**Introduction**

Medicinal plants have been used by all civilizations as sources of medicines since ancient times. 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. *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. *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 glycemia, hypertension, and cholesterol, to treat swollen glands, and is also credited with anticancer, antitumor, anti-inflammatory, diuretic, antithapotoxic, anti-fertility, anti-ulithiathic, and analgesic properties. 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. Principle polyphenol compounds in

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

Manosi Das¹, Achintya Mitra², Avijit Banerji³, Swaswati Roy⁴, Jayram Hazra⁵

**Abstract**

**Introduction:** Shigru or *Moringa oleifera* Lam. (Moringaceae) is a very useful medicinal plant in folklore 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.


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**Conflict of interest:** None

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*M. oleifera* leaves are flavonoids, mainly myricetin, quercetin, and kaempferol, and phenolic acids such as gallic acid.

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. Phytochemical 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. 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.
**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.

### 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.

### 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.

#### 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.

### Results

The crude *M. oleifera* (I) and *M. oleifera* (Tz) leaves powders were subjected to the physicochemical 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.

### Table 1: Physicochemical evaluation of *M. oleifera* leaves of Indian and Tanzanian varieties

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

1 The evaluation was carried out as per the WHO/API guidelines. NMT, not more than; NLT, not less than.
Phytochemical Screening and HPTLC Fingerprinting Profiles of Shigru (Moringa oleifera Lam.) Leaves from Tanzania and India

Table 2: Qualitative phytochemical screening of methanol extracts of M. oleifera leaves of Indian and Tanzanian varieties

<table>
<thead>
<tr>
<th>S. no.</th>
<th>Phytochemical class (tests)</th>
<th>M. oleifera (I) leaves</th>
<th>M. oleifera (Tz) leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids (Dragendorffs test)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavonoids (Shinoda test)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Steroids (LB test)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>Terpenoids (LB test)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Saponins (foam test)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>7</td>
<td>Carbohydrates (Molisch's test)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Reducing sugars (Benedict's test)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>9</td>
<td>Proteins (Biuret test)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Amino acids (ninhydrin test)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>S. no.</th>
<th>254 nm</th>
<th>366 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>2</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>3</td>
<td>0.50</td>
<td>0.46</td>
</tr>
<tr>
<td>4</td>
<td>–</td>
<td>0.64</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>0.88</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>S. no.</th>
<th>254 nm</th>
<th>366 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.29</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>3</td>
<td>0.52</td>
<td>0.52</td>
</tr>
<tr>
<td>4</td>
<td>0.67</td>
<td>0.67</td>
</tr>
<tr>
<td>5</td>
<td>0.73</td>
<td>0.73</td>
</tr>
<tr>
<td>6</td>
<td>0.82</td>
<td>0.85</td>
</tr>
<tr>
<td>7</td>
<td>0.92</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Figs 1A and B: HPTLC profiles of methanol extracts of M. oleifera (I, track 1) and M. oleifera (Tz, track 2), visualized at (A) 254 nm; (B) 366 nm

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.\(^\text{5,6}\) 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,\(^\text{5-6}\) 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

Figs 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

Figs 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

**REFERENCES**

हिंदी सारांश

tंजामिया और भारत से संग्रहित शिग्रु (मोरिगा ओलिफेरा लेम.) की पादपरासायनिक जांच और हाई परफॉर्म्स थिन लेयर क्रोमेटोग्राफी फिंगरप्रिटिंग प्रोफाइल्स

भूमिका: एशिया और अफ्रीका में वन्न, वुड्स, शेश, इदल रोग, आघाट, स्फूर्ति, लॉह अल्मता जनव रक्ताल्पता और लियर देमेज आदि रोगों के उपचार में शिग्रु या मोरिगा ओलिफेरा लेम. (मोरिगा ओलिफेरा) लोकद्वारे वाली औषधियों में बहुत ही महत्वपूर्ण औषधीय पादप है। इसके अतिरिक्त, पादप औषधियों कुपोषण हेतु एक बहुत अच्छी सहायक औषधि है और इसका उपयोग एक एंटीमाइक्रोबियल एजेंट के रूप में भी किया जाता है। वर्तमान अध्ययन पादप नमूनों हेतु फाइटोकेमिकल की भौतिकरासायनिक घटकों की जांच और हाई परफॉर्म्स थिन लेयर क्रोमेटोग्राफी (एचपीटीएल) फिंगरप्रिटिंग का मूल्यांकन करने के लिए किया गया।

सामग्री और विधियां: भारत और तंजामिया से संग्रहित एम. ओलिफेरा पत्तियों की दो किस्में के अपरिश्लेषण पाउडर भौतिकरासायनिक विश्लेषण के अध्ययन किए गए। संबंधित पत्तियों के मैथेनोलिक सत्र में उपस्थित फाइटोकेमिस्ट्रीय प्रोफाइल्स के बर्गों और एचपीटीएल की फिंगरप्रिटिंग प्रोफाइल्स को उपलब्ध करने हेतु फाइटोकेमिकल स्क्रीनिंग के अध्ययन किए गए।

परिणाम: फाइटोकेमिकल स्क्रीनिंग टेबल, प्लेगेनोइड्स, टर्पीनोइड्स, एल्कोनोइड्स, सेपोनिन्स, कार्बोहाइड्रेट्स, रिब्यूसिंग शुरु और प्रोटीन की उपस्थिति दोनों किस्मों की विभिन्न संदर्भों में पाई गई। तंजामिया से संग्रहित एम. ओलिफेरा के मैथेनोलिक सत्र की एचपीटीएल सी फिंगरप्रिटिंग प्रोफाइल भारतीय किस्म की तुलना में उच्च संदर्भ के साथ बैंड्स की अधिक संख्या को दर्शाती है।

निष्कर्ष: यह शोध पत्र एम. ओलिफेरा की दो निर्दिष्ट किस्मों की पत्तियों की एचपीटीएल सी प्रोफाइल के साथ-साथ भौतिकरासायनिक और फाइटोकेमिकल मापदंडों की तुलना करता है। पादप नमूनों को प्रमाणित करने के महत्व के अतिरिक्त, वर्तमान परिणाम यह शोधाती है कि विभिन्न प्रयोगों हेतु दोनों किस्मों का प्रयोग वातावरणिक औषधि के रूप में किया जा सकता है।

मुख्य शब्द: केमिकल कोल्स्ट्रूट्रूएंट्स, हाई परफॉर्म्स थिन लेयर क्रोमेटोग्राफी, मैथेनोल सत्र, मोरिगा ओलिफेरा, फाइटोकेमिकल।