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COMPARATIVE ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS AND PROXIMATE COMPOSITION OF *Moringa oleifera* SEEDS AND FLOWERS

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Abstract

Moringa oleifera, a drought-resistant tree native to the Indian subcontinent, is widely valued for its nutritional and medicinal properties. This study compared the phytochemical constituents and proximate composition of its flowers and seeds collected from Erode. Proximate analysis showed higher moisture content in flowers and higher ash content in seeds, indicating better storage stability and mineral richness of seeds. Both samples exhibited near-neutral pH and differences in bulk density. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, phenols, terpenoids, and steroids. Quantitative analysis showed higher flavonoid, iron, and amino acid content in flower extracts, whereas seeds contained higher alkaloid levels, with variations depending on the solvent used. The study highlights the significant nutritional and phytochemical potential of *Moringa oleifera* for applications in functional foods and therapeutics.

Keywords : *Moringa oleifera* flowers, seeds, phytochemical analysis, Proximate analysis, Biochemical analysis

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1. INTRODUCTION

Moringa oleifera Lam. is a fast-growing perennial species of the Moringaceae family, native to the Indian subcontinent and commonly known as the drumstick tree, horseradish tree, or ben oil tree. It is widely cultivated in tropical and subtropical regions and valued for its nutritional, medicinal, and industrial applications. Virtually every part of the plant including the leaves, flowers, seeds, bark, and roots contains high concentrations of bioactive compounds such as phenolics, flavonoids, alkaloids, terpenoids, tannins, glucosinolates, and isothiocyanates [1-3,5-7].

M. oleifera is recognized for its diverse pharmacological properties, exhibiting antioxidant, antibacterial, antihyperlipidemic, anti-inflammatory, antiulcer, and anticancer activities. Its leaves and young pods are widely consumed as nutrient-rich vegetables and serve as an important source of proteins, vitamins (A, C, and E), and essential minerals [6,8]. Owing to its high nutritional value and therapeutic potential, *M. oleifera* has been termed a “miracle tree” and is considered an effective resource for addressing malnutrition and promoting health in developing countries.

The seed oil of *M. oleifera*, often referred to as ben oil, is notable for its high content of unsaturated fatty acids (approximately 82%), predominantly oleic acid (around 70%), giving it a lipid profile similar to olive oil [1]. It is also rich in α -tocopherol and δ -tocopherol, known for their antioxidant properties and ability to protect cells from oxidative stress [9]. These attributes have led to the growing use of moringa seed oil in cosmetic, pharmaceutical, and food industries.

In traditional medicine systems such as Siddha and Ayurveda, *M. oleifera* holds a significant place. The flowers are traditionally used to treat inflammation, muscle ailments, cough, chronic bronchitis, urticaria, and skin diseases, and are incorporated in preparations like *Panca cuta meluku* for respiratory and dermatological disorders [10]. Phytochemical analyses of the flowers have demonstrated the presence of ascorbic acid, polyphenols, tannins, and flavonoids, which contribute to their strong free radical scavenging and antioxidant activities [11].

Economically, *M. oleifera* cultivation and trade contribute substantially to the agricultural and nutraceutical sectors across Asia. The global moringa market, valued at approximately USD 5.5 billion (₹ 456 billion) in 2018, is projected to reach USD 10 billion (₹ 830 billion) by 2025, reflecting increasing global demand for natural health products [12]. In India, the moringa ingredients market was valued at USD 1.12 billion (₹ 93 billion) in 2020 and is expected to grow to USD 2.55 billion (₹ 212 billion) by 2028, with an annual growth rate of nearly 10.8 %. [13–14].

2. MATERIALS AND METHODS

2.1. Collection of plant material

Fresh seeds and flowers of *Moringa oleifera* were collected from local farms in Erode District, Tamil Nadu, India. The plant material was authenticated by the Botanical Survey of India, Southern Regional Centre, Tamilnadu Agricultural University Campus, Coimbatore, Tamil Nadu. The samples were washed thoroughly with distilled water to remove adhering impurities and shade-dried at ambient temperature. The dried materials were pulverized into fine powder using a mechanical grinder and stored in airtight containers until further analysis.

2.2. Preparation of extracts

Powdered samples (10 g) of *M. oleifera* seeds and flowers were extracted separately using two solvents like distilled water and ethanol in a 1:10 (w/v) ratio. The mixtures were placed in 250 mL conical

flasks and agitated on a rotary shaker (150 rpm) at room temperature for 24 h. The extracts were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated and stored at 4 °C for subsequent phytochemical analyses [13].

2.3. Qualitative Phytochemical screening

The aqueous and ethanolic extracts of *M. oleifera* seeds and flowers were subjected to qualitative phytochemical tests for the detection of primary and secondary metabolites, following standard procedures [12–14]. The metabolites screened included carbohydrates, proteins, alkaloids, flavonoids, tannins, terpenoids, saponins, steroids, glycosides, phenols, quinones, coumarins, anthraquinones, carotenoids, fatty acids, and leucoanthocyanins.

2.4. Phytochemical test procedures

Standard qualitative tests were performed as follows:

- **Carbohydrates:** Molisch's, Fehling's, and Benedict's tests were used to confirm the presence of carbohydrates.
- **Proteins and amino acids:** Biuret, Millon's, and Ninhydrin tests were employed to detect proteins and amino acids.
- **Fats and steroids:** Extracts were treated with chloroform, acetic anhydride, and concentrated sulphuric acid; formation of violet to green coloration indicated steroids.
- **Saponins:** Foam and froth tests were conducted by vigorous shaking with distilled water; stable froth formation confirmed saponins.
- **Fatty acids:** Filter paper translucency test confirmed the presence of fatty acids.
- **Alkaloids:** Mayer's and Wagner's reagents were used to detect alkaloids, indicated by greenish or reddish-brown precipitates.
- **Flavonoids:** Acid tests using diluted sulphuric acid yielded orange coloration, indicating flavonoids.
- **Terpenoids:** Formation of blue or green rings with acetic anhydride and concentrated sulphuric acid indicated terpenoids.
- **Glycosides:** Liebermann's and Keller–Killiani tests were used to detect general and cardiac glycosides, respectively.
- **Coumarins:** Yellow coloration upon addition of sodium hydroxide confirmed coumarins.
- **Quinones:** Formation of a yellow precipitate with hydrochloric acid indicated quinones.
- **Carotenoids:** Reaction with concentrated sulphuric acid and chloroform produced a blue colour confirming carotenoids.
- **Phenols:** Formation of a deep blue colour with ferric chloride confirmed phenols.
- **Leucoanthocyanins:** Red coloration in the upper layer upon reaction with isoamyl alcohol indicated leucoanthocyanins.
- **Anthraquinones:** Bright pink coloration with ammonium hydroxide confirmed anthraquinones.

2.5 Proximate analysis

The proximate analysis of *Moringa oleifera* flower and seeds was carried out in accordance with standard laboratory procedures to quantify its primary nutritional components. The specific parameters assessed and the analytical methods adopted for their determination are detailed in the following section.

2.5.1 Estimation of Moisture Content

Approximately 5 g of the fresh sample was taken in triplicate and dried in a hot air oven at 105 °C for 8 hours until a constant weight was achieved. The dried samples, contained in China crucibles, were immediately transferred to a desiccator, cooled, and weighed. The moisture content was calculated using the following equation:

$$\% \text{ Moisture} = \text{Loss of weight (g)} \times 100 / \text{Weight of sample(g)}$$

2.5.2 Estimation of Ash Content

A 5 g portion of the sample was placed in a pre weighed crucible and incinerated in a muffle furnace at 550 °C for 4 hours. The crucibles were cooled in a desiccator and weighed. The ash content was determined using the formula:

$$\% \text{ Ash} = \text{Weight of ash(g)} \times 100 / \text{Weight of sample(g)}$$

2.5.3 Determination of pH

Approximately 0.5 g of Moringa leaf and flower powder was dissolved in 2 ml of an appropriate solvent. The pH was determined by immersing pH indicator paper into the solution, and the developed color was compared with a standard color chart to estimate the pH value.

2.5.4 Determination of Bulk Density

Bulk density of adsorbents was determined by gently filling in a cylindrical jar of known volume with the material and weighed. The electronic balance was used to measure the weight and it will measure to the nearest 0.001gm. The volume of the adsorbents was measured by a graduated cylindrical jar. In order to check the accuracy of the jar, certain quantity of water was taken in the jar and it was weighed in the electronic balance. The specific volume of water was verified and found correct. The bulk density was calculated.

$$\text{Bulk Density} = \text{Mass of Adsorbents} / \text{Volume of Adsorbents}$$

2.6 Biochemical Analysis

2.6.1 Estimation of Total Phenolic Content

Total phenolic content was determined using the Folin–Ciocalteu method. One milliliter of the alcoholic plant extract was mixed with 1 ml of Folin–Ciocalteu reagent, followed by the addition of 2 ml of 20% (w/v) sodium carbonate solution. The mixture was incubated in a boiling water bath for 1 min and then cooled to room temperature. Absorbance was measured at 650 nm using a colorimeter against a reagent blank. Catechol was used as the standard, and total phenolic content was calculated from the calibration curve and expressed as $\mu\text{g g}^{-1}$ fresh weight.

2.6.2 Estimation of Total Flavonoid Content

Total flavonoid content was estimated using the aluminium chloride colorimetric method. One milliliter of plant extract was mixed with 2 ml of distilled water and 1 ml of 5% (w/v) sodium nitrite solution. After 5 min, 1 ml of 1% (w/v) aluminium chloride solution was added, followed by the addition of 1 ml of 1 M sodium hydroxide after 6 min. The final volume was made up with distilled water, and absorbance was measured at 510 nm against a reagent blank. Total flavonoid content was calculated from the standard calibration curve and expressed as mg g^{-1} fresh weight.

2.6.3 Estimation of Iron Content

Iron content was estimated by Wong's method. One milliliter of the sample extract or iron standard (20–100 μg) was mixed with 1 ml each of acetate buffer, hydroxylamine hydrochloride, and 2,2'-dipyridyl reagent. The final volume was made up with distilled water, and absorbance was measured at 540 nm against a reagent blank. Iron concentration was calculated from the standard calibration curve and expressed as mg g^{-1} fresh weight.

2.6.4 Estimation of Total Free Amino Acids

Total free amino acids were determined by the ninhydrin method. To 0.1 ml of the sample

extract, 1 ml of ninhydrin reagent was added and the volume was made up to 2 ml with distilled water. The mixture was heated in a boiling water bath for 20 min, cooled, and diluted with 5 ml of diluent solvent. After 15 min, absorbance was recorded at 570 nm against a reagent blank. Leucine was used as the standard, and total free amino acid content was calculated from the calibration curve and expressed as $\mu\text{g g}^{-1}$ fresh weight.

3. RESULTS AND DISCUSSION

S. No.	Phytochemical Test	<i>Moringa .Oleifera</i> Seeds		<i>Moringa Oleifera</i> Flowers	
		Aqueous	Ethanol	Aqueous	Ethanol
1	Carbohydrates (Molisch's, Fehling's)	+	+	+	—
2	Alkaloids (Mayer's, Wagner's)	+	+	+	+
3	Saponins (Foam, Froth)	+	+	—	+
4	Flavonoids (Acid test)	—	—	+	+
5	Terpenoids (Acetic anhydride)	—	—	+	—
6	Amino acids (Ninhydrin)	+	+	+	+
7	Proteins (Millon's)	+	+	+	+
8	Glycosides (Libermann's)	—	—	—	—
9	Steroids	—	—	—	—
10	Coumarins	—	+	—	+
11	Quinones	+	+	+	+
12	Anthraquinones	+	+	+	+
13	Carotenoids	—	+	—	—
14	Fatty acids	+	+	+	+
15	Leucoanthocyanins	+	+	—	—
16	Phenols	—	—	—	—
17	Cardiac glycosides	—	+	—	—

3.1 Qualitative Phytochemical screening

Table 1. Qualitative phytochemical screening of aqueous and ethanolic extracts of *Moringa oleifera* seeds and flowers

The qualitative phytochemical screening of *Moringa oleifera* seeds and flowers revealed the presence of diverse bioactive constituents in both aqueous and ethanolic extracts (Table 1). Among the tested compounds, alkaloids, quinones, anthraquinones, fatty acids, and amino acids were consistently present in all extracts, indicating their abundant distribution across different plant parts. Flavonoids and terpenoids were predominantly detected in flower extracts, suggesting that floral tissues may serve as a richer source of antioxidant compounds compared to seeds.

The aqueous and ethanolic extracts of seeds showed strong positive reactions for carbohydrates, proteins, and amino acids, reflecting their nutritional and metabolic significance. The ethanolic extract of flowers exhibited the presence of flavonoids, coumarins, anthocyanins, and quinones, which are well

known for their antioxidant and antimicrobial activities. In contrast, phenols and steroids were absent in both seed and flower extracts, implying that these constituents might occur at low or undetectable levels in the tested solvents.

The presence of cardiac glycosides and cycloglycosides in selective extracts highlights the plant's potential pharmacological value, as these compounds are reported to exhibit cardiogenic and antimicrobial effects [15,16]. Overall, the results suggest that both seeds and flowers of *M. oleifera* possess a rich spectrum of secondary metabolites, though their qualitative profiles differ depending on the solvent and plant part. Such phytochemical diversity supports the traditional use of *M. oleifera* as a functional food and medicinal plant.

3.2 Proximate Analysis

S.No	Parameter	<i>M.Oleifera</i> Flowers	<i>M.Oleifera</i> Seeds
1	Moisture %	8.52 %	4.26 %
2	Ash %	15.5 %	24.4 %
3	pH	6.5	6.3
4	Bulk Density (g/cm ³)	0.48	0.253

Table 2. Proximate analysis of *Moringa oleifera* seeds and flowers

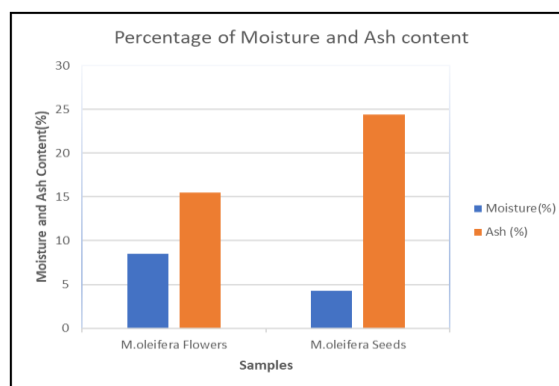
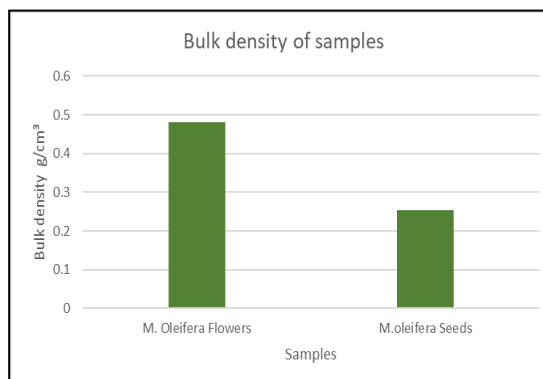
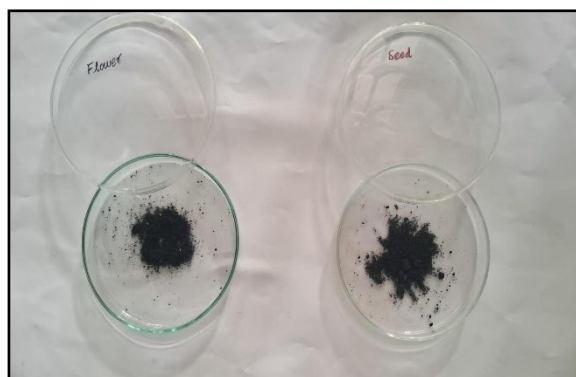


Fig 1. Proximate analysis of *Moringa oleifera* seeds and flowers



The proximate analysis of *Moringa oleifera* seeds and flowers showed that the flowers had higher moisture content (8.52%) than the seeds (4.26%), indicating that the seeds have better storage stability and lower susceptibility to spoilage. The pH values of both samples were slightly acidic, with flowers at

6.5 and seeds at 6.3, showing they are near neutral and suitable for consumption. The bulk density was higher in flowers (0.48 g/cm^3) than in seeds (0.253 g/cm^3), suggesting that flower powder is more compact, while the seeds are lighter and more porous.

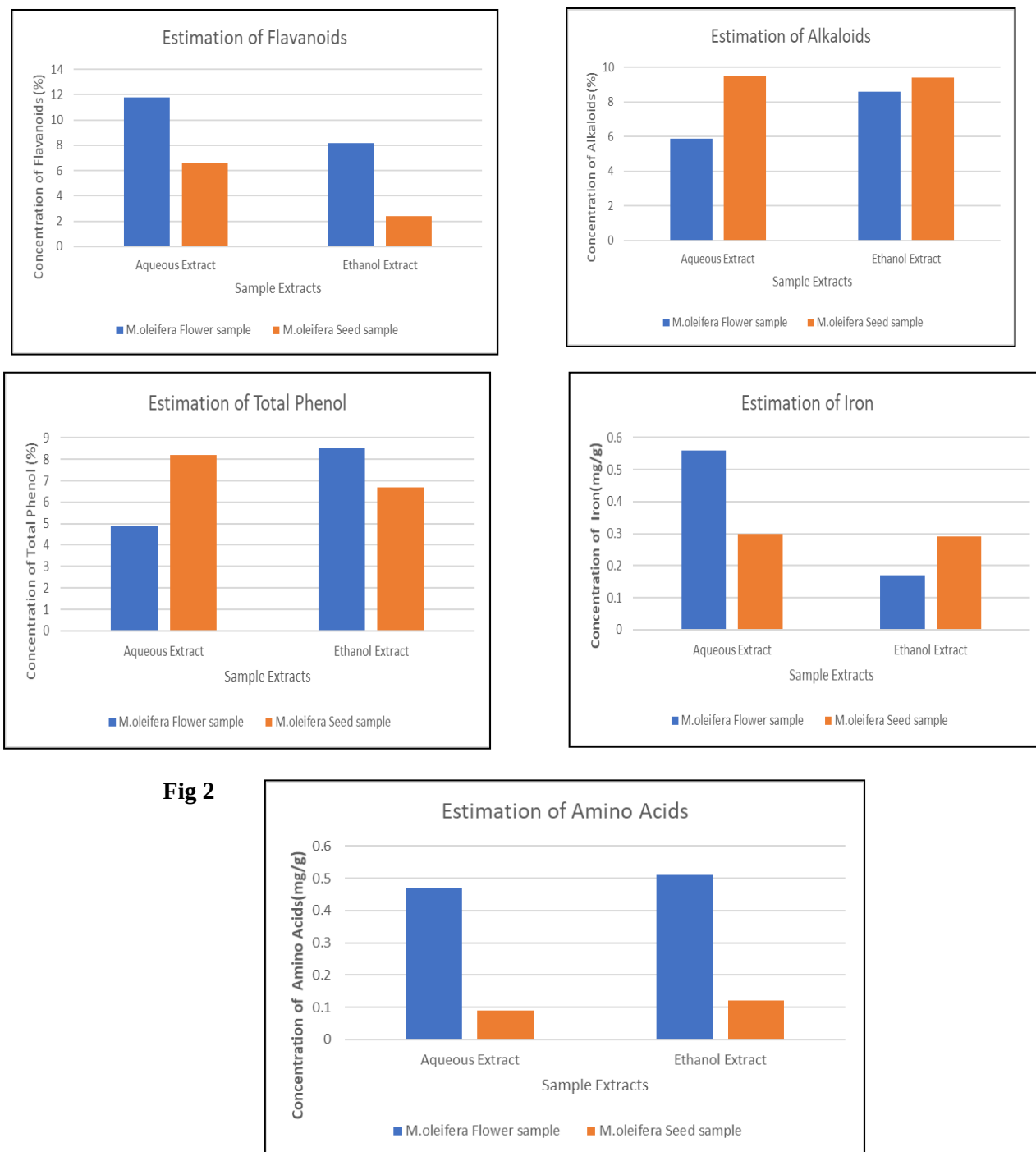
The ash content was higher in seeds (24.4%) compared to flowers (15.5%), indicating a higher mineral concentration in the seeds. Overall, the results show that *Moringa oleifera* seeds and flowers differ in composition, with seeds being richer in minerals and more suitable for long-term storage, while flowers have higher moisture and are more suitable for immediate or fresh use.

3.3 Biochemical Analysis

Phytoconstituents	<i>Moringa Oleifera</i> Flower Extracts		<i>Moringa Oleifera</i> Seed Extracts	
	Aqueous	Ethanol	Aqueous	Ethanol
Flavanoids (%)	11.8±0.23	8.2±0.24	6.6±0.33	2.4±0.14
Alkaloids (%)	5.9±0.11	8.6±0.43	9.5±0.28	9.4±0.37
Total Phenol (%)	4.9±0.29	8.5±0.17	8.2±0.16	6.7±0.26
Iron (mg/g)	0.56±0.01	0.17±0.005	0.30±0.03	0.29±0.04
Amino acids(mg/g)	0.47±0.02	0.51±0.01	0.09±0.04	0.12±0.04

Table 3: Biochemical analysis of *Moringa oleifera* seeds and flowers

The quantitative biochemical analysis of *Moringa oleifera* flower and seed extracts varied depending on the plant part and the solvent used. Flavonoid content was highest in the aqueous flower extract (11.8%), whereas seed extracts exhibited comparatively lower values. In contrast, alkaloid content was higher in seed extracts than in flower extracts, with maximum levels observed in both ethanolic and aqueous seed extracts. Total phenolic content showed solvent-dependent variation, with higher values recorded in the ethanolic flower extract (8.5%) and aqueous seed extract (8.2%). Iron content was highest in the aqueous extract of *Moringa* flowers (0.56 mg/g), indicating that water is a more effective solvent for iron extraction. Total free amino acids were also higher in flower extracts compared to seed extracts, with the maximum amount observed in the ethanolic flower extract (0.51 mg/g). Overall, *Moringa* flowers were richer in flavonoids, iron, and amino acids, whereas seeds contained higher levels of alkaloids, highlighting the influence of both plant part and extraction solvent on biochemical composition.

**Fig 2****Biochemical analysis of *Moringa oleifera* seeds and flowers****4. CONCLUSION**

This study highlights the significant phytochemical and nutritional potential of *Moringa oleifera* seeds and flowers, with variations influenced by plant part and extraction solvent. Both exhibited key bioactive compounds such as alkaloids, quinones, anthraquinones, fatty acids, and amino acids. Flower extracts were richer in flavonoids, iron, and amino acids, indicating strong antioxidant and nutritional properties, whereas seeds showed higher alkaloid content, suggesting distinct biological roles.

Proximate analysis revealed that seeds, with lower moisture content and higher ash values, possess better storage stability and greater mineral richness, while flowers, having higher moisture and bulk density, are more suitable for fresh use. Variations observed in phytochemical yield across different solvents further emphasize the importance of extraction methods in maximizing compound recovery.

Overall, *Moringa oleifera* seeds and flowers serve as valuable sources of bioactive and nutritional compounds with promising applications in functional foods and therapeutic formulations. Further studies focusing on isolation, characterization, and validation of bioactive constituents are recommended to enhance their practical utilization.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

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