Testing the Antioxidant Activity of Various Leaf Extracts from Young Moringa oleifera Lam. Plants Grown in a Temperate Climate Zone

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Article

Testing the Antioxidant Activity of Various Leaf Extracts from Young *Moringa oleifera* Lam. Plants Grown in a Temperate Climate Zone

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Abstract: Moringa (*Moringa oleifera* Lam.) is a tree that grows in tropical and subtropical regions. In this study, the plants were grown in a temperate climate zone from seeds collected at the Island of St. Lucia. Cultivation was carried out in the field and in a greenhouse in Prešov, East Slovakia. Leaf samples were taken from young plants and dried naturally. In the ethanol and hot water extracts of the leaves, the dry matter, total phenolic substances and antioxidant activity were determined using three methods: superoxide anion radical scavenging activity, hydroxyl radical scavenging activity and ferric reducing ability of plasma (FRAP) assay. The highest amount of total phenols was detected in the ethanolic extract of the leaves from the field, the lowest amount was noticed in the leaves from the greenhouse. The amount was significantly lower in the aqueous extracts. A high antioxidant activity of the leaves from the field was detected in all ethanolic and hot water extracts. Both types of leaf extracts from the greenhouse showed statistically significant lower antioxidant activity. The obtained results indicate that outdoor cultivation in a temperate climate zone was stressful for the plants, leading to an increased formation of phenolic substances, and consequently to higher antioxidant activity.

Keywords: antioxidant activity, ecological factors, free radicals, moringa, total phenols

1. Introduction

The genus *Moringa* belongs to the *Moringaceae* family, and include 13 species well as *Moringa oleifera*. It thrives in almost all tropical and subtropical regions, but is believed to be native to Afghanistan, Bangladesh, India and Pakistan [1]. The tree is also widespread in Africa, Southeast Asia and South America [2]. It is also grown in other countries such as Pakistan, Thailand and the Philippines [3] and has also been commercialized in Mexico, Hawaii, Cambodia and the Caribbean Islands [4]. Since ancient times, people all over the world have used this plant as part of their diet. Various medicines made from moringa have been described and have been used for ethnomedicinal purposes for centuries [1]. Many pharmacological studies report that various extracts of this plant show significant biological activities, such as anti-oxidant, anti-microbial, anti-inflammatory, anti-cancer, analgesic, cardiovascular, antiulcer, immunomodulatory, anti-hypertensive, hepatoprotective, diuretic, anturolithic, anthelmintic, hypoglycemic, anti-diabetic, anti-asthmatic, and anti-ageing potentials [6-10].

Moringa is referred to as a miracle tree, a source of raw materials for commercial functional food and non-food products. It is reported that this plant plays an important role in the UN Sustainable Development Goal 2 (SDG 2) on food security to ensure better, healthier and more diverse diets from sustainable agricultural systems [11]. All parts of the plants are used: wood, bark, roots, leaves, flowers, pods (fruits) and seeds [12]. The individual parts of this plant have a high content of proteins, lipids, vitamins, minerals and total polyphenols, including flavonoids. The seeds, in particular are, a rich source of proteins and lipids. The content of essential amino acids is considerable [9,13]. Due to

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its high nutritional value, it is a component of food in many countries. Moreover, it is used in the perfume industry, cosmetics industry, for the production of biofuels and for water purification, in the paper industry, the textile industry, the pharmaceutical industry, while in agriculture is utilized as an organic bio stimulant, an insecticide, as feed for farm animals and as green manure [14-18].

Moringa is a fast-growing tree with low environmental requirements. It is a robust plant that can grow in a variety of soils and climatic conditions. It grows successfully in dry to humid tropical or subtropical climates at altitudes from 0 to 1,400 meters above sea level and above. The annual rainfall required for growth is given as 760-1500 mm. The optimum temperature is 18-28°C [19,20]. It is reported to tolerate light frost and higher temperatures up to ~48°C in the shade [21]. It is adaptable to climatic conditions and can even grow in poor soils [22]. For optimal growth, it requires well-drained, sandy or loamy soils. Flooded or waterlogged conditions are not suitable. It has a high tolerance to soil pH values between 5.0 and 9.0 [23,24]. The tree begins to bear fruit at the age of 6 to 8 months [7].

Various *in vitro* methods are described in the literature to determine antiradical and total antioxidant activity. Different types of extracts are tested with different extraction agents. Different parts of the plant are used for this purpose [9,17,21,25]. Many studies have shown a significant positive linear correlation between antioxidant activity and total phenolic content [26,27]. A positive correlation between total phenolic content and antiradical activity was also found in the first study conducted with commercial samples of seeds and leaves from the Caribbean region, the Island of Saint Lucia [9]. Following this investigation, the antiradical and antioxidant activity of Moringa leaves from young plants (5 months old) was tested *in vitro*. They also come from the Island of Saint Lucia. Two types of extracts were studied, ethanolic and aqueous [28]. This study is followed by the present article, which evaluates the content of total phenols, the antiradical and antioxidant activity of ethanolic and aqueous extracts. The samples of young plant leaves used for the extraction were grown in a temperate climate zone in open ground and in a greenhouse in Prešov, East Slovakia.

2. Materials and Methods

2.1. Characteristics of the Cultivation Area

Moringa plants were cultivated at locality Presov - east Slovakia at the experimental field of Prešov University in Prešov (N 48° 59.382', E 21° 13.576', 253 m above sea level). Mean annual precipitation is 603 mm and mean annual temperature is 7.7 °C. The soil type of the site is a typical fluvi-soil formed by alluvial floodplains near the Torysa River. The soil is moderate with a neutral reaction and a good supply of acceptable nutrients, free of skeleton (skeleton content up to 0.6 m below 10%), deep (60 cm or more). The soil-forming substrate forms alluvial deposits near the Torysa River. The slope inclination is 0° -1°, the level without surface water erosion.

The soil and climatic characteristics are described on the basis of the BSEJ (Bonited Soil-Ecological Units) code 0757202 [29] and decoded according to [30]. Locality is a relatively warm, dry, basaltic and continental region. The average annual precipitation is 630 mm and the average annual temperature is 7.7 ° C. The average coldest month is January with an average monthly temperature of -3.5°C, the warmest month is August with an average monthly temperature of 19°C. In the last five years, the lowest average monthly temperature reached -5.6°C. The temperature for the growing season (TS ≥ 10 ° C) is 2800 - 2500. The average temperature of the air during the growing season (IV - IX. = T vegetation °C) is 14 - 15 ° C.

Soil acidity is neutral (pH 7.0) with medium phosphorus content, good potassium content and low magnesium content.

2.2. Plant Material

Seedlings were grown in the greenhouse from the seeds collected at the volcanic Caribbean island of St. Lucia. In May, the seedlings were separated and planted outside – in open-air field as well as in the greenhouse. Cultivation lasted until the end of September. The leaves were harvest-ed from young, five-month old plants, dried naturally and stored in a cool place until analysis.

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2.3. Extracts Preparation

Five grams of dried, crushed leaves were dissolved in 100 mL of 70% ethanol. The extraction was carried out for 72 hours at room temperature. The extracts obtained were filtered through filter paper type KA 1-M (very fast). The hot (distilled) water extracts ("tea") of moringa leaves were prepared by weighing one gram of dried crushed leaves and extracting in 100 mL of boiling water. The extraction time was 15 minutes. After cooling and filtration, the extracts were used for analysis.

2.4. Total Phenols and Antioxidant Activity

The total phenolic content of the ethanol and hot water extracts of the leaves was determined with the Folin-Ciocalteu reagent (FCR, Merck) according to a method described by [31]. The radical scavenging activity of superoxide anions was inspired by the work of [32]. The test for scavenging hydroxyl radicals was carried out according to [33]. The FRAP test (Ferric reducing ability of plasma) was carried out according to [34]. All methods are described in detail in [28]. The dry matter (DM) content was determined according [9].

2.5. Statistical Analysis

The data obtained were analyzed using analysis of variance ANOVA with the statistical program STATISTICA 12 using the LSD method (Least Significant Difference). The analysis of variance provided indications of differences between the various extracts.

3. Results

The results of total phenolic content, antiradical and antioxidant activity tested in vitro by three methods are shown in Table 1.

The ethanol extract from leaves grown in the field showed the highest content of phenolic substances and high activity against superoxide and hydroxyl radicals. The ethanol extract from leaves grown in the greenhouse had a significantly lower content of total phenols and the extracts showed significantly lower activity in all three methods. The aqueous extracts showed almost comparable amounts of phenolics, which is probably related to the extraction method. Although a lower content of total phenols was found, the aqueous extract from leaves grown in the field showed the highest activity in the FRAP method. Both types of aqueous extracts showed the same antiradical activity against hydroxyl (Table 1). The results obtained generally show that Moringa grown in the field as a tropical plant was under stress conditions to which it responded with an increased production of phenolic compounds in response to the stress. This also had a positive effect on antioxidant activity.

Table 1. Total phenol content, antioxidant activity against superoxide radical, hydroxyl radical and FRAP of different moringa leaves extracts.

Parameter	Cultivation in the field		Cultivation in a greenhouse		*p*
	Ethanol extract	Water extract	Ethanol extract	Water extract	
Phenols (mg GAE.L^{-1})	911.14 ±60.57^{c}	26.68 ±1.97^{a}	408.88 ±16.08^{b}	26.80 ±2.53^{a}	*p<0.001*
POI Superoxide (%)	62.35 ±5,58^{b}	65.27 ±1.84^{b}	52.00 ±2.31^{a}	48.33 ±2.38^{a}	*p<0.001*
POI Hydroxyl (%)	73.87 ±2.57^{c}	65.22 ±3.11^{b}	42.00 ±2.31^{a}	64.67 ±1.85^{b}	*p<0.001*
FRAP (μmol.L^{-1})	7372.47 ±663.14^{b}	9564.01 ±718.25^{c}	4011.54 ±387.65^{a}	4480.14 ±129.85^{a}	*p<0.001*

Data represent the mean ± s.d. (standard deviation); a, b, c values followed differences between extracts; significantly in $p < 0.05$, according the multiple range (ANOVA), test LSD 95 (least significant differences); POI = percentage of inhibition; GAE = Gallic acid equivalent.

The values of dry matter content and the calculated values of total polyphenols, antiradical and antioxidant activities are shown in Table 2.

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Table 2. Dry matter, total phenol content, antioxidant activity against superoxide radical, hydroxyl radical and FRAP of different moringa leaves extracts. Data recalculated on dry matter.

Parameter	Cultivation in the field		Cultivation in a greenhouse		*p*
	Ethanol extract	Water extract	Ethanol extract	Water extract	
DM (g.L^{-1})	15.96 ±0.13^{c}	4.32 ±0.01^{a}	13.07 ±0.08^{b}	3.70 ±0.01^{a}	
Phenols (mg GAE.g^{-1} DM)	69.70 ±4.63^{c}	6.18 ±0.46^{a}	25.62 ±1.01^{b}	7.24 ±0.69^{a}	*p<0.001*
POI - Superoxide (%)	3.98 ±0.18^{a}	15.11 ±0.43^{b}	3.91 ±0.35^{a}	13.063 ±0.64^{b}	*p<0.001*
POI - Hydroxyl (%)	5.65 ±0.14^{b}	15.10 ±0.72^{c}	2.63 ±0.20^{a}	17.48 ±0.50^{c}	*p<0.001*
FRAP (μmol.g^{-1} DM)	563.93 ±50.72^{a}	2213.89 ±166.26^{c}	251.35 ±24.29^{a}	1210.85 ±35.10^{b}	*p<0.001*

Data represent the mean ± s.d. (standard deviation); a, b, c values followed differences between extracts; significantly in $p < 0.05$, according the multiple range % (ANOVA), test LSD 95 (least significant differences); POI=percentage of inhibition; GAE=Gallic acid equivalent; DM=dry matter.

After conversion to dry matter, the ethanol extracts also showed a high content of total phenols, with the extract from leaves grown in the open field having a significantly higher content than the extract from leaves grown in the greenhouse. As with the non-converted values for the dry matter in aqueous extracts, a comparable phenol content was found. The situation is different after converting the results obtained for antioxidant activity per gram of dry matter. Both types of aqueous extracts, with a lower content of total polyphenols per gram of dry matter, showed a higher activity in all three methods compared to ethanol extracts, which had a significantly higher content of phenols.

4. Discussion

The total amount of phenolic compounds detected in the leaves depends on several factors. First of all, the accumulation of these secondary metabolites depends on the soil-climatic conditions under which the plant grows. In most plants, a change in a single factor can affect the secondary metabolite content, even if other factors are constant [35]. Under stress conditions, there is a decrease in the primary metabolism of plants due to unfavorable conditions. Under the influence of various abiotic and biotic stress factors, plants synthesize more secondary metabolites than under normal conditions [36]. An important factor influencing the amount of phenols is the extraction method used, the extraction reagent used and the method of analysis [37-40]. The polarity of the solvent plays an important role in increasing the solubility of phenolic compounds and therefore influences the extraction and thus their content, which are responsible for the antioxidant activity [41].

In Moringa oleifera, the influence of environmental conditions on the accumulation of total phenols was confirmed. These are environmental parameters such as annual rainfall, minimum and maximum temperature, soil type and location. Thus, the phenolic content in leaves of different samples from different locations ranged from 5.78 to 117.83 mg GAE.g^{-1} extract [42]. The total content of phenols in the aerial parts of the plant at the time of flowering is given as 38.33 mg GAE.g^{-1} extract [43]. The amount of total phenolic substances therefore varies depending on the factors described above.

The content of total phenols is positively correlated with antioxidant activity. Just as the influence of environmental conditions, analytical extraction procedures and the analysis itself affect the amount of phenols, these factors also affect antioxidant activity [9,17,21,25-27,42]. In the first study using leaf and seed samples from the island of St. Lucia, phenolic compounds and total phenolics, lipids, proteins, vitamin E and antioxidant activity were evaluated in ethanol extracts. Commercial samples of seed and leaf powder and crushed leaves were analyzed. The highest content was found in the extract of powdered leaves with 635.6 mg GAE.L^{-1} extract or 23.7 mg GAE.g^{-1} DM. Extracts from crushed leaves contained lower amounts of phenols 437.8 mg GAE.L^{-1} extract or 15.6 mg GAE.g^{-1} DM. The radical scavenging activity of superoxide anions and hydroxyl radicals correlated positively with the amount of phenolic compounds [9]. In a further study with leaf samples from young plants (5 months old), from Caribbean island St. Lucia. Ethanol extracts showed a high content of phenolic substances 727.5 mg GAE.L^{-1} extract or 46.2 mg GAE.g^{-1} DM. Aqueous extracts showed significantly lower amounts phenols 41.6 mg GAE.L^{-1} extract and 11.4 mg GAE.g^{-1} DM,

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respectively. The antiradical activity against superoxide and hydroxyl radicals was comparable for both types of extracts. In relation to the dry mass, the aqueous extracts showed a higher antioxidant activity. The FRAP method showed a significantly higher antioxidant activity of the aqueous extracts [28].

The results of this study are consistent with the results of previous studies [9,28]. The highest total phenols were detected in the ethanol extract of leaves grown outdoors. Significantly lower content of this biologically active substance was detected in the ethanol extract of leaves grown in the greenhouse. This confirms the findings from the literature that plants produce more secondary metabolites under stressful conditions, in this case outdoors [35,36,42]. The amount of phenols in an ethanol sample of Moringa leaves grown outdoors in a temperate climate zone was higher than in ethanol extracts of samples from the island of St. Lucia [9,28]. The greenhouse simulated to a certain extent the environmental conditions under which Moringa grows [19,20,23,24]. The plants were less stressed and produced lower secondary metabolites. We could not detect any difference in the amount of phenols between the aqueous extracts, which is probably related to the extraction method, where a comparable amount of this biologically active substance was extracted from both samples.

The antioxidant activity is also comparable to an earlier study [28]. First of all, a high activity of the aqueous extracts was found according to the FRAP method compared to the ethanol extracts. For the same amount of total phenolics extracted, the aqueous extract of leaves grown in the field showed significantly higher FRAP activity than the aqueous extract of leaves from the greenhouse and also compared to ethanol extracts. It can be assumed that the extraction with hot water mainly extracts compounds with a lower molecular weight. These are phenolic acids, but also other compounds that can easily exchange electrons in this method [44]. Ethanol is a less polar solvent than water, so higher molecular weight compounds such as flavonoids and less polar compounds are likely to be extracted [45]. As a result, ethanol extracts show significantly lower antioxidant activity, in spite of a higher content of total phenols.

5. Conclusions

The cultivation of *Moringa oleifera* in a temperate climate zone has shown that under stress conditions, i.e. when grown outdoors in open soil, there was an increased accumulation of phenolic compounds in the leaves. This amount was significantly higher than in extracts of leaves from a greenhouse, where the growing conditions were more similar to the conditions of natural occurrence of this species. The antioxidant activity was influenced depending on the leaf sample (open soil, greenhouse) and the extraction solvent (70% ethanol, hot distilled water).

The commercial annual cultivation of Moringa in a temperate climate zone appears to be a way to produce raw materials with a higher content of biologically active compounds for the pharmaceutical industry. However, a number of questions arise regarding the agrotechnology and economics of cultivation for this purpose.

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