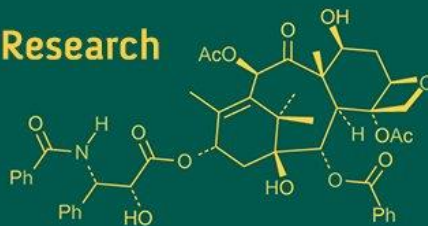
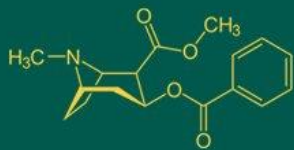


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## Biochemical and functional properties of (*Moringa oleifera*) drumstick leaf powder and its utilization

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### Abstract

*Moringa oleifera*, commonly known as the "drumstick tree" or "miracle tree," has gained global prominence due to its nutritional richness and versatile adaptability. *Moringa oleifera* is a perennial deciduous tropical plant from the Moringaceae family. It is rich in various bioactive compounds and is recognized as an effective remedy against malnutrition. *Moringa* exhibits multiple pharmacological properties, including anti-cancer, anti-diabetic, anti-inflammatory, and antioxidant effects. These beneficial properties are likely linked to the presence of its bioactive compounds, such as flavonoids, alkaloids, saponin etc. This study aimed to explore this biochemical and functional aspects of *Moringa oleifera* leaf powder and its integration into ready-to-serve soup preparations. In this study nutritional profiling of two *Moringa oleifera* varieties, ODC-3 and wild, resulted the ash (ODC-25%, Wild-33.6%), moisture (ODC-11.54%, Wild-7.94%), carbohydrate (ODC-8.1 gm/1000gm, Wild-8.0 gm/100gm) and protein (ODC-25.1 gm/100gm, Wild-23.2 gm/100gm) content. This research also revealed the presence of various antioxidants like phenolics, DPPH, flavonoids, tannins. Mineral composition, reconstitution properties, and phytochemical analysis were also performed. Gas-Chromatography-Mass-Spectrometry (GC-MS) analysis showed the presence of 40 bioactive compounds. Sensory evaluation of ready-to-serve soup of *Moringa oleifera* leaf powder showed overall acceptability for treatment 2 with 20% *Moringa oleifera* leaf powder. In conclusion, this research comprehensively explored the biochemical, functional, and nutritional aspects of *Moringa oleifera* leaf powder. The findings contribute valuable insight into its potential applications in food and pharmaceutical industries, highlighting its versatility and health promoting properties.

**Keywords:** *Moringa oleifera*, antioxidants, flavonoids, DPPH, GC-MS.

### Introduction

*Moringa oleifera*, commonly known as the "drumstick tree" or "miracle tree," (Koul & Chase, 2015) [28] originated from India but has spread to various regions, including Ethiopia, the Pacific islands, Florida, Sudan, the Caribbean, the Philippines, South Africa, Asia, and Latin America (Fahey, 2005) [18]. In the scientific classification, *Moringa oleifera* belongs to the family Moringaceae, genus *Moringa*, and species *oleifera* (Razis *et al.*, 2014) [52]. The moringa genus comprises a total of 13 identified species, with *Moringa oleifera* being one of the most prominent members of the Moringaceae family (Saini *et al.*, 2016) [53]. *Moringa oleifera* is categorized as a tropical plant and is well suited to a diverse range of agro-climatic environments. It is typically grown in regions that are tropical or subtropical and semi-arid. The optimal temperature range for its growth is between 25 and 35°C, but it can endure temperatures as high as 48°C (Palada *et al.*, 2012) [46]. *Moringa oleifera* is one of the most useful trees having great economic importance. Almost every plant part from roots to flowers of moringa are used for various purposes. Fresh, green and young pods, young shoots and leaves are used as vegetables (Ebert & Palada, 2017) [16, 47]. Epidemiological research has demonstrated that *M. oleifera* leaves are a valuable nutritional source and have shown various beneficial properties, including anti-tumour, anti-inflammatory, anti-ulcer, anti-atherosclerotic, and anti-convulsant activities (Chumark *et al.*, 2008; DanMalam *et al.*, 2001; Dahiru *et al.*, 2006) [12, 13, 14]. *Moringa* contains large amounts of  $\beta$ -carotene, ascorbate (vitamin C),  $\alpha$ -tocopherol (vitamin E), and iron, and ranking second in protein content (Freiberger *et al.*, 1998) [19]. *Moringa* contains seven times more vitamin C than oranges, ten times the vitamin A

of carrots, higher calcium and protein content than milk, fifteen times more potassium than bananas, and a remarkable twenty five times more iron than spinach (Liu *et al.*, 2018; Gopalkrishnan *et al.*, 2016) [31]. Moringa is also recognized as a rich source of polyphenols and antioxidants (Mishra *et al.*, 2011) [38]. The content of natural antioxidants, including total phenolics, and vitamins A, C, and E, ranged from 74–210  $\mu\text{mol/g}$  for phenolics, 70–100  $\mu\text{mol/g}$  for ascorbate (vitamin C), 1.1–2.8  $\mu\text{mol/g}$  for  $\beta$ -carotene, and 0.7–1.1  $\mu\text{mol/g}$  for  $\alpha$ -tocopherol (vitamin E) on a dry weight basis (Yang *et al.*, 2007) [61]. *Moringa oleifera* is used as an alternative to imported food supplements in developing countries to treat and combat malnutrition, especially among infants and nursing mothers, by virtue of its chemical constituents (Dhakar *et al.*, 2011) [15]. Various extracts were derived from moringa leaves, and these extracts exhibit the ability to inhibit the growth of bacteria and fungi (Mehmood *et al.*, 2022) [35]. Considering the significance of *Moringa oleifera* and the factors affecting its nutritional composition, this study aims to evaluate the biochemical and functional properties of *Moringa oleifera* leaf powder. The research focuses on elucidating its phytochemical makeup and nutritional benefits to optimize its utilization and enhance its application in diet formulation and medicinal use.

## Material and Methodology

### Collection of *Moringa oleifera* varieties

*Moringa oleifera* Var. (ODC 3) grown in Marathwada region and a wild genotype were selected for research based on visual appearance of fresh, dark green leaves, suitable for making dried leaf powder.

### Sample preparation

Fresh, dark green leaves of *M. oleifera* were air-dried until reaching a constant weight, then pulverized into fine powder using an electric blender. The resulting powder was stored in an airtight container at room temperature for biochemical analysis and further utilization, following the protocol by Stevel and Babatunde (2013) [58] with minor modifications.

### Nutritional qualities of *Moringa oleifera* leaf powder

#### Moisture content

5g of moringa leaf powder subjected to oven drying at 105<sup>o</sup> C for 4 hours, followed by cooling in a desiccator until a constant weight was achieved and the loss in weight was calculated as moisture content according to AOAC (2005) with minor modifications.

#### Ash content

A 5g sample was placed into a silica crucible over low flame and heated in a muffle furnace at 600°C for 4 hours. After cooling, consistent weight measurements were obtained to determine the ash content by calculating the difference between initial and final weights, following the procedure given by AOAC (2005) with minor modifications.

#### Carbohydrate

The total carbohydrate content of *Moringa oleifera* leaf powder was determined using the Anthrone method as proposed by Jayaraman in 1981 [24]. After hydrolysing 100 mg of leaf powder in HCl, the samples were neutralized, treated with Anthrone reagent, and quantified spectrophotometrically at 620 nm.

## Protein

Protein content was estimated using Lowry's method (Lowry *et al.*, 1951) [32] with minor modifications. Glass test tubes were filled with varying volumes of BSA solution and test samples, then adjusted to 4 ml with distilled water. After adding reagents and incubating, absorbance at 660 nm was measured, and protein concentrations were determined using a standard curve.

### Mineral composition of *Moringa oleifera* leaf powder

Determination of minerals was estimated by Raghuramulu *et al.*, (1983) with minor modification.

### Preparation of ash solution

Preparation of ash solution took place using the method given by Raghuramulu *et al.*, (1983). 1 g Ash was mixed with 0.5-1 ml distilled water, followed by addition of 5 ml hydrochloric acid and evaporation to dryness twice. Then, 4 ml hydrochloric acid and water were added, heated, filtered, and made up to 100 ml in a volumetric flask used for phosphorus, iron, and calcium determination.

### Iron estimation

1.5 ml of ash solution was mixed with 1 ml 30% H<sub>2</sub>SO<sub>4</sub> and 1 ml 7% potassium persulfate. Then, 1.5 ml 40% potassium thiocyanate solution was added. Red coloration was measured at 540 nm over 20 minutes. Standard aliquots (10-50  $\mu\text{g}$ ) were subjected to the same treatment.

### Calcium estimation

Amount of calcium was estimated using titrimetric method given by Raghuramulu *et al.*, (1983). The ash solution was treated with ammonium oxalate and ammonia was added, centrifuged, then titrated with KMnO<sub>4</sub> after adding H<sub>2</sub>SO<sub>4</sub> until a faint pink colour persisted.

### Phosphorous estimation

The amount of phosphorus was estimated by Fiske and Subba Raw method given by Raghuramulu *et al.* (1983). 2 ml of ash solution was mixed with 5 ml 10% trichloroacetic acid, centrifuged, and 3 ml of supernatant collected. To this, 0.4 ml of Aminonaphthol Sulphonic Acid (ANSA) was added. Standard aliquots (8-40  $\mu\text{g}$ ) were subjected to the similar treatment, volume adjusted to 10 ml, and incubated for 20 minutes before measuring color at 660 nm.

### Ascorbic acid (vitamin C)

Ascorbic acid content was determined by titrating a known sample weight with 2,6-dichlorophenol indophenol dye using oxalic acid using procedure given by AOAC 2000.

### Antioxidant composition in *Moringa oleifera* leaf powder

#### Preparation of extracts

Mature leaves of *Moringa oleifera* Var. ODC-3 and wild variety subjected to washing and shade drying, followed by grinding into powder. Extracts were obtained by mixing 10 gm leaf powder with ethanol and methanol followed by centrifugation, and evaporation. The resulting filtrate was dissolved in the same solvent for further analysis. Ethanolic extract was prepared following a method outlined by Olatunde & Dikwa in 2014 [42], while methanolic extract was prepared using a method described by (Pavithra *et al.*, 2009) [47].

### Total phenolics

The quantification of phenolic compounds in the leaf powder extracts were determined using the Folin-Ciocalteu reagent, following the method described by El Sohaimey & Masry in 2014 [17].

### DPPH (2,2-Diphenyl-1-picrylhydrazyl) activity

The assessment of the free radical scavenging activities of *Moringa oleifera* leaf powder extracts were carried out using procedure given by Rakesh *et al.*, (2010) [51]. To assess antioxidant activity, 1 ml of methanolic and ethanolic extracts were mixed with 0.5 ml of 0.15 mM DPPH solution, incubated for 30 minutes at 20°C, and absorbance measured at 517 nm to determine IC<sub>50</sub> value representing the concentration needed to scavenge 50% of DPPH free radicals.

### Flavonoids

Total flavonoid analysis was done by the method given by Benitez *et al.*, (2011). The determination of the total flavonoid content was conducted through a colorimetric assay. 100 µl of methanolic and ethanolic extracts were mixed with distilled water, followed by sodium nitrite, aluminium chloride, and sodium hydroxide. After dilution, absorbance was measured at 510 nm.

### Phytochemical analysis of *Moringa oleifera*

#### Quantitative analysis of phytochemicals

**Alkaloids:** Quantitative analysis of alkaloids was done by using procedures outlined by Krishnaiah *et al.*, in (2009) [29]. A 5 g sample was extracted with 200 ml of 10% acetic acid in ethanol for 4 hours, then concentrated. Alkaloids were precipitated with ammonium hydroxide, collected, washed, filtered, dried, and weighed.

### Flavonoids

Quantitative analysis of alkaloids was done by using procedures outlined by Krishnaiah *et al.*, in (2009) [29]. 10 gm leaf powder was subjected to repetitive extraction with 100 ml 80% aqueous methanol and ethanol at room temperature. The filtrate was evaporated to dryness in a crucible over a water bath until a constant weight was achieved.

### Total Tannins (TT)

Determination of tannin content was carried out through the Folin-Ciocalteu assay method. This method was in accordance with the procedure outlined by Tamilselvi *et al.*, in 2012 [59].

### Qualitative analysis of phytochemicals

The methanol and ethanol extracts were screened for active phytochemicals (tannins, alkaloids, triterpenoids, flavonoids, saponins, anthraquinone glycosides, carbohydrates, proteins, amino acids) using methods described by Harborne JB (1998) [21] and Kokate CK (2005) [27].

**Tannins:** The presence of tannins was confirmed by Ferric chloride test with the formation of a blue color indicating their presence.

**Alkaloids:** Confirmation of alkaloids presence was achieved by Wagner test and the formation of a yellow or brown precipitate indicated a positive test.

- **Triterpenoids:** For triterpenoids detection Salkowski test was carried out and resulted red-brown color interface confirming the presence of triterpenoids.
- **Flavonoids:** Alkaline reagent test and lead acetate test confirmed the presence of flavonoids. In Lead acetate test, formation of a yellow precipitate indicated the presence of flavonoids.
- **Saponins:** For confirmation of saponin Foam test is used. Formation of stable foam in the test tube confirmed the presence of saponins.
- **Anthraquinone glycosides:** Hydroxyanthraquinone test performed for Anthraquinone glycosides. If a red colour formed, it indicated a positive result for the test.
- **Carbohydrates:** Detection of monosaccharides was performed by using Barfoed's test where the formation of a red precipitate confirmed their presence. To test for glucose, Fehlings test was also used, where the formation of a brick-red precipitate indicated the presence of glucose.
- **Protein:** Proteins presence was confirmed by Biuret test, where the formation of a purple or violet color indicated their presence.
- **Fats and fixed oils:** The test for the presence of fats involved addition of copper sulphate solution, followed by sodium hydroxide. If a clear blue solution formed, it confirmed the presence of fats and fixed oils.

### Determination of Reconstitution properties of *Moringa oleifera*

- **Water Absorption Capacity (WAC):** The water absorption capacity (WAC) was determined following the procedure outlined by Adebawale *et al.*, in 2005 [3].
- **Water solubility index (WSI):** The water-solubility index (WSI) of the *M. oleifera* leaf powder determined by the method given by (Hernandez-Diaz *et al.*, 2007 [22].
- **Reconstitution Index (RI):** The reconstitution index of the samples was assessed using the procedure outlined by Onwuka in 2005 [44].
- **Bulk density (BD) determination:** The bulk density (BD) of the samples was assessed using the procedure outlined by Onwuka in 2005 [44].
- **Rehydration Ratio (RR):** The rehydration ratio (RR) of the samples was assessed using the procedure outlined by Krokida & Marinos-Kouris in 2003 [30].
- **Swelling index (SI):** The swelling index was determined by following the method described by Ukpabi & Ndimele in 1990 [60].
- **Antimicrobial activity screening of *Moringa oleifera*:** The stock solution was prepared for antimicrobial screening using the extract obtained from the above-mentioned method. 100 mg/ml samples were subsequently prepared by diluting them with ethanol, methanol, and water.
- **Micro-organisms for antimicrobial activity:** The microorganisms used in present study were obtained from Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. List of microorganisms shown in Table 1

**Table 1.** List of microorganisms used for detection of antimicrobial activity

Sr.No.	Name of Organisms	
	Fungal stains	Bacterial strains
1.	<i>Fusarium oxysporum</i>	<i>Bacillus subtilis</i>
2.	<i>Aspergillus niger</i>	<i>Pseudomonas fluorescens</i>
3.	<i>Trichoderma viridae</i>	<i>Escherichia coli</i>

**Antibacterial and Antifungal screening (in vitro)**

The antimicrobial efficacy of *Moringa oleifera* compounds was evaluated using the well diffusion method. Various strains of bacteria and fungi were inoculated onto prepared culture plates, followed by the introduction of extracts into wells created on the agar surface. After 24 hours at 37±2 °C for bacterial evaluation and 48 hours at 25±2 °C for fungal assessment, zones of inhibition were measured, including the well diameter, to determine efficacy.

**Gas Chromatography-Mass Spectrometry (GC-MS)**

**Analysis:** The GC-MS analysis of a methanolic extract of *Moringa oleifera* leaf powder was performed with Jeol Accut of GCv GSHRMS.

**Preparation of Ready to Serve Soup from *Moringa oleifera* Leaf Powder:**

*Moringa oleifera* leaves were air-dried at room temperature until a constant weight was achieved, then pulverized into a fine powder using an electric blender. The powder was stored in an airtight container at room temperature for later use in soup preparation. Additionally, other ingredients listed in Table 2 were cleaned, chopped into small pieces, and sun-dried for 5-6 hours before being utilized in soup preparation. Different formulations were prepared by mixing sugar, corn-starch, cumin, black pepper, salt, coriander, garlic, onion, tomato, lentil, sodium benzoate with 3 treatment including control sample. Only the most preferable of that combination was selected through sensory evaluation.

**Table 2:** Different Formulations of Ready to Serve Soup of *Moringa oleifera* Leaf Powder

Sr.No.	Ingredients (gm)	Formulations			
		Control	T1 MLP (18%)	T2 MLP (20%)	T3 MLP (22%)
1.	Onion	17	11	10	9
2.	Tomato	22	16	15	14
3.	Lentil	7.5	7	7	7
4.	Garlic	6	5	5	5
5.	Cumin	3	2	2	2
6.	Black pepper	4	3	3	3
7.	Coriander	1.5	1.5	1.5	1.5
8.	Corn flour	12.5	12.5	12.5	12.5
9.	Sugar	7.5	6	6	6
10.	Salt	18.5	17.5	17.5	17.5
11.	Sodium benzoate	0.5	0.5	0.5	0.5
12.	MLP	-	18	20	22
	Total (gm)	100	100	100	100

Nutritional analysis, mineral composition and reconstitution properties of Ready-to-Serve Soup of *Moringa oleifera* leaf Powder was carried out.

**Result and Discussion****Nutritional qualities of *Moringa oleifera* leaf powder**

The nutritional profiling of two varieties of *Moringa oleifera* was analysed comprehensively to evaluate their quality. Ash, moisture, carbohydrate, and protein content were estimated and reported in Table 3. The high amount of moisture content was observed in ODC 3 leaf powder (11.54%), than in wild variety (7.94%). Ash content in leaf powder of ODC 3 and wild variety was (25%) and (33.6%) respectively. Similar result for moisture and ash content was reported by Olusanya *et al.*, (2019) [43] and Madukwe *et al.* (2013) [33] in *Moringa oleifera* leaf powder. Looking on the carbohydrate, reveals a significant higher content present in ODC 3 and wild were 8.1gm/100gm and 8.0gm/100gm respectively. In addition, protein content in ODC 3 is 25.1 gm/100 gm and 23.2 gm/100 gm in the wild variety. Similar findings for carbohydrate and protein content in *Moringa oleifera* leaf powder were reported by (Gopalakrishnan *et al.*, 2016) [20].

**Table 3:** Nutritional composition of *Moringa oleifera* leaf powder (g/100g on dry weight basis)

Sr.No.	Parameters	Moringa varieties	
		ODC 3	WILD
1	Ash (%)	25	33.6
2	Moisture (%)	11.54	7.94
3	Carbohydrates (gm/100gm)	8.1	8.0
4	Protein (gm/100gm)	25.1	23.2

**Mineral composition of *Moringa oleifera* leaf powder**

The results revealed that *Moringa oleifera* leaf powder was found to be significantly rich in minerals viz., iron, calcium, phosphorus and data regarding mineral composition was mentioned in Table 4. Estimated iron content was 80 mg/100gm and 70mg/100gm in ODC-3 and wild varieties respectively. Similarly, Mikore and Mulugeta (2017) [37] reported iron contents of 80.03±2.50 mg/100gm on the basis their study. Calcium content was found to be higher in ODC -3 880.16mg/100gm whereas wild variety contains 760.12mg/100gm of calcium. The results of this study were less than the findings of (Raghavendra *et al.*, (2016) [49]; Abuye *et al.*, (2003); and Melesse (2011) [2, 36] reported Ca contents of 971 mg/100gm. In this study, phosphorus

content in *Moringa oleifera* leaf powder was found to be significantly higher in the ODC-3 variety (330 mg/100g) compared to the wild variety (290 mg/100g). This result findings were similar with Penalver *et al.* (2022).

**Table 4:** Mineral composition of *Moringa oleifera* leaf powder

Sr. No.	Parameters	<i>Moringa varieties</i>	
		ODC - 3	Wild
1.	Iron (mg/100gm)	80	70
2.	Calcium (mg/100gm)	880.16	760.12
3.	Phosphorus (mg/100gm)	330	290

**Ascorbic acid (Vitamin C):** Ascorbic acid content in wild variety was 500mg/100gm and 625 mg/100gm in ODC-3 mentioned in Table 5. These findings were in good agreement with the results of (Khawaja *et al.*, 2010) and (Arise *et al.*, 2014) [9, 26].

**Table 5:** Vitamin C content in *Moringa oleifera* leaf powder (mg/100gm)

Sr.No.	Parameters	<i>Moringa varieties</i>	
		ODC - 3	Wild
1.	Vitamin-C (mg/100gm)	625	500

#### Antioxidant composition of *Moringa oleifera* leaf powder

Antioxidants like total phenolics, DPPH, flavonoids, and total tannins were calculated and mentioned in Table 6. In this study, significantly higher antioxidant activity was observed in ethanolic extract than in methanol. The result of this study showed that the total phenolics in ethanolic

extract of ODC-3 and wild variety were 30 mg/g and 31 mg/g respectively, whereas total phenolics in methanolic extracts were 32 mg/g and 29mg/g in ODC-3 and wild variety respectively. All these findings were similar to the TPC value (45.4 mg /g) given by Sreelatha & Padma (2009) [57]. The DPPH scavenging activity of the ethanolic extract of ODC 3 variety of moringa was (71.14%) and in wild variety (60.69%) scavenging activity was observed which was higher than the methanolic extract of ODC-3 (46.26%) and wild (52.23%) respectively. These results were in a harmony with (Abdulkadir *et al.*, 2015) [1] who mentioned (58.62%) DPPH scavenging assay in the case of methanol solvent. In another study, ethanol extract was found to exhibit the highest DPPH activity (53.30% - 71.10%) reported by (Nobossé *et al.*, 2018) [39]. Flavonoid content present in the ethanolic extract of ODC 3 and wild variety were (30.7 mg/g and 30.6 mg/g) respectively which was higher than the methanolic extract of ODC 3 and wild were (30.4mg/100g and 30.8mg/100g). Similar findings were reported by Shanmugavel *et al.* (2018) [54], showing a total flavonoid content of 22.16 mg/g in ethanolic extract, consistent with previous studies (Pakade *et al.*, 2012) [45]. The ethanolic extracts of ODC-3 and wild varieties of *Moringa oleifera* leaf powder contained 12 mg/g and 10 mg/g of total tannin, respectively, higher than their methanolic extracts, which contained 10 mg/g and 8 mg/g of total tannin, respectively. Shakeela *et al.* (2023) reported 10.09 mg/g total tannin in methanolic extract and 13.08 mg/g in ethanolic extract which was similar to our current findings.

**Table 6:** Total antioxidants present in *Moringa oleifera* leaf powder

Sr. No.	Parameters	<i>Moringa varieties</i>			
		ODC - 3		Wild	
		Methanolic Extract	Ethanolic Extract	Methanolic Extract	Ethanolic Extract
1.	Total Phenolics (mg/g)	32	30	29	31
2.	DPPH (% inhibition)	46.26	71.14	52.23	60.69
3.	Flavonoid (mg/g)	30.4	30.7	30.8	30.6
4.	Total Tannins (TT) (mg/gm)	10	12	8	10

#### Phytochemical Analysis of *Moringa oleifera* leaf powder

**Quantitative analysis of phytochemicals presents in *Moringa oleifera* leaf powder:** The analysis of the various phytochemicals in *Moringa oleifera* leaf powder was conducted and mentioned in Table 7. Ethanolic extracts of both ODC-3 and wild varieties of *Moringa oleifera* showed high amounts of alkaloids (35 mg/100g) compared to methanolic extracts (25 mg/g for ODC-3 and 28 mg/g for wild varieties), similar to the findings of Adekanmi *et al.*

(2020) [4]. Ethanolic extracts of wild and ODC-3 *Moringa oleifera* had the highest flavonoid content (28 mg/g and 22 mg/g, respectively), while methanolic extracts showed 20 mg/g (wild) and 18 mg/g (ODC-3). Results were similar to Ajayi & Fadeyi (2015) [6]. Ethanolic extracts of ODC-3 and wild *Moringa oleifera* had higher saponin content (9 mg/g and 8 mg/g) compared to methanolic extracts (7 mg/g) in both wild and ODC-3, similar to findings by Ogbe & Affiku (2011) [40].

**Table 7:** Quantitative analysis of phytochemicals present in *Moringa oleifera* leaf powder

Sr.No.	Parameters	<i>Moringa varieties</i>			
		ODC - 3		Wild	
		Methanolic Extract	Ethanolic Extract	Methanolic Extract	Ethanolic Extract
1.	Alkaloids (mg/gm)	25	35	28	35
2.	Flavonoids (mg/gm)	18	22	20	28
3.	Saponin (mg/gm)	7	9	7	8

**Qualitative analysis of phytochemicals presents in *Moringa oleifera* leaf powder:** The phytochemical analysis of various phytoconstituents in ethanolic and methanolic extracts of the leaves of *M. oleifera* along with control was

represented in Table 8. On the basis of intensity of the colour the test result was demonstrated. Phytochemical tests showed hydrolysable tannins in both ethanolic and methanolic extracts of ODC-3 and wild varieties, with

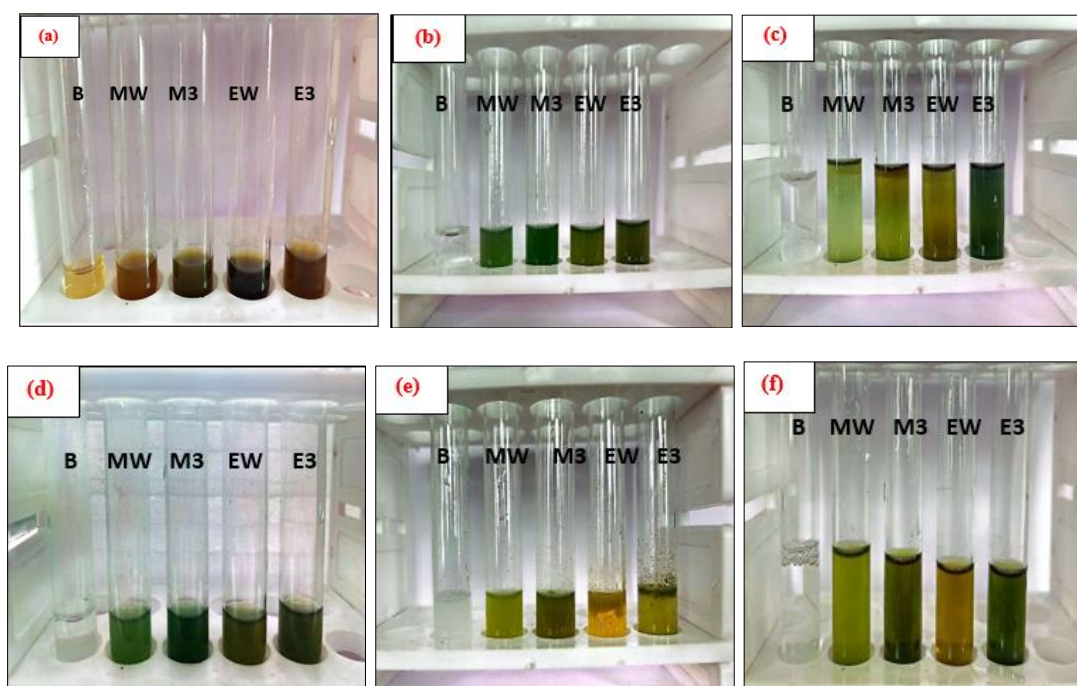
methanolic extracts showing higher tannin content detected by higher intensity of blue colour as shown in Fig 1(a). Formation of brown precipitate in Wagner's test showed a low amount of alkaloids in both ethanolic and methanolic extracts of ODC-3 and wild varieties as shown in Fig 1 (b). The Salkowski test showed a red-brown color was formed at the interface, indicating high triterpenoid content in both ethanolic and methanolic extracts of ODC-3 and wild varieties shown in Fig 1(c). Only the methanolic extracts of ODC-3 and wild *Moringa oleifera* varieties showed low amounts of flavonoids in the alkaline reagent test Fig1 (d). Both ethanolic and methanolic extracts of ODC-3 and wild *Moringa oleifera* varieties showed high flavonoid content with the formation of yellow precipitate in lead acetate test Fig 1 (e). Foam test results were nil in both ODC-3 and wild

varieties, indicating no stable foam formation as observed in the control Fig 1 (f), suggesting absent of saponin. High anthraquinone glycosides were found in the ethanolic extract of wild variety, while the methanolic extract showed low levels. ODC-3 ethanolic extract had low levels, with moderate levels of anthraquinone glycosides in the methanolic extract as shown in Fig 1 (g). In the present study, higher amounts of carbohydrates were observed in both varieties when subjected to the Fehlings test compared to Barfoed's test. as shown in Fig 1(h & i). The study found high protein content in both methanolic and ethanolic extracts of wild varieties, and low protein content in both methanolic and ethanolic extracts of ODC-3 varieties shown in Fig 1 (j).

**Table 8:** Qualitative analysis of phytochemicals present in *Moringa oleifera* leaf powder

Sr. No	Parameters	Test	Control	<i>Moringa</i> varieties			
				ODC 3		Wild	
				Ethanolic extract E3	Methanolic extract M3	Ethanoli c extract EW	Methanoli c extract MW
1.	Tannin	Ferric chloride	--	++	+++	+++	++
2.	Alkaloids	Wagner's test	++	+	+	+	+
3.	Triterpenoids	Salkowski Test	--	+++	+++	+++	+++
4.	Flavanoids	Alkaline reagent test	--	--	+	--	+
		Lead acetate test	--	+++	++	+++	++
5.	Saponin	Foam test	+++	--	--	--	--
6.	Anthroquin one glycosides	Hydroxyan thra-quinone Test	--	+	++	+++	+
7.	Carbohydr ates	Barfoed's Test	--	+	++	++	+++
		Fehlings test	--	++	+++	+++	++
8.	Protein	Biuret test	--	+	+++	+++	+
9.	Fats and fixed oils		--	+++	+++	+++	+++

\*The yield obtained was graded as high (+++); moderate (++); low (+); nil (--) based on the intensity of the color the test compared to control in each case.



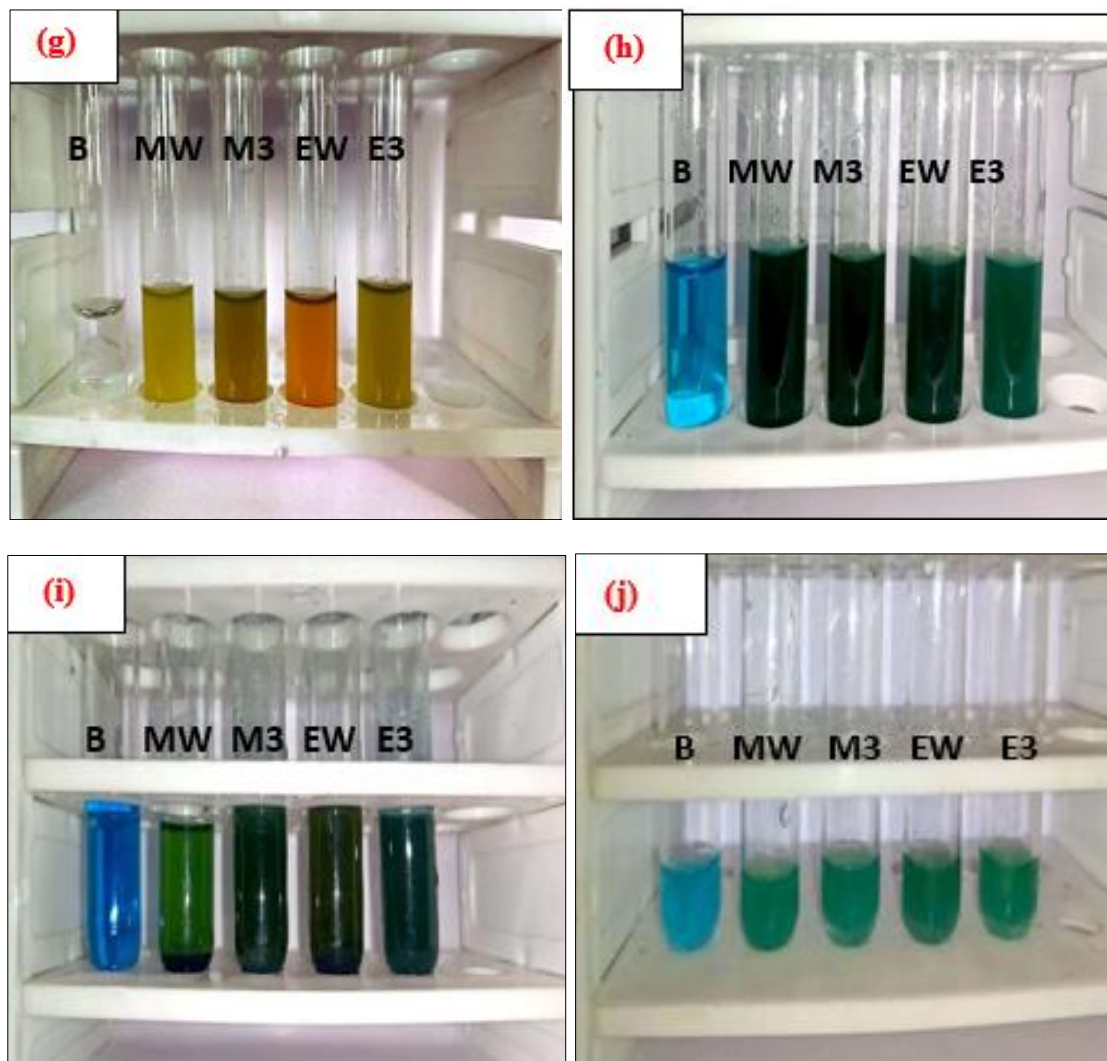


Fig 1 (a to j): Determination of phytochemicals in blank (B), ethanolic extract of ODC-3 and Wild variety (E3 and EW), methanolic extract of ODC-3 and Wild variety (M3 and MW) (a) Ferric chloride test for tannin (b) Wagner's test for alkaloids (c) Salkowski test for triterpenoids (d) Alkaline reagent test for flavonoids (e) Lead acetate test for flavonoids (f) Foam test for saponin (g) Hydroxyanthraquinone test for anthroquinone glycosides (h) Barfoed's test for carbohydrates (i) Fehlings test for carbohydrates (j) Biuret test for Protein

**Reconstitution properties of *Moringa oleifera* leaf powder:** The study examined various physicochemical properties of ODC-3 and wild varieties of *Moringa oleifera* as result mentioned in Table 9. The result of this study showed that ODC 3 had a higher water absorption capacity (59%) compared to the wild variety (57%). The findings aligned with previous studies, such as those reported by Singh *et al.* (2010) [56]. ODC 3 variety exhibited a higher water solubility index (10%) compared to the wild variety (8%), similar to the study conducted by (Singh *et al.*, 2012) [55]. The reconstitution index provides valuable insights into the ability of a substance to form a concentrated solution upon reconstitution. Slightly higher reconstitution index in ODC 3 (7g/ml) compared to the wild variety (6.9g/ml). Additionally, ODC-3 had a slightly higher bulk density (0.33 g/ml) compared to the wild variety (0.32 g/ml). Whereas higher swelling index in ODC-3 (3.6%) compared

to the wild variety (3.3%) implies that ODC-3 could absorb and retain more water.

**Table 9:** Reconstitution Properties of ODC-3 and Wild Variety of *Moringa oleifera* Leaf Powder

Sr.No.	Parameters	<i>Moringa</i> varieties	
		ODC - 3	Wild
1.	WAC (%)	59	57
2.	WSI (%)	10	8
3.	Reconstitution Index (g/ml)	7	6.9
4.	Bulk Density (g/ml)	0.33	0.32
5.	Swelling Index (%)	3.6	3.3

#### Gas Chromatography-Mass Spectrometry (GC-MS)

**Analysis:** The methanolic extract of *Moringa oleifera* leaf powder was used for chromatography separation. The GC-MS Chromatograph with various peaks of compounds detected in the methanolic extract of *Moringa oleifera* leaf powder is shown in Fig 2. The mass spectrum of the identified compounds with their retention time is shown in Fig 2. The report of area percent and retention time presented in Table 10. Table 11 presents the compounds, their common name, molecular formula, molecular weight, retention time, compound type and their biological activity. A total of 40 bioactive compounds were identified, showing the diverse chemical profile of the extract. These findings align with Karthika *et al.* (2016) [25], who identified 28 bioactive compounds in the ethyl acetate extract of moringa leaf powder.

**Table 10:** Retention time and Peak Area Percent report of methanolic extract of

Peak Number	Time [min]	PeakWidth	Area [Intens. *sec]	Height	Start Point		End Point	
		(FWH)[min]			Time [min]	Height	Time [min]	Height
1	10.57	0.0761	378641.40	69223.98	10.47	3205	10.81	3973
2	19.55	0.8256	780226.96	15042.53	19.13	810	21.19	1448
3	23.59	0.0419	22473.33	8256.07	23.53	1079	23.68	1254
4	28.86	0.0475	41899.00	14024.94	28.80	1017	28.93	1191
5	29.73	0.0993	384806.25	48289.86	29.55	1148	30.24	2044
6	34.40	0.1046	1871730.84	257423.14	34.27	3164	34.76	9483
7	36.56	0.1145	768191.82	88766.32	36.44	8893	37.01	9599
8	38.45	0.0525	96470.67	28646.03	38.37	4154	38.55	3469
9	43.12	0.1053	712443.32	107500.62	42.94	1730	43.29	2276

**Table 11:** Compounds identified by GC-MS in the methanolic extract of *M. oleifera* leaf powder with different retention time

Sr. No.	Compound Name	Common Name	Molecular Formula	MW (g/mol)	RT (min)	Compound Type	Biological Activity	References
1.	9-Octadecenoic acid (Z)-, 3-[(1- oxohexadecyl)oxy]-2-[(1- oxooctadecyl)oxy]propyl ester	-	C55H104O <sub>6</sub>	860	10.57	Triterpenoid saponin	Anti-cancer, anti-viral, and anti- bacterial properties	Zhang,J., & Wang,Y.(2011)
2.	Octadecanoic acid, 1-[[[(1 oxohexadecyl)oxy]methyl]-1,2-ethanediyl ester	-	C55H106O <sub>6</sub>	862	10.57	Steroidal saponin	Anti-inflammatory, Anti-viral properties	Li,Y.,& Wang,X. (2012)
3.	Diethyl 6-(1,5-dimethylhexyl)-3b,5a- dimethyl-2- oxohexadecahydrocyclopenta[a]cyclopropa[g]phenanthrene-3,3 (1H)-dicarboxylate	-	C34H54O <sub>5</sub>	542	10.57	Steroid saponin	Immunomodulatory, <i>Neuroprotective</i>	Sun, H., & Yang, X.(2015) <sup>[61]</sup>
4.	Octadecanoic acid, 3-[(1- oxohexadecyl)oxy]-2-[(1- oxotetradecyl)oxy]propyl ester	-	C51H98O <sub>6</sub>	335	10.57	Triterpenoid saponin	Anti-oxidant	Sun, H., & Yang, X. (2015) <sup>[61]</sup>
5.	Dodecanoic acid, 1a,2,5,5a,6,9,10,10a-octahydro-5a-hydroxy-4-(hydroxymethyl)-1,1,7,9-tetramethyl-6,11-dioxo	Oleanolic acid	C32H48O <sub>6</sub>	330	10.57	Triterpenoid	Hepatoprotective, <i>Antiviral and antibacterial</i>	Yang, S., & Zhang, L. (2018) <sup>[61]</sup>
6.	4a-Phorbol 12,13-didecanoate	Stearic acid	C40H64O <sub>8</sub>	325	10.57	Long-chain saturated fatty acid	Antitumor, <i>Antimicrobial</i>	Raihani <i>et al.</i> , (2010)

7.	9,19-Cyclolanostane-6,7-dione, 3-acetoxy-	Oleyl alcohol	C32H50O <sub>4</sub>	498	19.54	Monounsaturated fatty alcohol	Moisturizing, Hair care, Antioxidant, Anti-inflammatory	Vermesan <i>et al.</i> ,(2014)
8.	cis-Inositol tri-n-octaneboronate	Boronic acid	C30H57B <sub>3</sub> O <sub>6</sub>	319;	19.54	Triacontane glycol	Inhibit the growth of cancer cells <i>in vitro</i> and <i>in vivo</i>	Matsumoto <i>et al.</i> ,(2014)
9.	Cholestan-3-ol, 5-chloro-6-nitro-, acetate (ester), (3β,5α,6β)-	-	C29H48ClNO <sub>4</sub>	509	19.54	Atorvastatin calcium	Lowers cholesterol levels	Reed, A. S. <i>et al.</i> ,(2010)
10.	Lanosta-7,9(11),20-triene-3β,18-diol, diacetate	-	C34H52O <sub>4</sub>	296	19.54	Steroid hormone	Reducing the risk of heart disease	Shanthakumari, R., <i>et al</i> (2011)
11.	Cholestano[2,3-d]cinnoline-3',6'-dicarboxylic acid, 4',5'-dihydro-4'-(1-pyrrolidinyl)-, dimethyl ester	-	C37H59N <sub>4</sub>	283	19.54	Sphingolipid	Cellular signaling, Cell proliferation, Immune function, Angiogenesis	Spiegel, S., & Futerman, A. H. (2014)
12.	Tristearin	Alpha-linolenic	C57H110O <sub>6</sub>	283	19.54	Omega-3 fatty acid	Anti-inflammatory, Cardioprotective	Simopoulos, A. P. (2002).

		acid (ALA)					ective, Neuroprotec tive,	
13.	Acetic acid, 17-(4-chloro-5-methoxy-1,5-dimethylhexyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1-phenanthryl-	Clobetasol propionate	C33H55ClO3	534	23.59	Glucocorticoid	Anti-inflammatory Immunosuppressive, Vasoconstrictive	Pfahl, A., & Kreysel, H. W. (2007)
14.	7,8-Epoxy lanostan-11-ol, 3-acetoxy-	-	C32H54O4	502	23.59	Cholesterol	Precursor for Hormone Synthesis, Vitamin D Synthesis	Brown, M. S., & Goldstein, J. L. (1986)
15.	2β,4α-Epoxy methylphenanthrene-7-methanol, 1,1-dimethyl-2-methoxy-8-(1,3-dithien-2-ylidene)methyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-, acetate	-	C27H38OS2	490	23.59	Sulfated glycosaminoglycan	Anti-inflammatory Effects, Cell Signaling	Kiani, C., Chen, G., Woo, B. G., Rodeo, S. A., & Tohme, J. (2002)
16.	3-Phorbinepropanoic acid, 9-acetyl-14-ethyl-13,14-dihydro-21-(methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-,3,7,11,15-tetramethyl-2-hexadecenyl ester, [3s-[3.	-	C55H76N4O6	888	23.59	Non-protein iron-containing cofactor	Oxygen Transport, Heme Synthesis	Alayashi, A. M., & Adams, J. D. (2019)
17.	3H-Cycloprop(1,2)cholesta-1,4,6-trien-3-one, 1'-carboethoxy-1'-cyano-1β,2β-dihydro-	L-arginine	C32H45NO3	491	23.59	α-amino acid	Protein synthesis, blood	Boger, R. L. (2001)

							pressure regulation	
18.	3H-Cycloprop(1,2)-5-cholest-1-en-3-one, 1'-carboethoxy-1'-cyano-1,2-dihydro-	L-citrulline	C32H49NO3	495	29.72	α-amino acid	Muscle growth and recovery	Sureda, M., <i>et al.</i> (2004)
19.	Cholestane, 3,5-dichloro-6-nitro-, (3β,5α,6β)-	Ampicillin	C27H45Cl2NO2	485	29.72	Antibiotic	To treat bacterial infections	Walsh, C. T. (2003)
20.	Cholestan-3-ol, 5-chloro-6-nitro-, (3β,5α,6β)-	Amoxicillin	C27H46ClNO3	467	29.72	Antibiotic	They are used to treat various bacterial infections,	Chambers, H. F., & Pratt, R. F. (2001)
21.	Cholestan-3-ol, 5-chloro-6-nitro-, acetate (ester), (3β,5α,6β)-	Cefazolin	C29H48ClNO4	509	29.72	Antibiotic	To treat bacterial infections, and joint infections.	Chambers, H. F., & Pratt, R. F. (2001)
22.	Acetic acid, 17-acetoxy-3-hydroxyimino-4,4,13-trimethylhexadecahydrocyclopenta[a]phenanthren-10-ylmethyl ester	Metoclopramide	C25H39NO5	433	29.72	Antiemetic medication	Antiemetic, Prokinetic, Dopamine antagonist	Antiemetics: a review of current clinical practice. <i>Drugs</i> , 60(12), 2049-2061.
23.	Ethanaminium,2-[[[2,3-bis[(1-oxo-9-octadecenyl)oxy]propoxy]hydroxyphosphinyl]oxy]-N,N,N-trimethyl-,hydroxide, inner salt, (R)-	Lecithin	C44H84NO8P	785	29.72	Phospholipid	Emulsifier, Neurotransmitter Signaling	Phillips, M. C. (2014)

24.	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-(2-nitrophenyl) hexadecahydrocyclopenta[a]phenanthrene	Morphine	C33H51NO2	493	29.72	Alkaloid	Analgesic	Clemans, A., & Lyshia, D. C. (2011)
25.	5H-Cyclopropa(3,4)benz(1,2-e)azulen-5-one, 1,1α,1b-β,4,4a,7a-α,7b,8,9,9a-decahydro-7b-α,9,9a-α-trihydroxy	Beta-sitosterol	C41H66O8	686	34.39	Plant sterol	Reducing cholesterol levels, Reducing inflammation	Awad, A. B., & Fink, C. S. (2000)
26.	Docosanoic acid, 1,2,3-propanetriyl ester	Arachidonic acid	C69H134O6	1058	34.39	Polyunsaturated fatty acid	Prostaglandin Synthesis, Leukotriene Synthesis	Dennis, E. A., & Norris, R. C. (2003)

27.	Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxy phosphinyl]oxy]methyl]-1,2-ethanediyl ester	Sphingomyelin	C37H74NO8P	691	34.39	Sphingolipid	Myelin Formation, Lipid Raft Formation	Hannun, J. A., & Obeid, L. M. (2008)
28.	Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester	Phosphatidylcholine	C35H68O5	568	34.39	Phospholipid	Membrane Structure and Function, Signal Transduction	Vance, J. E., & Vance, D. E. (2004)
29.	2β,4a-Epoxyethylphenanthrene-7-methanol, 1,1-dimethyl-2-methoxy-8-(1,3-dithiin-2-ylidene) methyl-1,2,3,4,4a,4b,5,6,7,8,8a,9-dodecahydro-, acetate	Chondroitin sulfate	C27H38O4S2	490	36.57	Glycosaminoglycan	Anti-inflammatory Effects, Cell Signaling	Shibakawa, A., & Yoneda, Y

30.	Lanostane-7,11-dione, 3,18-bis(acetyloxy)-, cyclic 7-(1,2-ethanediyl mercaptole), (3β,20.xi.)-	Dermatan sulfate	C36H58O5S2	634	36.57	Glycosaminoglycan	Wound Healing, Water Retention	Wight, T. N. (2006)
31.	Octadecanoic acid, 2-[(1-oxohexadecyl)oxy]-1-[[[(1-oxohexadecyl)oxy]methyl]ethyl ester	Fucoidan	C53H102O6	834	36.57	Polysaccharide	Anticoagulant, Antioxidant, Antitumor	Kim, Y. N., <i>et al.</i> (2001)
32.	Milbemycin B, 5-demethoxy-5-one-6,28-anhydro-25-ethyl-4-methyl-13-chloro-oxime	Amoxicillin clavulanate	C32H44ClNO7	589	43.12	Antibiotics	Antibacterial	Chambers, H. F., & Pratt, R. F. (2001)
33.	Vitamin E	-	C29H50O2	430	43.12	Vitamin	Human	Brown, M. S., & Goldstein, J. L. (1986)
34.	(+)-α-Tocopherol acetate	Squalene	C31H52O3	472	43.12	Saturated hydrocarbon	Antioxidant, Immune-boosting, Emollient	Pan, F., <i>et al.</i> (2010)
35.	Tetracyclo[11.4.0.0(3,11).0(7,11)]heptadecan-1(13),14,16-triene-4-carboxylic acid, 14,17-dimethoxy-8-(2-hydroxy-1-methylethyl)-	Cholic acid	C23H32O5	388	43.12	Primary bile acid	Fat Emulsification, Antimicrobial Activity	Hofmann, A. F. (1994)
36.	4a,7a-Epoxy-5Hcyclopenta[a]cyclopropa[f]cycloundecan-4(1H)-one, 1a,6,7,10,11,11a-hexahydro-7,10,11-trihydroxy-1,1,3,6,9-pentamethyl-	Arachidonic acid	C20H28O5	348	43.12	Polyunsaturated fatty acid	Prostaglandin Synthesis, Leukotriene Synthesis	Dennis, E. A., & Norris, R. C. (2003)

37.	3-Acetyl-17-(1,5-dimethylhexyl)-10,13-dimethylhexadecahydrocyclopenta[a]phenanthren-2-one	Cholesterol	C29H48O2	428	43.12	Sterol	Hormone synthesis, Bile acid production	Brown, M. S., & Goldstein, J. L. (1986)
38.	2H-1-Benzopyran-6-ol, 3,4-dihydro-2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-, acetate, [2R-[2R*(4R*, 8R*)]]-	Squalene	C31H52O3	472	43.12	Saturated hydrocarbon	Pharmaceuticals, Food and beverage additives	Pan, F., <i>et al.</i> (2010)
39.	5,5'-Dimethoxy-3,3',7,7'-tetramethyl-2,2'-binaphthalene-1,1',4,4'-tetrone	Stearic acid	C26H22O6	430	43.12	Saturated fatty acid	Food Additive, Skin Barrier Function	Ziegenhagen, K. J., & Korting, H. C. (1991)
40.	3,4-Dimethoxy-5,7,8,13,13b,14-hexahydroindolo[2',3':3,4]pyrido[1,2-b]isoquinoline	Caffeine	C21H22N2O2	334	43.12	Psychoactive stimulant	Adenosine Receptor	Fredholm, B. B., <i>et al.</i> (2019)

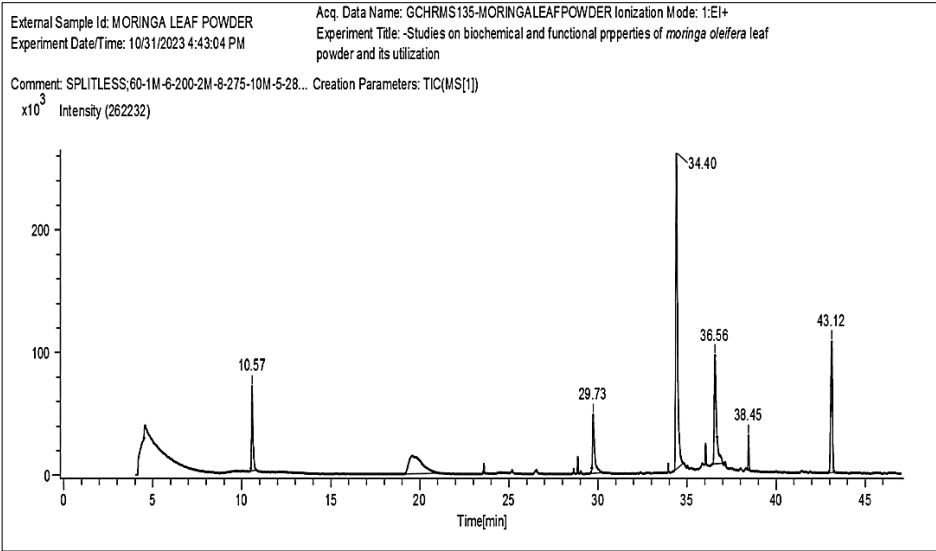


Fig 2: GCMS Chromatogram of methanolic extract of *Moringa oleifera* leaf powder

**Antimicrobial activity of different leaf extracts *Moringa oleifera*:** Antimicrobial activity of different leaf extracts of *Moringa oleifera* was tested against various fungal and bacterial strains as shown in Table 1.

**Antifungal activity of different leaf extracts *Moringa oleifera*:** Leaf extract of *Moringa oleifera* showed antifungal activity against *Fusarium oxysporum*, *Aspergillus flavus*, and *Aspergillus niger* as mentioned in Table 12. In case of aqueous leaf extract of *Moringa oleifera*, the results showed a similar zone of inhibition, measuring 3mm, for both *Aspergillus flavus* and *Fusarium oxysporum*, as illustrated in Fig., 3. However, aqueous extract did not exhibit any zone of inhibition against the *Aspergillus niger*, suggesting a lack of antifungal activity. The result obtained by (Bagheri *et al.*, 2020)<sup>[10]</sup> was also similar to this findings. He ethanolic extract of *Moringa oleifera* leaf powder showed varied zones of inhibitions as shown in Fig 3.

against different strains. both *Aspergillus flavus* and *Aspergillus niger* exhibited a similar zone of inhibition, measuring 3.5 mm at a concentration of 100mg/ml of the ethanolic extract and 3mm zone of inhibition was observed for *Fusarium oxysporum*. Oladeji *et al.* (2019)<sup>[41]</sup> reported a similar zone of inhibition for *Aspergillus flavus* ( $5.50 \pm 0.707\text{mm}$ ) and *Aspergillus niger* ( $4.50 \pm 0.707\text{mm}$ ) in the ethanolic extract, with a slight difference in the measurements. *Aspergillus niger* (fig., 3 (c) and *Fusarium oxysporum* (Fig., 3 (a) showed a similar zone of inhibition i.e. 4 mm for methanolic extract as shown in Plate. whereas, *Aspergillus flavus* showed a 3mm zone of inhibition with the methanolic extract. This observation is in agreement with the study of (Maqsood *et al.*, 2017)<sup>[34]</sup>. They observed the maximum zone of inhibition of ( $5.3 \pm 0.57 \text{ mm}$ ) at 100 mg/ml concentration of methanolic extract for *Aspergillus niger*.

Table 12: Zones of inhibition in (mm) of different extracts of *Moringa oleifera* leaves

Sr. No.	Name of Fungal strains	Zone of Inhibition (mm) of different leaf extracts			
		Control	Aqueous extract	Ethanolic extract	Methanolic extract
1.	<i>Fusarium oxysporum</i>	-	3	3	4
2.	<i>Aspergillus flavus</i>	-	3	3.5	3
3.	<i>Aspergillus niger</i>	-	-	3.5	4

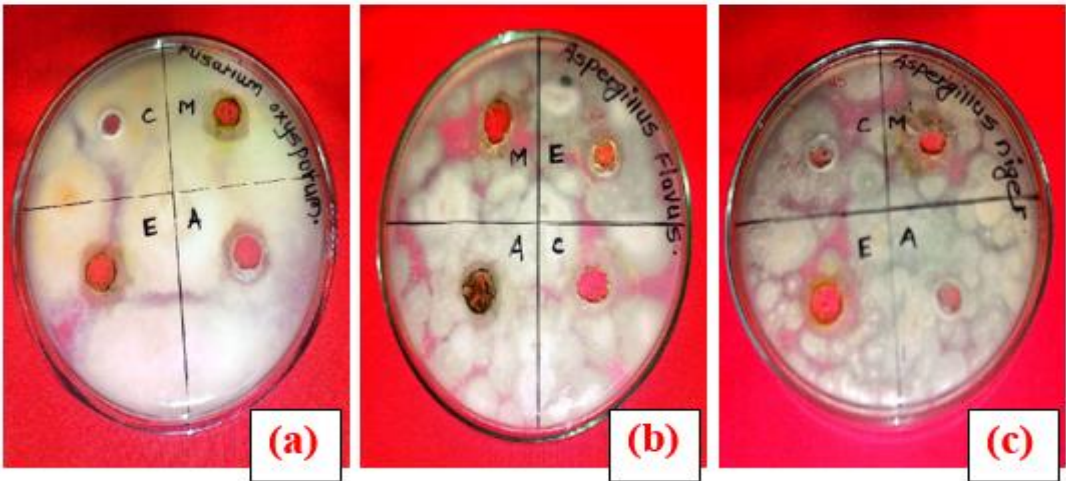


Fig 3 (a) (b) (c): Antimicrobial activity screening of ethanol, methanol, and aqueous leaf extracts *Moringa oleifera* (a) Antifungal activity of *Moringa oleifera* leaf extracts against *Fusarium oxysporum* (b) Antifungal activity of *Moringa*

*oleifera* leaf extracts against *Aspergillus flavus* (c) Antifungal activity of *Moringa oleifera* leaf extracts against *Aspergillus niger*

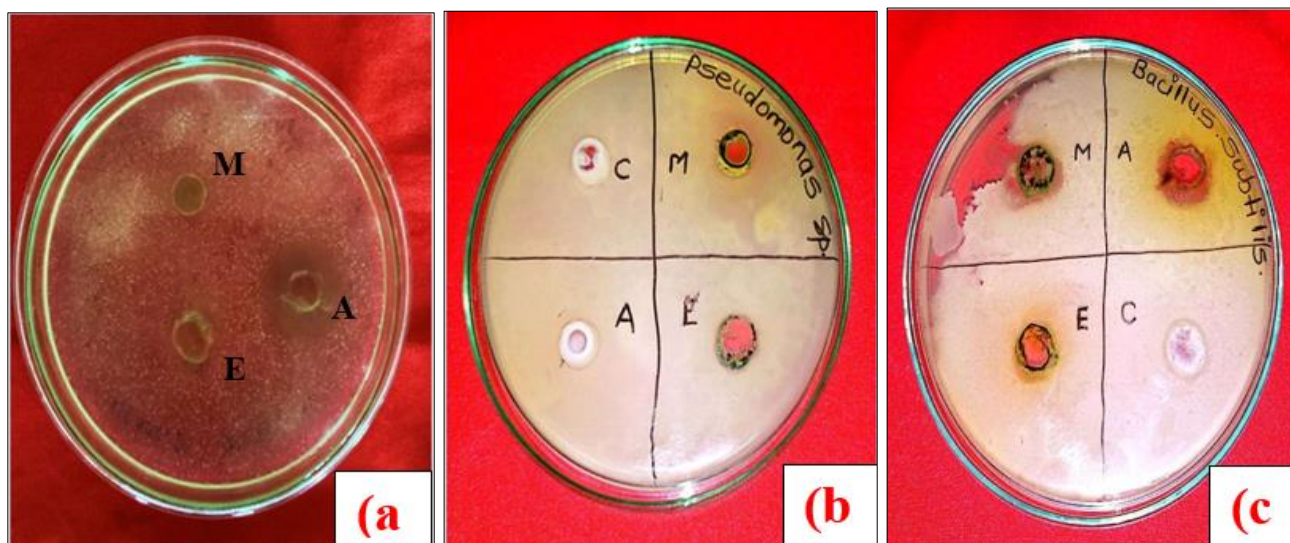


Fig 4 (a) (b) (c): Antimicrobial activity screening of Ethanol, Methanol, and Aqueous leaf extracts *Moringa oleifera* (a) Antibacterial activity of *Moringa oleifera* leaf extracts against *Escherichia coli* (b) Antibacterial activity of *Moringa oleifera* leaf extracts against *Pseudomonas fluorescens* (c) Antibacterial activity of *Moringa oleifera* leaf extracts against *Bacillus subtilis*

**Antibacterial activity of different leaf extracts of *Moringa oleifera*:** Leaf extract of *Moringa oleifera* showed antifungal activity against *Escherichia coli*, *Pseudomonas fluorescens*, and *Bacillus subtilis* as mentioned in Table 13 and Fig., 4. In case of aqueous extract, highest inhibition zone of 7mm against *E. coli*, 5mm against *Bacillus subtilis*; no zone observed for *Pseudomonas fluorescens* with aqueous extract, indicating no antibacterial activity. Adline & Devi (2014) [5] reported 0.6mm inhibition zone with

aqueous extract for *E. coli* and Issa *et al.* (2021) [23] found  $7.50 \pm 0.70$ mm inhibition at 100mg/ml, confirming antibacterial activity of *Moringa oleifera* against *E. coli*. *Escherichia coli* showed the largest zone of inhibition at 5mm (Fig., 4 (a), followed by *Bacillus subtilis* at 4mm (Fig., 4 (c), and *Pseudomonas fluorescens* at 2mm (Fig., 4 (b) for ethanolic extract. Similar results were obtained by Issa *et al.* (2021) [23], showed ethanolic extract exhibited  $10.00 \pm 1.41$ mm inhibition against *Escherichia coli* and  $8.00 \pm 1.21$ mm against *Pseudomonas aeruginosa* at 100mg/ml concentration. In the methanolic extract of *Moringa oleifera* leaf powder, *Escherichia coli*, *Pseudomonas fluorescens*, and *Bacillus subtilis* showed a similar zone of inhibition i.e. 3mm. This finding underscores the uniform inhibitory effect of the methanolic extract against the tested bacterial strains.

**Table 13:** Zones of inhibition in (mm) of different extracts of *Moringa oleifera*

Sr. No.	Name of Bacterial strains	Zone of Inhibition (mm) of different extracts of <i>Moringa oleifera</i>			
		Control	Aqueous extract	Ethanolic extract	Methanolic extract
1.	<i>Escherichia coli</i>	-	7	5	3
2.	<i>Pseudomonas fluorescens</i>	-	-	2	3
3.	<i>Bacillus subtilis</i>	-	5	4	3

**Nutritional composition of ready to serve soup of *Moringa oleifera*:** The nutritional analysis of ready-to-serve soup with various treatments of moringa leaf powder (Treatment 1, Treatment 2, and Treatment 3) was conducted and levels of ash, moisture, carbohydrate, and protein content were determined and reported in Table 14. Treatment 1, Treatment 2, and Treatment 3 showed varying ash content levels at (10%, 10%, and 12%), respectively. In contrast, control showed a relatively high ash content of (15%). Based on the findings of the current investigation, it was determined that the moisture content rises proportionally with the increasing quantity of moringa powder in the soup mixture. Treatment 3, containing (22%) moringa leaf powder (MLP), exhibited the highest moisture

content of (16%). In contrast, Treatment 2, Treatment 1, and the Control showed decreasing moisture content of (14%, 12%, and 10%), respectively. The study revealed that an increase in moisture content in the soup mix corresponds to an increase in the amount of carbohydrates. The highest carbohydrate content was observed in Treatment 3, amounting to 38gm/100gm. Treatment 2, Treatment 1, and the control showed carbohydrate levels of 37.5gm/100gm, 37gm/100gm, and 30gm/100gm, respectively. Protein content increased with higher amounts of moringa leaf powder in the soup mix: Treatment 3 had the highest at 36gm/100gm, followed by Treatment 2 (35gm/100gm), Treatment 1 (33gm/100gm), and the control (29gm/100gm).

**Table 14:** Proximate analysis of ready to serve soup of *Moringa oleifera* (g/100g on dry weight basis)

Sr.no.	Parameters	Formulations			
		Control	T1 MLP (18%)	T2 MLP (20%)	T3 MLP (22%)
1	Ash (%)	15	10	10	12
2	Moisture (%)	10	12	14	16
3	Carbohydrates (gm/100gm)	30	37	37.5	38
4	Protein (gm/100gm)	29	33	35	36

**Reconstitution Properties of ready to serve soup of *Moringa oleifera*:** Based on our research findings, it was determined that reconstitution properties such as water absorption capacity (WAC), water solubility index (WSI), reconstitution index, dehydration ratio, bulk density, and swelling index showed an increased amount corresponding to the rising quantity of moringa leaf powder (MLP) in the ready-to serve soup mix of *Moringa oleifera* as mentioned in Table 15. Treatment 3, with a (22%) moringa leaf powder (MLP) composition, demonstrated the highest water absorption capacity (WAC) at (36%). Conversely, WAC declined in Treatment 2 (20% MLP), Treatment 1 (18% MLP), and the control, with values of (34%, 32%, and 30%), respectively. In the present study, Treatment 3 (22% MLP) and Treatment 2 (20% MLP) showed the highest reconstitution index values of 3.5 each, while Treatment 1

(18% MLP) and the control had lower values of 3.4 and 3.1, respectively. The dehydration ratio was identical for Treatment 3 (22% MLP) and Treatment 2 (20% MLP), both measuring 4.8%. However, the dehydration ratio decreased in Treatment 1 (18% MLP) and the control, with values of 4.5% and 2.4%, respectively. According to the findings of the current study, the highest bulk density was noted in Treatment 3 (22% MLP), with a value of 0.5. Treatment 2 (20% MLP) and Treatment 1 (18% MLP) showed an identical bulk density of 0.4. The lowest bulk density was recorded in the control, measuring 0.3. The highest swelling index was observed in Treatment 3 (22% MLP) with a value of 1. A reduced swelling index was observed in Treatment 2 (20% MLP), Treatment 1 (18% MLP), and the control, with values of 0.9, 0.7, and 0.6, respectively.

**Table 15:** Reconstitution Properties of *Moringa oleifera* soup mix:

Sr.No.	Parameters	Formulations			
		Control	T 1 MLP (18%)	T 2 MLP (20%)	T3 MLP (22%)
1.	WAC (%)	30	32	34	36
2.	Reconstitution Index (g/ml)	3.1	3.4	3.5	3.5
3.	Dehydration Ratio	2.4	4.5	4.8	4.8
4.	Bulk Density	0.3	0.4	0.4	0.5
5.	Swelling Index	0.6	0.7	0.9	1

#### Sensory evaluation of *Moringa oleifera* leaf powder

Result of sensory evaluation by taking the mean of the scores shown in Table 16 and graphical representation shown in Fig 4.23. Treatment 2 (20% *Moringa oleifera* leaf powder) got the highest value followed by Control and T1 (18% *Moringa oleifera* leaf powder) and T3 (22% *Moringa oleifera* leaf powder) got the lowest value for all sensory

attributes. Scores of Treatments 2 for Colour and appearance, flavour, taste, Consistency, and overall acceptability were 7.9, 9.1, 8.1, 8.1, 9.1 respectively. The highest score for Treatment 2 suggested that a (20%) moringa leaf powder composition in the soup mix was favoured by the panellists in terms of colour, flavour, taste, consistency, and overall acceptability.

**Table 16:** Sensory attributes of instant soup mixes with moringa leaf Powder

Treatments	Color & appearance	Flavor	Taste	Consistency	Overall acceptability
Control	8.7	8.2	7.2	6.1	7.2
T1	6.9	7.2	8.1	7.1	7.1
T2	7.9	9.1	8.1	8.1	9.1
T3	6.9	8.4	6.1	6.1	6.1
SE (m)	0.146	0.267	0.171	0.139	0.180
SE (d)	0.206	0.377	0.242	0.197	0.255
CD	0.588	1.076	0.691	0.561	0.727
CV	2.704	4.87	3.270	2.858	3.434

#### \*Based on 9 point hedonic scale

- Control: Recipe without *Moringa oleifera* combination powder
- Sample T1: Recipe with 18 percent *Moringa oleifera* combination powder
- Sample T2: Recipe with 20 percent *Moringa oleifera* combination powder
- Sample T3: Recipe with 22 percent *Moringa oleifera* combination powder

#### Conclusion

*Moringa oleifera*, widely known as the "drumstick tree" or "miracle tree," is a remarkable plant with a broad global distribution and a significant impact on nutrition and health. Originating from India and now grown in various tropical and subtropical regions, *Moringa oleifera* thrives in diverse agro-climatic environments. Its leaves, rich in essential nutrients and bioactive compounds, offer numerous health benefits, including anti-tumour, anti-inflammatory, and antioxidant properties.

This study highlights the biochemical and functional properties of *Moringa oleifera* leaf powder, emphasizing its potential as a valuable resource for combating malnutrition, especially in developing countries. The findings underscore the plant's role as a natural source of vitamins, minerals, and antioxidants, making it a viable alternative to conventional food supplements.

Overall, the research supports the optimization of *Moringa oleifera*'s utilization in dietary formulations and medicinal applications, contributing to improved health outcomes and food security.

## References

1. Abdulkadir AR, Zawawi DD, Jahan MS. Proximate and phytochemical screening of different parts of *Moringa oleifera*. Russ Agric Sci. 2015;42(1):34-36.
2. Abuye C, Urga K, Knapp H, Selmar D, Omwega AM, Imungi JK, *et al.* A compositional study of *Moringa stenopetala* leaves. East Afr Med J. 2003;80(5):247-252.
3. Adebawale KO, Olu-Owolabi BI, Olawumi EK, Lawal OS. Functional properties of native, physically and chemically modified breadfruit (*Artocarpus artillis*) starch. Ind Crops Prod [Internet]. 2005 [cited 2025 Jul 15]. Available from: <http://dx.doi.org/10.1016/j.indcrop.2004.05.002>
4. Adekanmi AA, Adekanmi SA, Adekanmi OS. Qualitative and quantitative phytochemical constituents of moringa leaf. Int J Eng Inf Syst. 2020;4(5):10-17.
5. Adline J, Devi A. A study on phytochemical screening and antibacterial activity of *Moringa oleifera*. Int J Res Appl. 2014;2:169-176.
6. Ajayi AO, Fadeyi TE. Antimicrobial activities and phytochemical analysis of *Moringa oleifera* leaves on *Staphylococcus aureus* and *Streptococcus* species. Am J Phytomed Clin Ther. 2015;3:643-653.
7. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 2000.
8. Association of Official Analytical Chemists (AOAC). Official Methods of Analysis. 16th ed. Washington DC: AOAC; 2005.
9. Arise AK, Arise RO, Sanusi MO, Esan OT, Oyeyinka SA. Effect of *Moringa oleifera* flower fortification on the nutritional quality and sensory properties of weaning food. Croat J Food Sci Technol. 2014;6(2):65-71.
10. Bagheri G, Martorell M, Ramírez-Alarcón K, Salehi B, Sharifi-Rad J. Phytochemical screening of *Moringa oleifera* leaf extracts and their antimicrobial activities. Cell Mol Biol. 2020;66(1):20-26.
11. Benítez V, Mollá E, Martín-Cabrejas MA, Aguilera Y, López-Andréu FJ, Cools K, *et al.* Characterization of industrial onion wastes (*Allium cepa* L.): Dietary fiber and bioactive compounds. Plant Foods Hum Nutr. 2011;66(1):48-57.
12. Chumark P, Khunawat P, Sanvarinda Y, Phornchirasilp S, Morales PN, Phivthongngam L, *et al.* The *in vitro* and *ex vivo* antioxidant properties, hypolipidaemic and antiatherosclerotic activities of the water extract of *Moringa oleifera* Lam. leaves. J Ethnopharmacol. 2008;116:439-446.
13. Dahiru D, Obnubiya JA, Umaru HA. Phytochemical screening and antiulcerogenic effect of *Moringa*. Afr J Tradit Complement Altern Med. 2006;3(3):70-75.
14. DanMalam HU, Abubakar Z, Katsayal UA. Pharmacognostic studies on the leaves of *Moringa oleifera*. Niger J Nat Prod Med. 2001;5:45-9, 105.
15. Dhakar R, Pooniya B, Gupta M, *et al.* *Moringa*: The herbal gold to combat malnutrition. Chron Young Sci. 2011;2(3):119-125.
16. Ebert AW, Palada MC. *Moringa* - a vegetable tree for improved nutrition, health and income of smallholder farmers. Acta Hort. 2017;1158:309-316.
17. El Sohaimey S, Masry S. Phenolic content, antioxidant and antimicrobial activities of Egyptian and Chinese propolis. Am-Eurasian J Agric Environ Sci. 2014;14:1116-1124.
18. Fahey JW. *Moringa oleifera*: A Review of the Medical Evidence for Its Nutritional, Therapeutic, and Prophylactic Properties. Part 1. Trees Life J [Internet]. 2005 [cited 2025 Jul 15];1:5. Available from: <http://www.tfljournal.org/article.php/20051201124931586>
19. Freiburger C, Vanderjagt D, Pastuszyn A, Glew R, Mounkaila G, Millson M, *et al.* Nutrient content of the edible leaves of seven wild plants from Niger. Plant Foods Hum Nutr. 1998;53:57-69.
20. Gopalakrishnan L, Doriya K, Kumar DS. *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food Sci Hum Wellness. 2016;5(2):49-56.
21. Harborne AJ. Phytochemical methods: A guide to modern techniques of plant analysis. Springer Sci Bus Media. 1998.
22. Hernandez-Diaz JR, Quintero-Ramos A, Barnard J, Balandran Quintana RR. Functional properties of extrudates prepared with blends of wheat flour/pinto bean meal with added wheat bran. Food Sci Technol Int. 2007;13(4):301-308.
23. Issa SB, Muazu M, Rabi'u II. Phytochemical analysis and antibacterial activity of *Moringa oleifera* leaves extracts against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Asian J Biochem Genet Mol Biol. 2021;7(1):34-43.
24. Jayaraman J. Laboratory Manual in Bio-Chemistry. New Delhi: Wiley Eastern Limited; 1981. p.114-117.
25. Karthika CK, Yogeshwari G, Muruganantham, Kannaiyan, Manivannan S. Phytochemical analysis of *Ruellia patula* using gas chromatography-mass spectrometry. Asian J Pharm Clin Res. 2016;9:111-113.
26. Khawaja TM, Tahira M, Ikram UK. *Moringa oleifera*: a natural gift - A review. J Pharm Sci Res. 2010;2:775-781.
27. Kokate CK, Purohit AP. A Textbook of Practical Pharmacognosy. New Delhi: Vallabh Prakashan; 2005.
28. Koul B, Chase N. *Moringa oleifera* Lam.: Panacea to several maladies. J Chem Pharm Res. 2015;7:687-707.
29. Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. J Med Plants Res. 2009;3(2):67-72.
30. Krokida MK, Marinos-Kouris D. Rehydration kinetics of dehydrated products. J Food Eng. 2003;57(1):1-7.
31. Liu Y, Wang XY, Wei XM, Gao ZT, Han JP. Values, properties and utility of different parts of *Moringa oleifera*: An overview. Chin Herb Med. 2018;10(4):371-8. doi:10.1016/j.chmed.2018.09.002

32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193(1):265-275.
33. Madukwe EU, Ugwuoke AL, Ezeugwu JO. Effectiveness of dry *Moringa oleifera* leaf powder in treatment of anaemia.
34. Maqsood M, Qureshi R, Arshad M, Ahmed MS, Ikram M. Preliminary phytochemical screening, antifungal and cytotoxic activities of leaves extract of *Moringa oleifera* Lam. from Salt range, Pakistan. *Pak J Bot.* 2017;49(1):353-359.
35. Mehmood A, Naveed K, Khan SU, Haq NU, Shokat MF, Iqbal M, *et al.* Phytochemical screening, antioxidant properties, and antibacterial efficacy of *Moringa* leaves. *J Xi'an Shiyu Univ Nat Sci Ed.* 2022;18(10):59-70.
36. Melesse A, Tiruneh W, Negesse T. Effects of feeding *Moringa stenopetala* leaf meal on nutrient intake and growth performance of Rhode Island Red chicks under tropical climate. *Trop Subtrop Agroecosyst.* 2011;14(2):485-492.
37. Mikore D, Mulugeta E. Determination of proximate and mineral compositions of *Moringa oleifera* and *Moringa stenopetala* leaves cultivated in Arbaminch Zuria and Konso, Ethiopia. *Afr J Biotechnol.* 2017;16(15):808-818. doi:10.5897/ajb2017.15919
38. Mishra G, Singh P, Verma R, *et al.* Traditional uses, phytochemistry, and pharmacological properties of *Moringa oleifera* plant: an overview. *Der Pharm Lett.* 2011;3(2):141-164.
39. Nobossé P, Fombang EN, Mbofung CMF. Effects of age and extraction solvent on phytochemical content and antioxidant activity of fresh *Moringa oleifera* L. leaves. *Food Sci Nutr.* 2018;6(8):2188-2198. doi:10.1002/fsn3.783
40. Ogbe AO, Affiku JP. Proximate study, mineral and anti-nutrient composition of *Moringa oleifera* leaves harvested from Lafia, Nigeria: potential benefits in poultry nutrition and health. *J Microbiol Biotechnol Food Sci.* 2011;1(3):296-308.
41. Oladeji OA, Taiwo KA. Impact of incorporating *Moringa oleifera* seed on the nutritional, physicochemical, and sensory properties of fermented maize porridge 'Ogi' flour. *Coast J Sch Sci OAUSTECH Okitipupa.* 2019;1(1).
42. Olatunde A, Dikwa MA. Qualitative and quantitative analysis of phytochemicals.
43. Olusanya RN, Kolanisi U, Van Onselen A, Ngobese NZ, Siwela M. Nutritional composition and consumer acceptability of *Moringa oleifera* leaf powder (MOLP)-supplemented mahewu. *S Afr J Bot.* 2019;129:175-180.
44. Onwuka GI. Bulk density determination of food materials. *J Food Sci Technol.* 2005;42(2):123-127.
45. Pakade V, Cukrowska E, Chimuka L. Metal and flavonol contents of *Moringa oleifera* grown in South Africa. *S Afr J Sci.* 2012;109(3):1-7.
46. Palada MC, Wu DL, Ebert AW. Horticultural characterization and propagation of moringa germplasm at AVRDC - The World Vegetable Center. *Proc Reg Symp High Value Veg Southeast Asia: Production, Supply and Demand*; 24-26 Jan 2012; Chiang Mai, Thailand.
47. Pavithra PS, Sreevidya N, Verma RS. Antibacterial and antioxidant activity of *Moringa oleifera*.
48. Peñalver R, Martínez-Zamora L, Lorenzo JM, Ros G, Nieto G. Nutritional and antioxidant properties of *Moringa oleifera* leaves in functional foods. *Foods.* 2022;11(8):1107.
49. Raghavendra HL, Kekuda TRP, Vijayananda BN, Duressa D, Solomon D. Nutritive composition and antimicrobial activity of *Moringa stenopetala* (Baker f.) Cufod. *J Adv Med Pharm Sci.* 2016;10(3):1-9. doi:10.9734/jamps/2016/29987
50. Raghuramalu N, Nair MK, Kalyanasundaram SA. Manual of laboratory techniques. 1st ed. Hyderabad: National Institute of Nutrition, KMR; 1983. p.31-32.
51. Rakesh SU, Patil PR, Salunkhe VR. Free radical scavenging activity of hydroalcoholic extracts of dried flowers of *Nymphaea stellata* Willd.
52. Razis AF, Ibrahim MD, Kntayya SB. Health benefits of *Moringa oleifera*. *Asian Pac J Cancer Prev.* 2014;15(20):8571-6.
53. Saini RK, Sivanesan I, Keum YS. Phytochemicals of *Moringa oleifera*: a review of their nutritional, therapeutic and industrial significance. *3 Biotech.* 2016;6:203.
54. Shanmugavel G, Prabakaran K, George B. Evaluation of phytochemical constituents of *Moringa oleifera* (Lam.) leaves collected from Puducherry region, South India. *Int J Zool Appl Biosci.* 2018;3(1):1-8.
55. Singh GP, Sharma SK. Antimicrobial evaluation of leaf extract of *Moringa oleifera* Lam. *Int Res J Pharm.* 2012;3:1-4.
56. Singh HP. *Moringa-A crop of future.* Keynote address for Brain Storming session on Moringa; 23 Mar 2010.
57. Sreelatha S, Padma PR. Antioxidant activity and total phenolic content of *Moringa oleifera* leaves in two stages of maturity. *Plant Foods Hum Nutr.* 2009;64(4):303. doi:10.1007/s11130-009-0141-0
58. Stevel IO, Babatunde OI. Chemical compositions and nutritional properties of popcorn-based complementary foods supplemented with *Moringa oleifera* leaves flour. *J Food Res.* 2013;2(6):117-132.
59. Tamilselvi N, Krishnamoorthy P, Dhamotharan R, Arumugam P, Sagadevan E. Analysis of total phenols, total tannins and screening of phytocomponents in *Indigofera aspalathoides* (Shivanar Vembu) Vahl EX DC. *J Chem Pharm Res.* 2012;4(6):3259-3262.
60. Ukpabi UJ, Ndimele C. Evaluation of the quality of gari produced in Imo State. *Niger Food J.* 1990;8:105-10.
61. Yang RY, Chang LC, Hsu JC, Weng BB, Palada MC, Chadha ML, *et al.* Nutritional and functional properties of moringa leaves from germplasm to plant, to food, to health. In: *Moringa leaves: strategies, standards and markets for better impact on nutrition in Africa.* Paris: Moringa News, Moringa and Plant Resources Network; 2007. 9 p.