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Evaluation of physico-chemical properties and antioxidant capacity of leaf powder of Moringa (*Moringa oleifera*, Lam) grown in Sri Lanka

P. K. Perera[1*], U. R. Subasinghe[1], L.D.A.M. Arawwawala[2]
1.Department of DrvyagunaVignana (Ayurveda Pharmacology and Pharmaceutics), Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka.
2.Industrial Technology Institute, Bauddhaloka Mawatha, Colombo 7, Sri Lanka

ABSTRACT

Moringa oleifera, Lam is widely used in Sri Lankan traditional system of medicine for various disease condition including dyslipidemia, diabetes and inflammatory conditions. Aim of the present study was to evaluate physico-chemical, phytochemical parameters and antioxidant properties of tea bags consist of M. *oleifera* leaves powder. Physico-chemical properties, phytochemical screening, DPPH radical scavenging activity, total flavonoid content and polyphenolic content were investigated for *M. oleifera* leaf powder using standard protocols. According to the results, 11.2 ± 0.3% of total ash, 3.4 ± 0.1% of water soluble ash and 0.7 ± 0.0% of acid insoluble ash were present in the leaf powder. Phytochemical screening reveals the presence of tannins, flavonoids, steroid glycosides, coumarins, saponins and cardiac glycosides in both hot water and hot methanolic extracts of *M. oleifera* leaf powder. TLC fingerprint profile of the methanol and dichloromethane (1:1 v/v) extract of *M. oleifera* consists of 5 and 10 prominent spots under the UV light and after spraying vanillin sulphate respectively. DPPH radical scavenging activity, total polyphenol content and total flavonoid content of water extract of *M. oleifera* leaf were 12.58 ± 0.82 mg trolox equivalents/100 ml, 57.63 ± 3.80 mg gallic acid equivalents/100 ml, 20.25 ± 0.54 mg quercetin equivalents/100 ml respectively. Selected *M. oleifera* leaf powder was effective source for develop functional teabags which comply with good standards.

Key words: *Moringa oleifera*, Standardization, antioxidant

*Corresponding Author Email: drkamalperera@yahoo.com
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INTRODUCTION

Moringa oleifera Lam is belonging to plant family Moringaceae. It is a perennial angiosperm plant and exhibits variety of nutritional and medicinal benefits [1,2,3]. It occurs in the forests of Western Himalaya and frequently cultivated in India, Sri Lanka, Burma, Malaya, Philippine Islands and East Africa. In Sri Lanka, it is often grown in the dry zone specially in Jaffna, Mannar and Puttalam. It is known as Drumstrick Tree or Indian Horse Radish in English and Murunga in Sinhalese [4]. Phytochemical analyses have shown that its leaves are particularly rich in potassium, calcium, phosphorous, iron, vitamins A and D, essential amino acids, as well as such known antioxidants such as β-carotene, vitamin C, and flavonoids [5,6]. Scientific investigations have been shown pharmacological activities such as antitumor [7], antipyretic [8], antiurolithiatic [9], antiulcer [10], analgesic [11,12], anti-inflammatory [13], antifungal and antibacterial activities [14] and cardiovascular [15] protection. *M. oleifera* leaves are widely used in Sri Lankan traditional medicine and Ayurveda system of medicine for various disease conditions [16,17].

M. oleifera is a popular remedy for snake-bite poisoning in Sri Lanka. The leaves are used as a poultice to reduce glandular swellings. The juice of the leaves has purgative properties. The fresh root is regarded as a stimulant diuretic. In India and Indo-China, the roots are regarded antiscorbutic, rubefacient and counter irritant. The juice of the root is useful as a decoction for asthma, gout, rheumatism, enlarged spleen and liver, internal and deeply seated inflammations and calculus ailments. In Nicaragua, a decoction of the root is used for dropsy. The ben oil from seeds has no smell, no taste and no colour. It has the property of not undergoing oxidation for a long time when exposed and is hence valuable for ointments as an external application for rheumatism [4].

Most of countries are used dried leaves for preparation of herbal tea. In Sri Lanka this plant is abundantly found and still it is not much popularized as herbal functional tea. Therefore, one of our aims is to develop a user friendly marketable dosage forms viz. herbal tea bags using *M. oleifera* leaves. Therefore, an attempt was made to evaluate phyto and physico-chemical properties and potential antioxidant activity by determination of total flavonoid content of, total polyphenolic content and DPPH radical scavenging activity of *M. oleifera* leaf powder which intended to develop tea bags.

MATERIALS AND METHOD

Leaf powder of *Moringa oleifera*

Leaves of *M. oleifera* were collected from Western Province of Sri Lanka during the period of June - August 2014. The plant material was identified and authenticated by Senior Lecturer of Institute of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka. A voucher specimen (specimen number**:** PGD/2013/2014/A/62) was deposited at the above

Institute. Leaves of *M. oleifera* were washed with tap water, dried under the shade and powdered by using a domestic grinder and kept in an air tight container until used.

Hot water extract

Sample (5 g) was taken into a round bottom flask and distilled water (100 mL) was added. The contents were shaken well and a reflux condenser was attached to the flask and boil gently for 2 h, allowed to cool, and filtered rapidly using a dry filter paper (Qualitative filter paper, 90 mm Diameter Whatman ®). Then the filtrate was transferred to a round bottom flask and evaporated to dryness under reduced pressure (at 70 ^{o}C) using a rotor vapor and stored at 4 ^{o}C until use.

Hot methanol extract

Sample (5 g) was taken into around bottom flask and methanol (100 mL) was added. The contents were shaken well and a reflux condenser was attached to the flask and boiled gently for 2 h, allowed to cool and filtered rapidly using a dry filter paper (Qualitative filter paper, 90mm Diameter Whatman ®). Then the filtrate was transferred to a round bottom flask and evaporated to dryness under the reduced pressure (at 40 ^{o}C) using a rotor vapor and stored at 4 ^{o}C until use.

Determination of physico-chemical parameters of *Moringa oleifera* leaves

Physico-chemical parameters such as total ash, water soluble ash and acid insoluble ash content of leaf powder were determined according to the WHO guide lines [18].

Screening of phyto-chemicals of *Moringa oleifera* leaves

Presence or absence of phytochemicals such as tannins, flavonoids, steroid glycosides, coumarins and saponins were screened according to the standard protocols [19] using hot water and hot methanolic extracts of the *M. oleifera* leaf powder.

Development of Thin Layer Chromatography (TLC) of *Moringa oleifera* leaves

Sample preparation

Leaves of *M. oleifera* (~1 g) was extracted into 10 mL of methanol: dichloromethane in a ratio of 1:1 v/v, filtered and concentrated in vacuo and spotted on a TLC plate using Hexane: Dichloromethane: Methanol in a ratio of 1: 3.75: 0.25 as the solvent system. Observations were carried out before (wave lengths at 254 nm & 366 nm) and after spraying vanillin sulphate.

DPPH radical scavenging activity water extract of *Moringa oleifera* leaves

1,1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging assay was performed according to the method described by Blois [19], in 96-well micro-plates. Reaction volumes of 200 µl, containing 125 µM of DPPH radical and 50 µl of different concentrations of water extract of *M. oleifera* leaves (200, 100, 50, 25, 12.5 and 6.25 µl/ml) were incubated at 25 ± 2 ^{o}C for 15

minutes and the absorbance was recorded at 517 nm. Five different concentrations of Trolox (40, 20, 10, 5 and 2.5 µg/ml) were used to construct the standard curve. Results were expressed IC_{50} and Trolox equivalents antioxidant capacity as mg of Trolox equivalents per 100 ml of water extract of *M. oleifera* leaves.

Determination of total polyphenolic content of water extract of *Moringa oleifera* leaves

Total poly phenol content of water extract of *M. oleifera* leaves were determined by the Folin-Ciocalteu [20] using 96-well micro-plates. Twenty microliters of 250 and 125 µl/ml of water extract of *M. oleifera* was added separately to 110 µl of ten times diluted freshly prepared Folin-Ciocalteu reagent. Seventy microliters of sodium carbonate solution was added to the mixture and incubated at room temperature (25 ± 2 ^{o}C) for 30 minutes and absorbance was recorded at 765 nm. Five different concentrations of gallic acid (1, 0.5, 0.25, 0.12 and 0.06 mg/ml) were used to construct the standard curve. Total poly phenol content of water extract of *M. oleifera* leaves were expressed as mg gallic acid equivalents per 100 ml of water extract of *M. oleifera* leaves.

Determination of total flavonoid content of water extract of *Moringa oleifera* leaves

Total flavonoid content of water extract of *M. oleifera* leaves were determined by aluminium chloride method [21] using 96 well micro-plates. One hundred microliters of 2 % aluminium chloride in methanol solution was added to 100 µl of 250 and 125 µl/ml of water extract of *M. oleifera* in methanol. The mixture was incubated at room temperature (25 ±2 ^{o}C) for 10 minutes and absorbance was recorded at 415 nm. Pre-plate reading was recorded before adding the aluminium chloride solution. Six different concentrations of quercetin (125, 62.5, 31.25, 15.62 and 7.81 mg/ml) were used to construct the calibration curve. Total flavonoid content of water extract of *M. oleifera* leaves were expressed as mg quercetin equivalents per 100 ml of water extract of *M. oleifera* leaves.

Statistical Analysis

All assays were performed in triplicate. Values were expressed as Mean±S.E.M., and statistical nalysis was carried by one way ANOVA followed by Tukey test. $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Over the years, a great demand was developed towards the plant based products. *M. oleifera* has been identified as a multifunctional versatile plant with enormous economic, nutritional and health potentials [22]. In this study, Moringa leaf powder was evaluated for its physicochemical properties and antioxidant potency to develop natural product viz. tea bags. Results of the physicochemical parameters of *M. oleifera* are shown in Table 1. Total ash is particularly important in the evaluation of purity and quality of a plant product. The ash value

was determined by 3 different methods, which measured total ash, acid insoluble ash, and water soluble ash. The total ash method is employed to measure the total amount of material remaining after ignition [23]. The total ash usually consists of carbonates, phosphates, silicates, and silica, which include both physiological ash and non-physiological ash. The physiological ash comes from the mineral components of the plant itself. A high ash value is indicative of contamination, substitution, adulteration, or carelessness [23]. Acid insoluble ash indicates contamination with silica, for example, earth and sand. Water soluble ash is that part of the total ash content, which is soluble in water. It is a good indicator of the water soluble salts in the plant [24]. In the present study, very low amount of acid insoluble ash content indicates the purity of the powder of *M. oleifera*. Similar to the present study, physico-chemical parameters were investigated for many medicinal plants grown in Sri Lanka such as *Alpinia calcarata* [25], *Trichosanthes cucumerina* [26], *Mallotus philipinensism* [27] and *Phyllanthus niruru* [28] in order to establish the monographs for standardization of medicinal plants in Sri Lanka.

Phytochemical screening reveals the presence of tannins, flavonoids, steroid glycosides, coumarins, saponins and cardiac glycosides in both hot water and hot methanolic extracts of *M. oleifera.* TLC fingerprint profile of *M. oleifera* consists of 5 and 10 prominent spots under UV light (at 254 nm and 366 nm) and after spraying vanillin sulphate respectively (Table 2).

Antioxidant capacity was explored by DPPH radical scavenging activity (Table 3). A number of methods are used to determine the radical scavenging effects of antioxidants. The DPPH method is a preferred method because it is fast, easy and reliable and does not require a special reaction and device. DPPH is a stable, synthetic radical that does not disintegrate in water, methanol, or ethanol. The free radical scavenging activities of extracts depend on the ability of antioxidant compounds to lose hydrogen and the structural conformation of these components [29].

Water extract of *M. oleifera* leaf powder was rich in polyphenols and flavonoids (Table 3). A similar study was carried out by Pakade and co-workers [30] in South Africa using methanolic extract of *M. oleifera*. According to the results, total phenolic content and total flavonoid content of *M. oleifera* were in a range of 19.5 ±3.5 to 31.9 ±4.9 g gallic acid equivalents/100 g extract and 24.0 ±5.9 to 58.7 ±3.0 g quercetin equivalents/100 g extract respectively. Variation of total phenolic content and total flavonoid content may be due to the exposure of the plants to different agroclimatic conditions. However, total phenolic content and total flavonoid content of *M. oleifera* is rich than that of some selected vegetables such as cabbage, spinach, peas, cauliflower and broccoli [31].

Plant materials rich in phenolics are increasingly being used in the food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food [31]. Phenolic compounds of plants are also very important because their hydroxyl groups confer scavenging ability. Phenolic compounds of plants fall into several categories; chief among these are the flavonoids which have potent antioxidant activities [32]. Flavonoids are naturally occurring in plants and are thought to have positive effects on human health. Studies on flavonoid derivatives have shown a wide range of antibacterial, antiviral, anti-inflammatory, anticancer, and anti-allergic activities [33, 34]. Flavonoids have been shown to be highly effective scavengers of most oxidizing molecules, including singlet oxygen, and various free radicals [35] implicated in several diseases. Therefore, our results suggested that high DPPH radical scavenging activity, total polyphenol content and total flavonoid content may be the major contributors for the antioxidant capacity of *M. oleifera* leaves.

Table 1: Physico-chemical parameters of powder of *Moringa oleifera* leaves

Physico-chemical parameters	Percentage (%) in dry weight basis
Total ash	11.2±0.3
Water soluble ash	3.4±0.1
Acid insoluble ash	0.7±0.0

Table 2: R_f values and colours of extract of *Moringa oleifera* leaves

Before spraying (at 254 nm & 366 nm)	After spraying vanillin sulphate
0.04	0.04 (Light brown)
0.08	0.08 (Light brown)
0.39	0.23 (Dark purple)
0.44	0.39 (Purple)
0.98	0.44 (Purple)
	0.49 (Purple)
	0.71 (Purple)
	0.83 (Dark purple)
	0.93 (Pink)
	0.98 (Pink)

Table 3: DPPH radical scavenging activity, total polyphenol content and total flavonoid content of water extract of *Moringa oleifera* leaves

1,1-diphenyl-2-picryl-hydrazyl Assay (mg trolox equivalents/100 ml water extract)	**Total Polyphenol Content** (mg gallic acid equivalents/100 ml water extract)	**Total Flavonoid Content** (mg quercetin equivalents/100 ml water extract)
12.58 ± 0.82	57.63 ± 3.80	20.25 ± 0.54

CONCLUSION

Physico-phytochemical properties and antioxidant capacity of *M. oleifera* grown in Western province of Sri Lanka was investigated for the first time and these results can be used as a

reference standard for quality control of *M. oleifera* grown in Sri Lanka. Further high capacity of antioxidant constituents of *M. oleifera* leaf powder can be used as functional tea in the management of various chronic diseases.

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