



## Evaluation of Bioactive Compounds and Antioxidant Activity of Various Parts (Pod, Flower and Leaves) of Drumstick (*Moringa oleifera*)

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### ABSTRACT

*Drumstick (Moringa oleifera) is also commonly known as the "tree of life" or "miracle tree". It has been categorised as a significant plant due to its various medicinal and non-medicinal benefits. It is globally found in all tropical and subtropical regions. As a folk medicine, different parts of this plant are used to cure wounds, pain, ulcers, heart disease, liver disease, cancer, and inflammation. Several scientific studies have stated that the drumstick plant has a wide range of phytochemicals and antioxidants which play a crucial role in nutrition and various medicinal purposes. This investigation involves the quantitative determination of phytochemicals and antioxidant activity of the various parts of aqueous and isopropanol extracts of Moringa oleifera. The findings of the present study indicate that the highest moisture content was found in the Moringa oleifera pod (82.96%), followed by the flower (80.02%) and leaves (77.7%). The solvent extract of Moringa oleifera leaves exhibited the highest TPC of 46.4 mg GAE/g, and flowers exhibited the highest TFC, with 54.85 mg QE/g. The highest % inhibition of DPPH radical scavenging activity was found in the aqueous extract of drumstick pod (91.45%). The greater TAC value for both aqueous (168.72 mg AAE/g) and isopropanol (98.21 mg AAE/g) extract was found for the flowers of Moringa oleifera compared to the pods and leaves. These results established that different parts of Moringa oleifera of both aqueous and isopropanol extracts have significant quantities of phytochemicals and possess good antioxidant activity.*

**Keywords:** *Moringa oleifera*, leaves, pod, bioactive compounds, antioxidant activity

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### Abbreviations

TPC- Total phenolic content, TFC- Total flavonoid content, DPPH- 2,2'-diphenyl-1-picrylhydrazyl, TAC- Total antioxidant capacity,

### INTRODUCTION

Throughout the world, *Moringa oleifera*, a member of the Moringaceae family, is referred to in English as "Drumstick tree," "Horseradish tree," or "Ben tree." It is often known as the Miracle Tree [1]. Out of the 13 species in the *Moringa* genus, *Moringa oleifera* and *Moringa concanensis* are the two that are most frequently found in India [2]. *Moringa oleifera* is the most widely cultivated of the native plants of South Asia; specially, various regions in India, Pakistan, Afghanistan, Bangladesh, Sri Lanka, Southern Florida, East and West Africa, the West Indies [3]. In many countries, almost every part of this plant, including the leaves, pods, and flowers, is consumed as vegetables, which has a variety of nutritional benefits. In addition, it also finds extensive application in non-food goods such as biogas, fertilizer, animal feed, charcoal, textiles, fence, water purification, fine machine lubricating oil, lumber, and hair care products [5]. *Moringa oleifera* is used as a viable treatment for malnutrition due to its ease of cultivation. Children are treated with different parts of drumsticks in several nations like Senegal and Benin for the prevention of malnutrition. Few studies have stated that *Moringa oleifera* is abundant in hormone precursors, such as stigmaterol, sitosterol, and campesterol. These substances help to raise the production of oestrogen, which in turn encourages the growth of the mammary gland ducts, causing lactating mothers to produce milk [6]. Several

bioactive substances, particularly secondary metabolites such as alkaloids (two different types of alkaloid chemicals found in *Moringa oleifera* (moringine and moringinine), phenolic compounds, terpenoids, tannins, and phytosterols, are also found to be abundant in drumstick. Due to the presence of all of these bioactive compounds, drumstick has various types of therapeutic properties such as antipyretic, anti-ulcer, anti-inflammatory, anti-convulsant, antioxidant, immunomodulatory, analgesic, anti-diabetic, anti-fertility and anti-malaria, as well as anti-hypertensive, anti-carcinogenic, anti-microbial, anti-ageing [7,8]. In addition to being a great source of vitamins C and E, the leaves also include minerals, a significant amount of rough protein (20–29%), phenolic acid, calcium, iron,  $\beta$ -carotene, and riboflavin. It is claimed that moringa contains seven times more vitamin C than oranges, ten times more vitamin A than carrots, nine times more protein than yoghurt, seventeen times more calcium than milk, twenty-five times more iron than spinach, and fifteen times more potassium than bananas [9]. The leaves of Moringa plants contain a variety of chemicals, including flavonoids, ascorbic acid, phenolics, and carotenoids, which are excellent sources of naturally occurring antioxidants [8]. Drumstick flower is rich in nine amino acids, sugar, D-glucose, wax, and quercetin; some flavonoid pigments including kaempferol, rhamnetin, isoquercitrin, kaempferitrin, and the ash are high in potassium, calcium. The flower is also used in the human diet as it has an abundance of calcium, potassium, and antioxidants ( $\alpha$  and  $\gamma$  tocopherol) [9]. Studies have shown that the pod of drumsticks helps to lower blood cholesterol, triglycerides, low-density lipoprotein (LDL), phospholipids, and atherogenic index lipids levels [7]. *Moringa oleifera* is becoming more popular as a food fortifier in various regions of the world, including Africa, Central and South America, Sri Lanka, India etc. Numerous research studies have demonstrated the possible uses of *Moringa oleifera* in food applications, including the creation of herbal cookies, bread, cakes, yoghurt, cheese, Amala (stiff dough made from yam flour), soups, and foods for weaning [10]. There are various studies have already been conducted on various parts of *Moringa oleifera* for their nutritional composition and antioxidant activities worldwide. However, different results can be seen from those studies may be due to geographical variation, and agroclimatic conditions. The present investigation involves the collection of samples (pods, leaves and flowers) from the same plant of *Moringa oleifera*. Two solvents such as, aqueous and isopropanol were used for the extraction process and compared in the current study. Hence, the present investigation aims to determine the physicochemical, phytochemicals and antioxidant activity of aqueous and isopropanolic extracts of different parts (pods, leaves and flowers) of the *Moringa oleifera* plant.

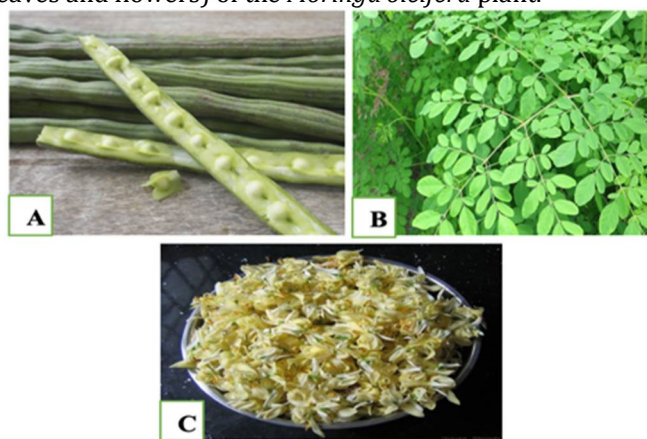


Fig 1: A) Drumstick Pod B) Drumstick Flower C) Drumstick Flower [4]

## MATERIAL AND METHODS

**Sample Collection:** Drumstick pods, leaves and flowers were collected from one healthy tree in Rajarhat, West Bengal (India) in the period of February-March 2023. The fully matured pods were taken out. Blooming flowers and well-grown leaves were gently removed from the stem. Following collection, the samples were cleaned, sliced, and weighed. They were then dried in a hot air oven at 55°C until two subsequent weight readings were equivalent. For further study, each dried sample was ground into a fine powder, put in an airtight glass container, and kept in the refrigerator.

### Sample Preparation:

**Preparation of the aqueous extract:** 7.5 g of each sample was weighed with the help of a weighing balance before being transferred to a beaker. 100 ml of distilled water was used to measure by a measuring cylinder, which was then put into the beaker. The solution was swirled for three hours with the help of a magnetic stirrer. Then, the aqueous solution was centrifuged at 6000 rpm for 10 minutes. For further activities, the aqueous extract was collected and kept in the refrigerator at a temperature of - 4°C [11].

**Preparation of the solvent extract:** The same procedure (preparation of aqueous extract) was followed only isopropanol was used instead of aqueous solvent [11].

**Quantitative Estimation of Physicochemical Properties:**

**Total Moisture Content:** The AOAC standard method was used to calculate the total moisture content of each sample [12]. At first, weighed fresh samples were equally spread out on a tray and dried at  $55^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for five hours. Then, the samples were subsequently cooled to room temperature, and their weight was verified. Until the difference between two consecutive measurements was less than 1 milligram, the same procedure was repeated. This formula was used to determine the total moisture content.

$$\text{Moisture \% by weight} = \frac{\text{initial weight of sample} - \text{final weight of sample}}{\text{initial weight of sample}} \times 100 \quad (1)$$

**Total Ash Content:** The AOAC standard method was used to calculate the total ash content of each sample [12]. All the samples that had previously been dried in a hot air oven were weighed in a crucible and heated for five hours at  $600^{\circ}\text{C} \pm 10^{\circ}\text{C}$  in a muffle furnace to produce grey ash and weighed after cooling in the desiccator. The provided formula was used to get the total ash content.

$$\text{Ash \% by weight} = \frac{\text{final weight of sample}}{\text{initial weight of sample}} \times 100 \quad (2)$$

**Quantitative analysis of phytochemicals:**

**Total Phenolic Content** - To ascertain the total phenolic content Folin-Ciocalteu's method was used with slight modification [13]. 50  $\mu\text{l}$  of the sample was mixed with 9.5 ml of distilled water. A final volume of 0.5 ml of Folin-Ciocalteu's reagent was then added. 2.5 ml of a 20% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) solution was then added. The contents of the test tube were mixed in a vortex mixer. After exposing the sample to room temperature and darkness for 40 minutes, the absorbance at 725 nm was measured using a UV-visible spectrophotometer (Labtronics, Model: LT-2201). A standard for the calibration curve was used in a range of concentrations of gallic acid. The results were calculated as (mg GAE/g).

**Total Flavonoid Content**- The method of aluminium chloride was slightly modified to assess the flavonoid content [14]. After combining 100  $\mu\text{l}$  of the sample with 4 ml of distilled water, 0.3 ml of 5% sodium nitrite ( $\text{NaNO}_2$ ) was added, and the combination was allowed to stand for 5 minutes. The mixture was then given 0.3 ml of 10% aluminium chloride ( $\text{AlCl}_3$ ) and let a further 6 minutes to stand before getting 1 ml of 1(M) sodium hydroxide ( $\text{NaOH}$ ). The final volume was adjusted to 10 ml by adding a small amount of distilled water. The wavelength of 510 nm was used to measure the absorbance. The number of flavonoids was calculated as mg quercetin equivalents (mg QE/g) from the standard curve.

**Determination of *In Vitro* Antioxidant Activity:**

**DPPH radical scavenging assay**- Using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), the antioxidant activity assay was conducted using slight modification [15]. 50 ml of ethanol was mixed with 0.002 g of DPPH to create the ethanolic solution of DPPH. A mixture of 0.5 ml of the sample and 2.5 ml of the DPPH solution was prepared, and the mixture was left at room temperature in the dark for 20 minutes. The mixture's absorbance was measured at 517 nm. The formula was used to calculate the DPPH radical scavenging activity.

$$\% \text{ of inhibition activity} = \frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100 \quad (3)$$

**Total antioxidant capacity (Phosphomolybdenum assay)**

The phosphomolybdenum method was used to evaluate the antioxidant capacities of different parts of moringa plants, with a few changes [16]. 4.704 ml of sulphuric acid ( $\text{H}_2\text{SO}_4$ ), 0.392 g of ammonium molybdate, 0.288 g of sodium phosphate, and 80 ml of distilled water were combined to create the molybdate reagent solution. In a glass test tube, 300  $\mu\text{l}$  of sample and 3 ml of molybdate reagent were combined. The test tubes were incubated in a water bath for 90 minutes at  $90^{\circ}\text{C}$ . After the samples were cooled to room temperature, the absorbance at 695 nm. Ascorbic acid was employed as the standard for the standard curve. The result was represented (mg AAE/g) of dry material.

**Statistical analysis:**

All the data are expressed as mean  $\pm$  SD by SPSS application.

## RESULTS AND DISCUSSION

### Quantitative estimation of physicochemical parameters:

**Table 1: Quantitative estimation of physicochemical parameters of pod, leaves, and flower powder of drumstick (*Moringa oleifera*) (per 100 g of fresh weight)**

Parameters	<i>Moringa oleifera</i> Pod	<i>Moringa oleifera</i> Leaves	<i>Moringa oleifera</i> Flower
Moisture %	82.96±0.58	77.7±0.71	80.02±0.87
Ash %	10.3±0.32	7.01±0.54	10.43±0.39

**Note: Data are expressed as mean ± SD, (n=3)**

The total moisture and ash content is shown in Table 1. In the present investigation, it has been noted that the pod of *Moringa oleifera* has the highest moisture content (82.96%) followed by the flower of *Moringa oleifera* (80.02%) and leaves of moringa (77.7 %). The findings suggest that the leaves of moringa would have a long shelf life due to its low moisture content compared to the pod and flower [17]. The ash content is usually considered as to evaluate any food's quality and determine its functional characteristics [17]. In this study, both the pod and flower of *Moringa oleifera* possess a good amount of ash 10.3 % and 10.43% respectively; which indicates the presence of inorganic part i.e., mineral content, including essential minerals like calcium, potassium, and magnesium. The leaves of moringa have (7.01%) of the total ash.

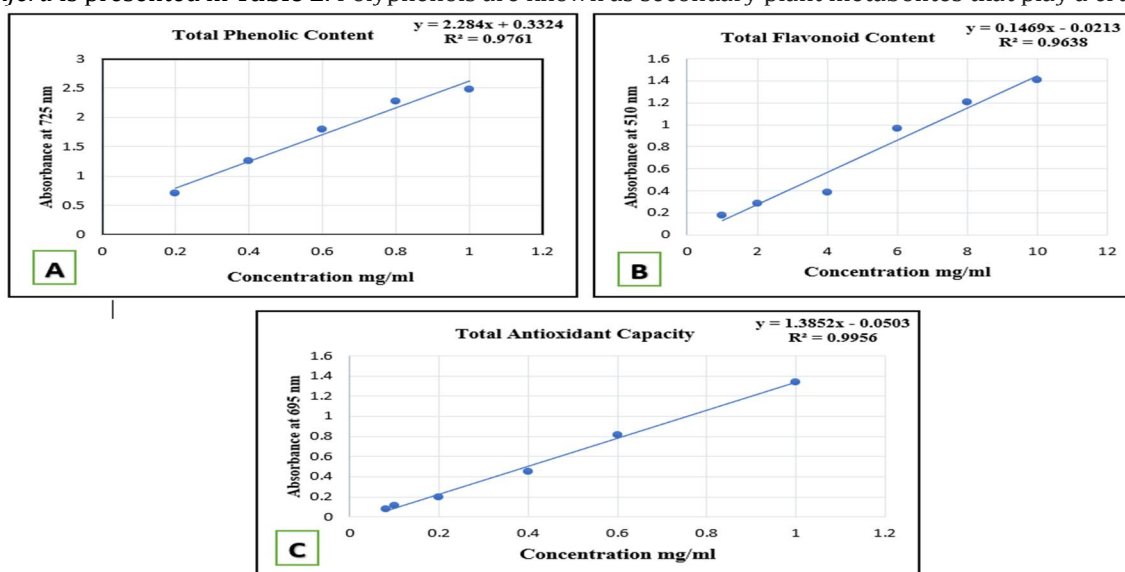
### Determination of phytochemicals and antioxidant activity:

**Table 2: Antioxidant activity analysis of water and isopropanol extract of the pods, leaves, and flower of drumstick (*Moringa oleifera*)**

Assay type	<i>Moringa oleifera</i> Pod		<i>Moringa oleifera</i> Leaves		<i>Moringa oleifera</i> Flower	
	Aqueous	Isopropanol	Aqueous	Isopropanol	Aqueous	Isopropanol
TPC (mg GAE/g)	1.05±0.39	3.09±0.79	24.5±0.93	46.4±0.69	11.22±0.96	41.52±0.30
TFC (mg QE/g)	7.10±0.25	26.04±0.71	11.95±0.81	35.90±0.52	31.38±0.49	54.85±0.87
DPPH (% of inhibition/g of dried sample)	91.45±0.76	87.39±0.95	83.76±0.39	55.25±0.58	78.95±0.76	87.33±0.71
TAC (mg AAE/g)	6.21±0.82	17.39±0.87	29.37±0.42	37.18±0.48	168.72±0.82	98.21±0.97

**Note: Data are expressed as mean ± SD, (n=3)**

The total phenolic content (TPC) of both (aqueous and isopropanol) extracts of different parts of *Moringa oleifera* is presented in Table 2. Polyphenols are known as secondary plant metabolites that play a crucial



**Fig 2: (A) Standard curve of Total Phenolic Content (B) Standard curve of Total Flavonoid Content (C) Standard curve of Total Antioxidant Capacity**

role in human physiology such as, antioxidant, antimicrobial, anti-inflammatory and anti-ageing activity [15]. The gallic acid was used as standard and the results were derived from a calibration curve ( $y = 2.284x + 0.3324$ ,  $R^2 = 0.9761$ ) (Fig 2 A). In the present investigation, *Moringa oleifera* leaves exhibited the highest phenolic content, with 24.5 mg GAE/g in the aqueous extract and 46.4 mg GAE/g in the solvent extract. Whereas, the flower of *Moringa oleifera* exhibited lower phenolic content, with 11.22 mg GAE/g in the aqueous extract and 41.52 mg GAE/g in the solvent extract followed by pod of *Moringa oleifera* has the lowest phenolic content i.e., 1.05 mg GAE/g in aqueous extract and 3.09 mg GAE/g in the solvent extract. A similar study on the same sample collected from Nigeria found the total phenolic content of the leaves and flowers 113.3 mg GAE/g and 175.6 mg GAE/g respectively [18]. The differences in the obtained values of phenolic content may be due to the geographical and agroclimatic conditions and the use of different solvents for extraction. Flavonoid belongs to the polyphenolic compounds and possesses various therapeutic prosperities including anticancer, antiviral, antioxidant, neuroprotective, anti-inflammatory and cardio-protective effects [19,20]. The total flavonoid content was calculated by the calibration curve equation of the standard quercetin ( $y = 0.1469x - 0.0213$ ,  $R^2 = 0.9638$ ) (Fig 2B). In this study, the total flavonoid content (TFC) of aqueous extracts from pod, leaves, and flowers of *Moringa oleifera* was obtained to be as follows: 7.10 mg QE/g, 11.95 mg QE/g, and 31.38 mg QE/g. The isopropanol extracts from the same plant parts have the following TFC values: pod- 26.04 mg QE/g, leaves- 35.90 mg QE/g, flower- 54.85 mg QE/g. Among all the extracts, the isopropanolic extract of the flower of *Moringa oleifera* has the highest value of TFC value (54.85 mg QE/g). The same experiment that was conducted in Nigeria showed the total flavonoid content of the leaves and flower of *Moringa oleifera* 91.2 mg QE/g and 84.3 mg QE/g respectively [18]. Whereas, the TFC value was obtained from an India-based study that (Leaves- 4.44 mg QE/g), (Flower- 4.41 mg QE/g) [18]. It has been already proven that phenolic and flavonoid content is directly associated with the antioxidant activity of any compound. To determine the antioxidant activity of any substance; the DPPH radical scavenging activity is one of the simple and popular methods. In the present investigation, the DPPH radical scavenging activity per g of dried in aqueous extract of *Moringa oleifera* pod, leaves, and flowers was found to be 91.45%, 83.73% and 78.95 %. Whereas, for isopropanolic extract (Pod- 87.39 %), (Leaves- 55.25 %) (Flower – 87.33 %). Among the aqueous extract pod of *Moringa oleifera* (91.45%) has obtained the highest % of inhibition. However, for the isopropanolic extract, both the pod and leaves have found almost the same % of inhibition 87.33% and 87.39 respectively. An antioxidant is a molecule that is capable enough to donate an electron to a free radical and neutralize it. This neutralized molecule can inhibit cellular damage caused by free radicals in the human body [20]. The total antioxidant capacity (TAC) of different parts of *Moringa oleifera* was derived by the standard calibration curve of ascorbic acid ( $y = 1.3852x - 0.0503$ ,  $R^2 = 0.9956$ ) (Fig 2C). According to the findings, the aqueous extract of the dried pod, leaves, and flower powder of *Moringa oleifera* has a TAC of 6.21 mg AAE/g, 29.37 mg AAE/g, and 168.72 mg AAE/g. In contrast, the TAC for the isopropanolic extract of the dried pod, leaves, and flower powder of *Moringa oleifera* is 17.39 mg AAE/g, 37.18 mg AAE/g, and 98.21 mg AAE/g, respectively. The greater TAC value for both aqueous and isopropanolic extract is found for the flower *Moringa oleifera* compared to the pod and leaves.

## CONCLUSION

The present study has shed light on the variability in bioactive compound content and antioxidant activities among various parts of the *Moringa oleifera* plant such as pods, leaves, and flowers. Drumstick leaves demonstrated the highest total phenolic content, followed by flowers and pods. Comparatively to the other components of the moringa plant, the isopropanol extract of the moringa flower contains a significantly larger level of total flavonoid content. Aqueous extract drumstick flower exhibits the greatest TAC value and moringa fruit aqueous extract exhibits the highest % inhibition of DPPH radical scavenging activity. These findings emphasize the potential of Moringa as a valuable source of natural antioxidants, which can have significant implications for various applications in the food, pharmaceutical, and nutraceutical industries. *Moringa oleifera* has a positive prognosis for the future. The great potential for health advantages, preservation, and oxidation prevention of drumstick leaves, flowers, and pods can be unlocked by incorporating them into a variety of applications and could be utilized in the formulation of pharmaceuticals or nutraceutical products targeting specific health conditions.

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