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Pharmacognostic and Phytochemical Comparison of *Moringa oleifera* and *Moringa concanensis*

Aleda M.H. Chen[1*], Yong Chen[2]

[1]Cedarville University, School of Pharmacy, Cedarville, OH, United States.
[2]Institute for Advanced Study, Nanchang University, Nanchang, Jiangxi, China.

***E-mail** ✉ amchen@cedarville.edu

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ABSTRACT

Moringa oleifera (*M. oleifera*) and *Moringa concanensis* (*M. concanensis*), both belong to the Moringaceae family and are commonly recognized as drumstick trees. *M. oleifera*, often referred to as the "miracle tree," has been valued for its extensive health benefits for centuries. *M. concanensis*, also known locally as Kattumurungai, is another vital medicinal plant used for treating conditions such as skin tumors, fatigue, high blood pressure, jaundice, and diabetes. This study compares the pharmacognostic and phytochemical properties of both species. Preliminary phytochemical screening of the plants revealed the presence of alkaloids, glycosides, flavonoids, steroids, tannins, carbohydrates, proteins, and lipids. *M. oleifera* showed the highest yield for these compounds. Physicochemical parameters, including ash content and extractive values, were also found to be superior in *M. oleifera*. Thin layer chromatography (TLC) was employed to evaluate key phytochemicals such as alkaloids, steroids, and flavonoids in methanolic extracts. Both species showed distinct spots on TLC plates, some with similar Rf values, while others differed, indicating the presence of both shared and unique alkaloids, steroids, and flavonoids. The total phenolic and flavonoid content was determined using the Folin-Ciocalteu and Aluminum Chloride methods. *M. oleifera* showed significantly higher concentrations of total phenols (453 µg/ml) and flavonoids (365 µg/ml) compared to *M. concanensis*. These results indicate that *M. concanensis* has comparable macroscopical and phytochemical characteristics to *M. oleifera* but is more similar in terms of alkaloid, steroid, and flavonoid composition. Further research is needed to identify the specific compounds associated with the TLC spots using reference standards.

Keywords: *Moringa oleifera*, Thin layer chromatography (TLC), *Moringa concanensis*, Phytochemical analysis

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Introduction

Moringa oleifera (*M. oleifera*) and *Moringa concanensis* (*M. concanensis*) are two species in the Moringaceae family, both well-known for their wide range of health benefits and distinct properties. These species are widely studied for their bioactive compounds, which have made them valuable in medicinal and traditional practices across various cultures [1, 2].

M. oleifera, often called the "drumstick tree" or "miracle plant," is native to regions in South Asia, including India, Bangladesh, Pakistan, and Afghanistan [3, 4]. It is renowned for its high nutritional value, offering a variety of essential vitamins, minerals, antioxidants, and amino acids. Due to its diverse applications, *M. oleifera* has been widely used in both food and medicine [5, 6].

M. concanensis, also known as the "West Indian Moringa," "Kattumurugai," or "Monga," is a lesser-known species native to the Western Ghats of India. Though it belongs to the same genus as *M. oleifera*, it features distinct botanical characteristics and a unique chemical profile [7, 8].

While *M. oleifera* is well-recognized globally, *M. concanensis* also has medicinal value and is utilized by local communities for various therapeutic purposes [9-11]. Comparing these two species offers valuable insights into

their morphological and chemical differences, as well as their ethnobotanical significance. Both species contain a variety of bioactive substances such as alkaloids, terpenoids, flavonoids, tannins, and reducing sugars, present in the leaves, flowers, seeds, bark, roots, and pods of the plants [12, 13]. Studies have reported various pharmacological activities of both species, including anti-inflammatory, antimicrobial, anti-diabetic, analgesic, antioxidant, and hepatoprotective effects [14-26]. This study aims to explore the comparative pharmacognostic characteristics of these two species, focusing on phytochemical composition, physicochemical properties, TLC analysis, and the assessment of total phenolic and flavonoid content in their leaves.

Materials and Methods

Collection and authentication of plants

M. oleifera and *M. concanensis* were gathered from agricultural fields located near Miyapur, Hyderabad. The identification and authentication of the plants were conducted by a botanist from the Government Degree College, Kukatpally, Medchal district, Hyderabad.

Macroscopic evaluation

Both species were evaluated based on their morphological and organoleptic characteristics to establish their identity.

Preparation of plant extracts

Leaves of the plants were separated and dried at room temperature under shade. The dried leaves were ground into a coarse powder. For extraction, 250 grams of the powdered material was subjected to methanol in a Soxhlet apparatus for 2 hours. The methanolic extract was then concentrated by evaporating the solvent in a water bath, and the percentage yield was calculated [27].

Preliminary phytochemical screening

Sugar detection

Molisch's test: The extract was treated with alcoholic α-naphthol and concentrated sulfuric acid. A reddish-violet ring at the interface confirmed the presence of sugars.

Fehling's test: Hydrolysis of the extract with dilute hydrochloric acid, followed by the addition of Fehling's solutions A and B, resulted in a reddish-brown color upon heating, indicating reducing sugars.

Protein detection

Biuret test: The extract was mixed with Biuret reagent, and a pink or purple color upon heating indicated the presence of proteins.

Ninhydrin test: The addition of Ninhydrin reagent to the extract followed by heating in a water bath resulted in a pinkish-red color, confirming the presence of proteins.

Fixed oils and fats detection

The extract was rubbed between two filter papers. If oil stains appeared, it indicated the presence of fixed oils and fats [28, 29].

Saponin detection

Froth test: A small quantity of the plant powder (0.1 g) was shaken with distilled water for five minutes and allowed to stand. The appearance of froth after 15 minutes indicated the presence of saponins.

Flavonoid detection

Shinoda test: A magenta color developed upon adding magnesium ribbon pieces and concentrated hydrochloric acid to the extract, signaling the presence of flavonoids.

Alkali test: The extract was treated with a dilute ammonia solution, resulting in a yellow color in the ammonia layer, confirming the presence of flavonoids.

Steroid detection

Salkowski test: When concentrated sulfuric acid was added to the chloroform extract, a red or golden-yellow color formed in the lower layer, indicating the presence of sterols or triterpenes [30].
Liebermann-Burchard test: Acetic anhydride was added to the chloroform extract, followed by the careful addition of concentrated sulfuric acid from the sides of the test tube [31].
Keller-Kiliani test: A mixture of acetic acid, ferric chloride, and concentrated sulfuric acid was added to the extract, and the appearance of a reddish-brown ring turning bluish-green confirmed the presence of deoxy sugars.

Alkaloid detection
Dragendroff's reagent test: A few drops of Dragendroff's reagent were added to the extract, and a reddish-brown precipitate was formed, indicating the presence of alkaloids.
Hager's reagent test: The addition of Hager's reagent resulted in a yellow precipitate, confirming the presence of alkaloids [32].

Tannin detection
Ferric chloride test: The methanolic extract was treated with ferric chloride, and the appearance of a blue or brownish-green color indicated tannins.
Lead acetate test: When the extract was treated with 10% lead acetate, the formation of a creamy yellow or white precipitate confirmed the presence of tannins [33-35].

Physicochemical parameter analysis
The physicochemical properties of *M. oleifera* and *M. concanensis* were assessed according to standard methods [36, 37].

Total Ash content
Approximately 2 g of the powdered plant material was placed in a pre-weighed silica crucible and heated at 500-600 °C in a muffle furnace (Proto-tech, Mumbai) until the sample turned white, indicating complete combustion. The crucible was then weighed again to calculate the percentage of total ash on a dry-weight basis.

Acid-Insoluble Ash
After adding 25 mL of dilute hydrochloric acid to the total ash, the mixture was boiled gently and filtered through ashless paper. The residue was washed with hot water until the filtrate was neutralized. The residue was then incinerated to constant weight, and the acid-insoluble ash was calculated.

Alcohol-soluble extractives
Five grams of powdered plant material were macerated in 100 mL of 90% alcohol and left for 24 hours with periodic shaking. After 18 hours, the mixture was filtered, and 25 mL of the filtrate was evaporated in a pre-weighed porcelain dish to dryness. The alcohol-soluble extractive percentage was determined based on the dried plant sample.

Water-soluble extractives
A similar procedure to the alcohol-soluble extractives was followed, but 90% alcohol was replaced with chloroform water to determine water-soluble extractives.

Petroleum ether-soluble extractive value
The same method as for alcohol-soluble extractives was followed, but instead of alcohol, petroleum ether was used.

Thin layer chromatography (TLC)
TLC analysis was performed using handmade Silica Gel G plates. Different mobile phases were used for detecting specific phytochemicals: toluene: methanol (9:1) for steroids, toluene: diethylamine: ethyl acetate (7:1:2) for alkaloids, and ethyl acetate: glacial acetic acid: formic acid: water (100:11:11:20) for flavonoids.

Sample preparation

1 mg of the methanolic extract of *M. oleifera* and *M. concanensis* was dissolved in methanol for alkaloids, in 80% methanol for flavonoids, and petroleum ether for steroids before application onto TLC plates. After spotting, the plates were placed in their respective mobile phases. The plates were examined under UV light at 245 nm or 365 nm, and the Rf values were calculated by dividing the distance traveled by the sample by the solvent front distance [38-40].

Quantification of total phenolic content

To measure the total phenolic content, a calibration curve was generated using gallic acid. A 100 µg/ml stock solution of gallic acid was prepared, and from this, several dilutions (10-50 µg/ml) were made. One milliliter of each concentration was added to a volumetric flask, followed by 3 ml of distilled water. Next, 0.5 ml of phenol reagent was introduced, and then 2 ml of a 2% sodium carbonate solution was added. The volume was then adjusted to 10 ml with distilled water. Absorbance was measured at 765 nm using a UV-visible spectrophotometer (Shimadzu UV-1800, Japan), and distilled water was used as a blank.

For the plant extract preparation, 100 mg of *M. oleifera* and *M. concanensis* were dissolved separately in 50 ml of 50% methanol. The extracts were filtered, and 1 ml from each filtrate was taken into a 10 ml volumetric flask, followed by the same reagents and procedures used for the calibration curve preparation. The phenolic content was calculated using the gallic acid calibration curve [41].

Total flavonoid content determination

The flavonoid content was estimated using the Aluminum chloride method with quercetin as the standard. A 100 µg/ml stock solution of quercetin was prepared, and dilutions (10-50 µg/ml) were made. One milliliter of each concentration was transferred to a volumetric flask. The following were then added to each flask: 0.2 ml of 10% aluminum chloride, 3 ml of 95% methanol, and 0.2 ml of 1 M potassium acetate. The solutions were diluted to 10 ml with distilled water and allowed to stand at room temperature for 30 minutes. Absorbance was measured at 415 nm using a UV-visible spectrophotometer.

For the plant extracts, 100 mg of *M. oleifera* and *M. concanensis* were dissolved in 80% methanol. One milliliter of each extract was pipetted into a 10 ml volumetric flask, and the same reagents and procedures used for the standard preparation were followed. The flavonoid content was then calculated from the quercetin calibration curve [42].

Results and Discussion

The morphological and organoleptic features of *M. oleifera* **(Figure 1a)** and *M. concanensis* **(Figure 1b)** are outlined in **Table 1**.

Table 1. Morphological and organoleptic characters

Macroscopic characters	***M. oleifera***	***M. concanensis***
Leaves	Tripinneately compound	Bipinneately compound
Shape	Oval	Obovate
Size	1.4-5 cm	2.2-5 cm
Texture	Smooth to touch	Hard to touch
Fruits	Long, slender, and green	Long and slender and green with brownish tinge
Color	Light green color	Dark green color
Odor	Unpleasant odour	Unpleasant odour
Taste	Bitter	Bitter

a)

b)

Figure 1. a) *M. oleifera*, and b) *M. concanensis*

Preliminary phytochemical study

The therapeutic effects of the plant are primarily due to the presence of various bioactive compounds, which can be identified through a preliminary phytochemical analysis. The investigation of *M. oleifera* and *M. concanensis* extracts revealed that both contain several key phytochemicals such as carbohydrates, lipids, proteins, alkaloids, flavonoids, tannins, saponins, and steroids. However, a positive result was observed in the Kellar-Kiliani test for *M. oleifera*, while *M. concanensis* showed no response.

Evaluation of physicochemical parameters

Although physicochemical properties may vary, they are critical for assessing the authenticity, purity, and quality of medicinal plants [43]. Ash values reflect the inorganic content, which could either be naturally present or possibly a result of adulteration. Extractive values help estimate the chemical composition of the plant material [28, 37]. The extract underwent multiple physicochemical tests, with the outcomes displayed in **Table 2**. *M. oleifera* exhibited higher ash and extractive values than *M. concanensis*. The alcohol extractive value was the highest among the extracts, followed by the water and petroleum ether extracts for both plant species.

Table 2. Physicochemical properties

Physicochemical parameters	*M. oleifera*	*M. concanensis*
Total ash	4.5 ± 0.42	3.5 ± 0.23
Acid insoluble ash	1.1 ± 0.22	0.8 ± 0.35
Water-soluble extractives	18.7 ± 0.39	16.5 ± 0.43
Alcohol-soluble extractives	27.2 ± 0.13	25.5 ± 0.26
Petroleum ether-soluble extractives	5.3 ± 0.21	4.9 ± 0.32

Note: n represents the number of readings.

Thin layer chromatographic (TLC) analysis

TLC is a widely used method for identifying the phytochemicals present in plant extracts. This technique provides a "fingerprint" of the constituents in the sample, aiding in the isolation of bioactive compounds [44]. The method offers several advantages, such as reducing both analysis time and sample cost and allowing the simultaneous analysis of multiple samples with minimal mobile phase usage [45, 46]. Additionally, TLC, when combined with different analytical detection techniques, proves effective in the qualitative and quantitative separation of crude mixtures [47].

TLC of *M. oleifera* and *M. concanensis* was performed using three distinct solvent systems: Toluene: Methanol (9:1) for steroids, Toluene: Diethylamine: Ethyl acetate (7:1:2) for alkaloids, and Ethylacetate: Glacial acetic acid: Formic acid: Water (100:11:11:20) for flavonoids. The results of the TLC analysis are summarized in **Table 3**.

- For steroids, *M. oleifera* produced 4 spots, exhibiting brownish to pink fluorescence under UV 365 nm, while *M. concanensis* displayed 6 spots with pink fluorescence. In visible light, both species showed light brown to greenish spots, with *M. oleifera* showing 3 spots and *M. concanensis* showing 4.
- For alkaloids, both extracts from *M. oleifera* and *M. concanensis* exhibited 4 spots with pink fluorescence under UV 365 nm, while in visible light, both species displayed 5 spots ranging from light brown to green, with identical Rf values.

- For flavonoids, *M. oleifera* revealed 8 spots, and *M. concanensis* displayed 5, all of which fluoresced blue, pink, or purplish under UV 365 nm. In visible light, both species presented 3 spots ranging from light brown to greenish.

Table 3. TLC study of *M. oleifera* and *M. concanensis*

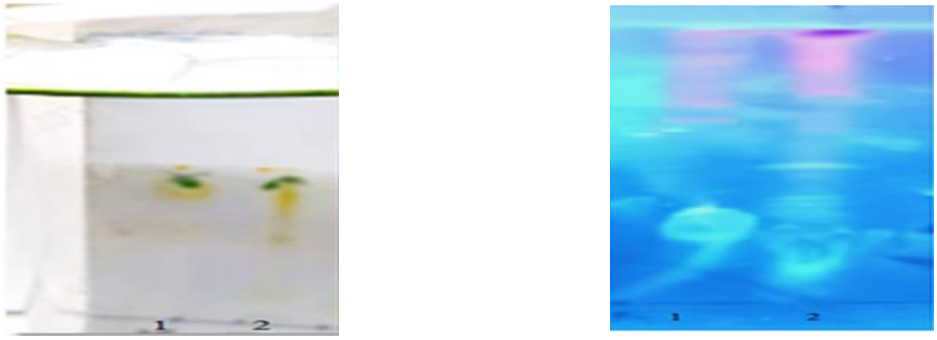
Phytochemical groups	Rf values	
	M. concanensis	***M. oleifera***
Steroids (Figure 2)	0.16, 0.28, 0.32, 0.44, 0.59, 0.76, 0.85 (7)	0.24, 0.28, 0.40, 0.73, 0.83 (5)
Alkaloids (Figure 3)	0.54, 0.59, 0.70, 0.82 (4)	0.54, 0.59, 069, 0.82 (4)
Flavonoids (Figure 4)	0.12, 0.55, 0.66, 0..80, 0.92 (5)	0.17, 0.23, 0.27, 0.34, 0.45, 0.66, 0.80, 0.92 (8)

a) b)

Figure 2. TLC of steroids: a) in developing chamber, and b) Under UV 365 nm; track 1: *M. concanensis*, and track 2: *M. oleifera*

a) b)

Figure 3. TLC of alkaloids: a) in developing chamber, and b) under UV 365 nm; track 1: *M. concanensis*, and track 2: *M. oleifera*



a) b)

Figure 4. TLC of flavonoids: a) in developing chamber, and b) under UV 365 nm; track 1: *M. concanensis*, and track 2: *M. oleifera*

Total phenolic and flavonoid content

Phenolic compounds, including flavonoids, are common secondary metabolites in plants, playing a vital role in various biological activities such as antioxidation, anti-inflammatory, anticancer, and antibacterial effects [41, 42]. The total phenolic content was assessed using the Folin-Ciocalteu method, while the total flavonoid content was determined by the Aluminum chloride method.

Standard calibration curves for gallic acid (for phenols) and quercetin (for flavonoids) were constructed across concentrations ranging from 10 to 50 µg/ml. The coefficient of determination (R^2) for gallic acid was 0.991, and for quercetin, it was 0.998. Among the two species, *M. oleifera* showed significantly higher levels of both phenolic and flavonoid content. Specifically, *M. oleifera* contained 453 µg/ml of phenols and 365 µg/ml of flavonoids, whereas *M. concanensis* contained 393 µg/ml of phenols and 263 µg/ml of flavonoids.

Conclusion

M. oleifera and *M. concanensis* are two notable species in the *Moringa* genus, with *M. oleifera* being more extensively studied regarding its medicinal properties and phytochemical content. In this comparison, both species exhibited similar phytochemical profiles, with a few distinctions. Both species were found to contain substantial amounts of phenols and flavonoids, which are key contributors to their antioxidant properties. Future research is encouraged to isolate and explore the active compounds in *M. concanensis*, which may offer therapeutic potential for various health conditions.

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