Phytochemical Screening and High Performance Thin Layer Chromatography Fingerprinting Profiles of Shigru (*Moringa oleifera* Lam.) Leaves from Tanzania and India

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**Abstract**

Introduction: Shigru or *Moringa oleifera* Lam. (Moringaceae) is a very useful medicinal plant in folkloric medicines of Africa and Asia for the treatment of diseases like ulcers, wounds, inflammation, heart problem, stroke, obesity, anemia, and liver damage. Moreover, the plant leaves are very good supplements for malnutrition and are also used as an antimicrobial agent. The present work was carried out for assessment of physicochemical constants screening of phytochemicals and high performance thin layer chromatography (HPTLC) fingerprinting of the plant samples.

Materials and methods: The crude powders of *M. oleifera* leaves of two varieties, collected from India and Tanzania, were subjected to the physicochemical analysis. The methanolic extracts of the respective leaves were subjected to phytochemical screening to determine the classes of phytoconstituents present and generate HPTLC fingerprinting profiles.

Results: Phytochemical screening showed the presence of tannins, flavonoids, terpenoids, alkaloids, saponins, carbohydrates, reducing sugars, and proteins in both the varieties, but in different concentrations. The HPTLC finger printing profile of the methanolic extract of the *M. oleifera* leaves collected from Tanzania showed more number of bands with higher concentrations as compared to that of the Indian variety.

Conclusion: This research article compares the physicochemical and phytochemical parameters as well as HPTLC profiles of the leaves of the two designated *M. oleifera* varieties. Besides the importance for authenticating the plant samples, the present results also show that both the varieties can be used as herbal drugs for different purposes.

Keywords: Chemical constituents, High performance thin layer chromatography, Methanol extract, *Moringa oleifera*, Phytochemicals.

**Introduction**

Medicinal plants have been used by all civilizations as sources of medicines since ancient times.¹ In recent times, there has been a growing interest in exploiting the biological activities of different Ayurvedic medicinal herbs, due to their cost-effectiveness and lesser side effects. The medicinal plants reported in traditional medicinal systems including Ayurveda have been the lead sources for the development of several modern drugs.² *Moringa oleifera*, commonly known as drumstick or horse radish tree, is native to Asia but is also spread in most parts of Africa. It is the sole genus in the Moringaceae flowering plant family. This genus is made of 12 species in which *M. oleifera* constitutes one of the most economically important species.³ *M. oleifera* leaves are traditionally used as purgatives, for the treatment of headache, hemorrhoids, fever, sore throat inflammation, bronchitis, eye and ear infections, and to combat vitamin C deficiency. The *M. oleifera* leaf juice is used to control glyceria, hypertension, and cholesterol, to treat swollen glands, and is also credited with anticancer, antitumor, anti-inflammatory, diuretic, anesthetic, antioxidant, anti-diabetic, and analgesic properties.⁴ Besides being good sources of carotenoids with provitamin A activity and vitamin E, fresh leaves of *M. oleifera* are reported to contain 11,300–23,000 IU of vitamin A and approximately 200 mg/100 g of vitamin C, which is more than that in orange. Among the group B vitamins, only thiamine, riboflavin, and niacin are present in the leaves. The leaves are also well-known for the antioxidant activity, essentially due to the presence of high amounts of polyphenols.⁵ Principle polyphenol compounds in *M. oleifera* leaves are flavonoids, mainly myricetin, quercetin, and kaempferol, and phenolic acids such as gallic acid.⁶

In view of the above, formulations of *M. oleifera* leaves are expected to offer various medicinal benefits. However, these require proper authentication of the plant materials. Physicochemical screening and high performance thin layer chromatography (HPTLC) fingerprinting play vital roles for authentication and identification of herbs/plants as well as assessing the quality of the herbal drugs.⁷ In this paper, we assessed these two aspects of *M. oleifera* leaves of two varieties, collected from India and Tanzania, and compared their physicochemical and phytochemical properties.

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Materials and Methods

Plant Collection
The fresh mature healthy leaves of the *M. oleifera* plant of one variety were collected from CARIDD garden, Kolkata, West Bengal, India. Another variety was collected from Dar-es-Salaam city center, Tanzania. The selected plants were authenticated by the Pharmacognosy Department, CARIDD, Kolkata. The Indian and Tanzanian varieties of the plant samples are designated as *M. oleifera* (I) and *M. oleifera* (Tz), respectively.

Plant Samples Processing
The plant materials were washed with distilled water, and shade-dried at room temperature to avoid loss of essential oil, and milled with the aid of a grinding machine. The powdered plant samples were stored at room temperature in airtight, light-resistant containers as per guidelines of Ayurvedic Pharmacopoeia.1,9

Extraction of the Plant Materials
The powdered *M. oleifera* leaves of both varieties (about 20 g) were extracted by soaking overnight in methanol (100 mL) at room temperature. The individual extracts were filtered and concentrated on a water bath to obtain the respective powders.

Physicochemical Analysis
The crude leaf powders were used for the physicochemical analysis as per the WHO/API guidelines. For determining the aqueous and alcohol extractive values, the leaf powder (4 g) of both varieties were soaked in the respective solvents (100 mL) with occasional shaking up to 6 hours, followed by keeping the mixture overnight at room temperature. The extracts were subsequently concentrated on a hot water bath to constant weights and the extractive values calculated.

Phytochemical Analysis
The methanol extracts (both variety) were used for phytochemical screening to detect the secondary metabolites. The individual extracts were evaporated to dryness and stored at ~4°C in air-tight containers for screening the presence of secondary metabolites.10

HPTLC Study

**Equipment**
A CAMAG HPTLC system (Switzerland), comprising of a CAMAG Linomat V applicator, CAMAG TLC scanner 3, CAMAG Wincats Software, Version 1.44, CAMAG Reprostar 3, a CAMAG TLC plate heater, and a CAMAG UV cabinet, was used for the study.11,12

**Preparation of Samples**
The suspensions of powdered *M. oleifera* leaves of both varieties (1 g each) in methanol (30 mL) were separately refluxed for 1 hour. The individual extracts were filtered using filter papers, the filtrates concentrated on a water bath up to 10 mL and used for the HPTLC analyses.

**Stationary Phase**
Preactivated 5 × 10 cm aluminum-supported precoated silica gel 60 F254 plates (Merck, India, Batch No. 1.05554.0007) were used.

**Application of Samples**
The individual sample solutions (5 μL each) were applied at 12 mm from the base of the plate as 8-mm bands with the help of a 100 μL Hamilton syringe using a Linomat V applicator.

**Mobile Phase**
Toluene:ethyl acetate:methanol (6.5:3:0.5) v/v (GR grade solvents, MERCK, India) were used.

**Plate Development and Detection of Spots**
The TLC plate was developed up to 80 mm in a presaturated CAMAG twin trough chamber, after preconditioning the plate at 27°C and a relative average humidity of 48%. Images of the developed plate were captured at 254 nm and 366 nm and densitometric scanning was performed.13

Results
The crude *M. oleifera* (I) and *M. oleifera* (Tz) leaves powders were subjected to the phytochemical analysis, which helps to compare their qualities. Evaluation of the physicochemical parameters of the *M. oleifera* (I) powders showed (Table 1) the total ash value as 10.7% and acid-insoluble ash contents as 0.35%, while its weight loss on drying (LOD) was 7.0%. The corresponding values for the same parameters of *M. oleifera* (Tz) powders were 7.9%, 0.15%, and 11.0%, respectively. The extraction yields of the *M. oleifera* (Tz) sample in water as well as alcohol were slightly higher than that of *M. oleifera* (I).

The chemical constituents of plants/herbs contribute to their physiological properties and consist of primary metabolites, viz., sugars, amino acids, and proteins, along with secondary metabolites such as alkaloids, terpenoids, flavonoids, tannins, etc. In the present study, different qualitative tests were carried out with the methanol extracts of the *M. oleifera* (I) and *M. oleifera* (Tz) leaves powders. The results of the phytochemical screening (Table 2) revealed the presence of alkaloids, saponins, tannins, and proteins but absence of steroids and amino acids in the extracts of both the samples. The *M. oleifera* (Tz) sample had significantly higher amounts of flavonoids, saponins, and reducing sugars.

The methanol extracts of *M. oleifera* (I) and *M. oleifera* (Tz) leaves were analyzed by HPTLC to generate their fingerprint profiles. The HPTLC conditions for the best separation of the phytoconstituents were optimized using preactivated HPTLC silica gel 60 F254 plates and different combinations of polar and nonpolar solvents as the mobile phases (data not shown). Best result was obtained with toluene:ethyl acetate:methanol (6.5:3:0.5, v/v) solutions. The *M. oleifera* (I) extract showed three spots when visualized at 254 nm and five spots at 366 nm. But only two of these spots had same Rf values (Table 3). The *M. oleifera* (Tz) sample showed seven

| S. no. | Parameters                | Indian variety (%) | Tanzania variety (%) | API limit (%) |
|--------|---------------------------|--------------------|----------------------|--------------|
| 1      | Loss on drying            | 7.0                | 11.0                 | Not mentioned|
| 2      | Ash value                 | 10.7               | 7.9                  | 16 (NMT)     |
| 3      | Acid-insoluble ash        | 0.35               | 0.15                 | 4 (NMT)      |
| 4      | Water-soluble extractive  | 27.5               | 30.3                 | 22 (NLT)     |
| 5      | Alcohol-soluble extractive| 20.3               | 24.5                 | 8 (NLT)      |

1The evaluation was carried out as per the WHO/API guidelines. NMT, not more than; NLT, not less than.
Table 2: Qualitative phytochemical screening of methanol extracts of M. oleifera leaves of Indian and Tanzanian varieties

| S. no. | Phytochemical class (tests) | M. oleifera (I) leaves | M. oleifera (Tz) leaves |
|--------|----------------------------|-----------------------|------------------------|
| 1      | Alkaloids (Dragendorffs test) | +                      | +                      |
| 2      | Flavonoids (Shinoda test)  | +                      | ++                     |
| 3      | Tannins                     | +                      | +                      |
| 4      | Steroids (LB test)          | −                      | −                      |
| 5      | Terpenoids (LB test)        | +                      | +                      |
| 6      | Saponins (foam test)       | +                      | ++                     |
| 7      | Carbohydrates (Molisch’s test) | +                  | +                      |
| 8      | Reducing sugars (Benedict’s test) | +                  | ++                     |
| 9      | Proteins (Biuret test)     | +                      | +                      |
| 10     | Amino acids (ninhydrin test) | −                      | −                      |

Note: (+) trace amount, (+++) higher amount, (−) absent

Table 3: $R_f$ values of the M. oleifera (I) leaves extract

| S. no.  | 254 nm $R_f$ value | 366 nm $R_f$ value |
|---------|--------------------|--------------------|
| 1       | 0.21               | 0.21               |
| 2       | 0.33               | 0.33               |
| 3       | 0.50               | 0.46               |
| 4       | 0.64               | 0.64               |
| 5       | −                  | 0.88               |

Table 4: $R_f$ values of the M. oleifera (Tz) leaves extract

| S. no. | 254 nm $R_f$ value | 366 nm $R_f$ value |
|--------|--------------------|--------------------|
| 1       | 0.29               | 0.29               |
| 2       | 0.35               | 0.35               |
| 3       | 0.52               | 0.52               |
| 4       | 0.67               | 0.67               |
| 5       | 0.73               | 0.73               |
| 6       | 0.82               | 0.85               |
| 7       | 0.92               | 0.92               |

spots both at 254 nm and 366 nm; six of these spots had the same $R_f$ values (Table 4). The HPTLC profiles of the above leaves samples, as visualized at 254 nm and 366 nm, are shown in Figure 1. The corresponding densitograms are shown in Figures 2 and 3.

Discussion

This is the first report on the comparative physicochemical and phytochemical parameters of M. oleifera leaves from India and Tanzania as well as HPTLC fingerprinting of their methanol extracts. In the physicochemical assay, the ash value and the acid-insoluble ash value of M. oleifera (Tz) were lower than that of M. oleifera (I), suggesting the presence of higher amounts of inorganic solids such as silica in the latter. Nevertheless, the results of LOD, ash value, acid-insoluble ash and extractive values (water and alcohol) of both the varieties complied with the API limits. The lesser LOD value of M. oleifera (I) leaves vis-à-vis M. oleifera (Tz) leaves suggested that the former contains less moisture and, hence, may be stored for a longer period at an ambient temperature without much spoilage.

In the phytochemical screenings, both the varieties showed the presence of several classes of secondary metabolites except steroids and amino acids. But the content of flavonoids, saponins, and reducing sugars was higher in the methanol extract of M. oleifera (Tz) than in M. oleifera (I). Presence of several alkaloids such as Nα-L-rhamnopyranosyl vincosamide, 4-(α-L-rhamnopyranosyloxy) phenylacetamide (niazirin), pyrrolemarumine, 4′-O-α-L-rhamnopyranoside, 4′-hydroxy phenylethanamide-α-L-rhamnopyranoside (marumoside A) and its 3-O-β-D-glucopyranosyl derivative (marumoside B), and methyl 4′-α-L-rhamnopyranosyloxy)-benzylcarbamate has been earlier confirmed in M. oleifera leaves.5,6 Also, polyphenols including flavonoids and tannins were earlier found in the M. oleifera leaves.5,6 Thus, our phytochemical results are consistent with the previous reports but show both qualitative and quantitative differences between the M. oleifera (Tz) and M. oleifera (I) varieties. This is confirmed from the higher extractive values of M. oleifera (Tz) in water and alcohol than of M. oleifera oleifera (I), suggesting higher contents of the polar phytoconstituents in the former.

We also developed a convenient HPTLC method for generating fingerprinting profiles of both the M. oleifera varieties, which showed significantly more number of secondary metabolites and with higher concentrations in M. oleifera (Tz) than in M. oleifera (I). The respective HPTLC profiles of the samples are in accordance with their corresponding extraction yields in alcohol and water.

Conclusion

Overall, we compared the physicochemical and phytochemical parameters of the leaves of M. oleifera (I) and M. oleifera (Tz) varieties and generated their HPTLC fingerprinting profiles. These may be very useful in their quality assessment. In particular, the HPTLC fingerprints may help in identifying M. oleifera leaves of different sources, and assist in isolation and identification of the bioactive compounds from them. Compliance of the physicochemical parameters of both the varieties with the API guidelines indicates their quality. Given that M. oleifera (I) leaves are credited with several health benefits,5,6 the additional chemical compounds in M. oleifera (Tz) may not contribute substantially in these aspects.
Phytochemical Screening and HPTLC Fingerprinting Profiles of Shigru (Moringa oleifera Lam.) Leaves from Tanzania and India

Fig. 2A and B: Densitogram display for the constituents in the methanol extract of (A) M. oleifera (I); (B) M. oleifera (Tz) visualized at 254 nm

Fig. 3A and B: Densitogram display for the constituents in the methanol extract of (A) M. oleifera (I); (B) M. oleifera (Tz) visualized at 366 nm

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हिंदी सारांश

तंजानिया और भारत से संग्रहित शिग्रु (Moringa oleifera Lam.) की पादपरासायनिक जांच और हाई परफर्मेंस थिन लेयर क्रोमेटोग्राफी फिंगरप्रिंटिङ फ्रोफाइल्स

भूमिका: एशिया और अफ्रीका में वन, वुद्ध, शैक्षणिक, इतंत रोग, आधार, स्थलीय, लौह औसत्त जन्म रक्तपत्रमता और लिवर डेमेज आदि रोगों के उपचार में शिखर या शिखर ओलिफेरा लेम (Moringa oleifera) लोकप्रिय औषधियों में बहुत ही महत्वपूर्ण औषधीय पादप है। इसके अतिरिक्त, पादप औषधियों कुपोषण हेतु एक बहुत अच्छी सहायक औषधि है और इसका उपयोग एक एंटिभॉक्सियल आजिहेट के रूप में भी किया जाता है। वर्तमान अध्ययन पादप नमूनों हेतु फाइडेटेक्सिमल की भौतिकरासायनिक घटकों की जांच और हाई परफर्मेंस थिन लेयर क्रोमेटोग्राफी (एचपीटीएलसी) फिंगरप्रिंटिङ का मूल्यांकन करने के लिए किया गया।

सामग्री और विधि: भारत और तंजानिया से संग्रहित एम. ओलिफेरा पत्तियों की दो किस्में के अपरिष्कृत पाउडर भौतिकरासायनिक विश्लेषण के अध्ययन है। संबंधित पत्तियों के मेधनाष्ट रत्न उपस्थित फाइडेटेक्सिमल प्रोफाइल के वागी और एचपीटीएलसी फिंगरप्रिंटिङ फ्रोफाइल्स का उपयोग करने हेतु फाइडेटेक्सिमल स्क्रीनिङ के अध्ययन है।

परिणाम: फाइडेटेक्सिमल स्क्रीनिङ टेलिभिज़न, फ्लेवोनाइड्स, टर्पिनाइड्स, अल्कोहोलाइड्स, सुपरमिन्स, कार्बोहाइड्रेट्स, रिज्वुसिंग शुगर और प्रोटीन की उपस्थिति पत्तियों की विश्लेषण सांदर्भिक में एक गड़बड़ है। तंजानिया से संग्रहित एम. ओलिफेरा के मेठतनालिक सत्ता एचपीटीएलसी फिंगरप्रिंटिङ प्रोफाइल भारतीय किस्म की तुलना में उच्च सांदर्भिक के साथ बेड्रस की अधिक संख्या को दर्शाती है।

निष्कर्ष: यह शोध पत्र एम. ओलिफेरा की दो निर्दिष्ट किस्में की पत्तियों की एचपीटीएलसी प्रोफाइल के साथ-साथ भौतिकरासायनिक और फाइडेटेक्सिमल मापदंडों के तुलना करता है। पादप नमूनों को प्रमाणित करने के महत्व के अतिरिक्त, वर्तमान परिणाम यह भी दर्शाते हैं कि विश्लेषण प्रयोजनों हेतु दोनों किस्मों का प्रयोग वातावरणस्तिक औषधि के रूप में किया जा सकता है।

मुख्य शब्द: कॉमिकल कॉल्स्ट्रूट्रांग्स, हाई परफर्मेंस थिन लेयर क्रोमेटोग्राफी, मेठीनाल सत्ता, मोरिङा ओलिफेरा, फाइडेटेक्सिमल।