Total phenolic and antioxidant capacity of flower, leaf and seed of *Moringa oleifera*
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ABSTRACT

The *Moringa oleifera* it is considered one of the most useful trees in the world because almost all parts of this plant can be used as food, in medicines and for industrial purpose. Is versatile as a medicine, functional food and nutraceutical. Thus, the objective was to estimate the total phenolic content and antioxidant capacity of the flower, leaf and seeds of *Moringa oleifera* Lam. Were purchased flowers, leaves and seeds of *Moringa oleifera* Lam., in the city of Seropédica, Rio de Janeiro, Brazil and harvested in January 2013. The determination of total phenolic content was assessed following to the Folin-Ciocalteu assay and the antioxidant capacity was determined by the method of DPPH (2,2-defenil-1-picrilidrazil). It has been verified that the content of phenolic compounds from the leaf of *Moringa oleifera* (170.07 ± 0.43 mg /100 g gallic acid) were better when compared to the results found in the flower and seed (114.49 ± 3.95 mg / 100 g gallic acid and (22.43 ± 2.35 mg / 100 g gallic acid), respectively. The leaf presented a kidnap largest free radical antioxidant compared in flower and seed. The present study suggests that *M. oleifera* could be a potential source of compounds with strong antioxidant potential. Hence consumption of diet supplemented with *M. oleifera* could protect against diseases induced by oxidative stress.

**Keywords:** *Moringa oleifera*, Phenolic, Antioxidant Capacity, Functional Food
Introduction

In 2012, *M. oleifera* leaf was approved as a new food resource by ministry of health of the people’s republic of China (MOH) for its high nutritional value. It was reported that the vitamin E content is up to 116.79 mg/100 g dry weight (1).

*Moringa oleifera* Lam (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an impressive range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, β-carotene, amino acids and various phenolics (2). A wide variety of nutritional and medicinal virtues have been attributed to its roots, bark, leaves, flowers, fruits, and seeds (3).

Flowers of *Moringa oleifera* are rich in calcium, potassium and antioxidants (α and γ-tocopherol), and are used in human diet, mainly in the Philippines (4). *Moringa oleifera* leaf powder is said to give a child the following recommended daily allowances: protein 42%, calcium 125%, magnesium 61%, potassium 41%, iron 71%, vitamin A 272%, and vitamin C 22%. Gram for gram, *Moringa oleifera* leaves contain seven times the vitamin C in oranges, four times the calcium in milk, four times the β-carotene in carrots, twice the protein in milk and three times the potassium in bananas (5).

Seeds of *M. oleifera* have antihyperlipidemic and antitumor activities, are used in liver dysfunction, cardiovascular and hematological disorders having significant antioxidant and diuretic activities (6). The oil extracted from the seeds of *M. oleifera* is composed of 82% unsaturated fatty acids, 70% of which is oleic acid. This oil contains the same fatty acid profile as olive oil except for linoleic acid (7).

Various preparations of *M. oleifera* exhibited antibiotic, hypotensive, anti-ulcer, anti-inflammatory, anti-cancer properties (8), diuretic, antimicrobial, antioxidant, antidiabetic, antihyperlipidemic, antineoplastic, antiulcer, cardioprotectant, and hepatoprotectant activities (9;10). The edible leaves of *M. oleifera* tree have been known as an anti-diabetic food for centuries (8).

The main purpose of this work was to estimate the total phenolic content and antioxidant capacity of the flower, leaf and seeds of *Moringa oleifera* Lam.

Material and methods

Obtaining the flowers, leaves and seeds of Moringa oleifera, were purchased flowers, leaves and seeds of *Moringa oleifera* Lam., in the city of Seropédica, Rio de Janeiro, Brazil and harvested in January 2013.

Antioxidant Properties

Extracts preparation

The extracts were obtained according to (14;15) with minor modifications. 6 g of sample were diluted with ethanol 70%, in volumetric flasks (100 mL). These solutions were subjected to magnetic stirrer at 25 °C for 1 h, and then vacuum filtered using a sintered filter funnel (n. 3).

Determination of Total Phenolic Compounds

The total phenolic content was assessed following to the Folin-Ciocalteu assay (16;17). The results were expressed in gallic acid equivalents (GAE; mg/100 g fresh mass) using a gallic acid (0.05 to 1.2 mg/mL) standard curve. All analysis was performed in triplicate.

DPPH Scavenging Activity

The antioxidant capacity was assessed following to the DPPH method described by (18), which is based on the quantification of free radical-scavenging, with modifications. A methanol solution containing 0.06 mM DPPH was prepared. After adjusting the blank with methanol, an aliquot of 100 μL of samples extract was added to 3.9 mL of this solution. The absorbance was measured using an UV Spectrophotometer NEW 2000 (São Paulo, Brazil) at the 517 nm. The amount antioxidant capacity was expressed...
Figure 1. Flowers (a), leaves (b) and seeds (c) of *Moringa oleifera*.

Table 1. Phenolic compounds and antioxidant capacity of *Moringa oleifera* *

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<th>Flower</th>
<th>Leaf</th>
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<tr>
<td><strong>Phenolic (mg/100g of gallic acid)</strong></td>
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<td></td>
<td>114.49±3.95&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170.07±0.43&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.43±2.35&lt;sup&gt;c&lt;/sup&gt;</td>
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<td><strong>Antioxidant capacity (µM Eq. Trolox/g)</strong></td>
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<td></td>
<td>21.96±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.25±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.13±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>%FRS</td>
<td>31.41±0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.03±1.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>13.25±0.17&lt;sup&gt;c&lt;/sup&gt;</td>
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*Average ± RI= reliable interval for a statistical probability of 95%; Where same letter on the same line do not present significant differences (p<0.05) among each other. The results presented in FRS (Percent of free radical-scavenging); Eq. (Equivalent); µM (micromolar). Each value is presented as mean ± standard deviation (n = 3); Means within each row with different letters (a–c) differ significantly (p<0.05); *in natura.
as µM of Trolox Equivalent per 100 g of sample (dry basis). The free radical-scavenging (%FRS) of each sample was calculated according to Eq. 1. Where: \( A_c \) and \( A_s \) are absorbance values of blank and sample, respectively. All analysis was performed in triplicate.

\[
\text{%FRS} = \left( \frac{A_c - A_s}{A_c} \right) \times 100
\]

Statistical Analysis

The results were verified by variance analysis (ANOVA). The chemical and physical tests were analyzed by variance and Tukey test at 5% of significance level for averages comparison.

Results and discussion

Table 1 shows the results of analyzes of phenolic compounds and antioxidant capacity of Moringa oleifera.

The phenolic content and antioxidant capacity of the Moringa oleifera leaf was higher (170.07±0.43 mg/100g of gallic acid; 29.25±0.42 µM Eq. Trolox/g) than that found in the flower and seed. It is also observed that the greatest free radical-scavenging was of leaf of Moringa oleifera (36.03±1.37%).

The results of present study was higher (170.07±0.43 mg/100g of gallic acid) than found by other autors, where in the extract of Moringa oleifera leaf found total phenolics content of 105.04 mg gallic acid equivalents (19). The results of our study corroborate the results of other studies discussed below.

Sohaimy et al. (20) showed that among the different extracts of the Moringa oleifera leaves, methanol extract had the highest amount of TPC (48.35 mg GAE/g). Similarly, the results revealed significant variation in total phenolic contents in different extracts of Moringa oleifera samples collected from different regions of Punjab province of Pakistan . The highest TPC was present in methanol extract (53.3±0.169 mg GAE/g ), while lowest in chloroform extract (14.1 mg GAE/g) (21). Alhakmani et al. (22) reported low TPC (19.31 mg GAE/g) in ethanol extract of Moringa oleifera flower pods. The comparable results were also recorded in the study (22) as low TPC (25.93 mg GAE/g) in ethanol extract was noticed compared to methanol extract (48.35 mg GAE/g) (23).

Although the samples showed a significant antioxidant activity, it was verified that they obtained a percentage of free radical sequestration (% SRL) considered low. That may be related to the product collection period (summer), once studies have shown, that the samples collected in the cold period has a better nutritional quality.

Agroclimatic locations and seasons have profound effects on the antioxidant activity of M. oleifera leaves from Pakistan. Antioxidant activity of samples from cold areas was relatively higher than those from temperate regions. Similarly, from all the samples, antioxidant activity was highest in December (cold month) and lowest in June (hot month), with few exceptions. These findings suggest that environmental temperature has a significant effect on antioxidant activity evaluation. However, there is still a need to investigate effects of soil properties on antioxidant activity of Moringa leaves (24).

Other question, in the present research was used 70% ethanol to extraction of antioxidant compounds from moringa leaves, suggesting new studies aiming at a better extraction of the antioxidant potential present in Moringa.

Considering various factors involved in the extraction process, maceration with 70% ethanol was advantageous to other methods with regards to simplicity, convenience, economy, and providence of the extract containing maximum contents of total phenolics and total flavonoids with the highest antioxidant activity. Maceration and 70% ethanol were recommended as the extraction method and solvent for high quality antioxidant raw material extract of M. oleifera leaves for pharmaceutical and nutraceutical development (25). According other autors both methanol (80%) and ethanol (70%) were found to be the best solvents for the extraction of antioxidant compounds from moringa leaves (26).

Thus, M. oleifera leaves, fruits, roots, and seeds are sources of biologically important phytochemicals and may be used as a consummate nutraceutical molecule, because of its innumerable desirable
biological properties. There is an extended interest in using natural antioxidant compounds, as the consumer’s pressure on food industry augments, to avoid chemical preservatives, due to the increasing evidence implies that synthetic antioxidant produce toxicity (19).

**Conclusão**

The present study suggests that M. oleifera could be a potential source of compounds with strong antioxidant potential, being that the antioxidant potential may be attributed to the presence of polyphenolic compounds, both in leaf, seed or flower. Hence consumption of diet supplemented with M. oleifera could protect against diseases induced by oxidative stress. Where the protective effect of M. oleifera may explain its extensive use in life and to possible health benefits. It is suggested with this research, to investigate the antioxidant potential of the moringa in other seasons, as in winter; In addition the use of other solvents would be necessary, aiming for a better extraction power.

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