extracts from distinct plant tissues are potential sources of antioxidants.

The antioxidant activity was stronger in the ethanolic than in the saline extracts. The leaf tissue ethanolic extract showed the best scavenging capacity to DPPH radical.

The antioxidant properties of *M. oleifera* tissues provide more useful applications of many parts of this plant in the human diet.

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Conflict of Interest

The authors have declared that there is no conflict of interest.

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