Comparative Studies of Phytochemical Constituents of Different Parts of *Moringa oleifera*

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Abstract: *Moringa oleifera* is widely cultivated throughout India, belongs to family *Moringaceae*. It is widely used as a nutritive herb and possesses valuable pharmacological activities. The present study deals with the evaluation and comparison of nutrients, Phytochemicals and antioxidant content of different parts of *M. oleifera*. It is one of the rich sources of vitamin A, vitamin C, milk protein, etc. Present study shows that, in *M. oleifera*, polyphenols and flavonoids are present in the significant amount in leaves. This is the reason it is used traditionally to treat many oxidative related diseases. Every part of Moringa is said to have beneficial properties that can serve humanity so the whole plant can be extensively studied for further research aspects.

Keywords: *Moringa oleifera*, polyphenols, flavonoids, alkaloids, tannins, antioxidants.

1. Introduction

The most widely cultivated species throughout the tropics is *Moringa oleifera*, a multipurpose tree native to the foothills of the Himalayas in northwestern India. It can also be used for water purification and hand washing and is sometimes used in herbal medicine [1]. Many parts of *Moringa* are edible. Regional uses of the *Moringa* as food vary widely and include the immature seed pods, called 'drumsticks', are popular in Asia & Africa, leaves are eaten, particularly in Cambodia, Philippines, India, Sri lanka and Africa, mature seeds, flowers, oil pressed from the mature seeds and roots.

In some regions, the young seed pods are most commonly eaten, while in others, the leaves are the most commonly used part of the plant. The flowers are edible when cooked and said to taste like mushrooms [2].

The leaves are the most nutritious part of the plant, being a significant source of vitamin C, pro-vitamin A as beta carotene, vitamin K, manganese and protein, among other essential nutrients. Hence compared with common foods particularly high in certain nutrients /100g fresh weight, cooked *Moringa* leaves are considerable sources of these same nutrients. Some of the calcium in *Moringa* leaves is bound as crystals of calcium oxalate though at levels $1/25^{th}$ to $1/45^{th}$ of that found in spinach, which is a negligible amount. The leaves are cooked & used like spinach & are commonly dried & crushed into a powder used in soups & sauces [3].

Phytochemicals such as tannins, sterols, terpenoids, flavonoids, saponins, anthraquinones, alkaloids and reducing sugar present along with anti-cancerous agents like glucosinolates, isothiocyanates, glycoside compounds and glycerol-1-9-octadecanoate [4] are present in *Moringa sp*. *Moringa* leaves also have a low calorific value and can be used in the diet of the obese. The pods are fibrous and are valuable to treat digestive problems and thwart colon cancer [5]. A research shows that immature pods contain around 46.78% fiber and around 20.66% protein content. Pods have 30% of amino acid content, the leaves have 44% and flowers have 31%. The immature pods and flowers showed similar amounts of palmitic, linolenic, linoleic and oleic acids [6].

The main aims and objectives of this present study are to assess the phytochemical constituents of the different parts of *M. oleifera*, to assess the antioxidant contents of the different parts of *M. oleifera* and to compare the phytochemical constituents and antioxidant contents of these studied parts of *M. oleifera*.

2. Materials and methods

All the chemicals, reagents and solvents used in this study were of AR/GR grade and obtained from E. Merck and SRL.

The parts of *M. oleifera* (leaves, stem & bark) were collected from Ichhapur, West Bengal, India. Those were kept in sterile zip lock plastic bag and preserved in ice bag during transportation from the field to the laboratory.

2.1 Preparation of sample extract

The fresh leaves, stem and bark of *Moringa sp*. were collected and weighed by using pan balance. To 0.5g of fresh sample 10ml (70% ethanol w/v) was mixed and was kept overnight in refrigerator. The mixture was then allowed to cold centrifuge at 5000 rpm for 30 min. Then the extracts were filtered using glass wool and was kept for further analysis.

2.2 Moisture Analysis

5g of fresh *Moringa* samples were taken and weighed using pan balance. The samples were kept in Petri plates with their respective lids and the measurements were taken in triplicates. Petri plates were marked as leaves, stem and bark. Then those were dried in hot air oven at 70 °C for consecutive 3days until they reached a constant weight. On 3rd day the final weight was taken [7].

The moisture content of leafy vegetables was calculated by using the following formula:

$$\text{Moisture Content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Weight of the sample}} \times 100$$

2.3 Phytochemical screening

The phytochemical screening for the extract of *M. oleifera* was carried out by using standard protocols **[**8].

2.3.1 Carbohydrate test

a. Molish's Test:

To 3ml of aqueous extract 2-3 drops of alpha napthol and concentrated sulphuric acid (H_2SO_4) was added carefully along the side of the test tube. Presence of violet ring indicated that carbohydrate is present.

b. Fehling's Test:

To 2 ml of aqueous extract, Fehling A and Fehling B was added in the test tube. The extract was mixed and placed in water bath for 10 min. A red or orange precipitate showed the presence of carbohydrate.

c. Barfoed's Test:

To the Barfoed reagent 3-4 drops of sample was added and placed in water bath. Red precipitate indicated the presence of monosaccharide.

2.3.2 Protein

2 ml of the extract was taken in a test tube and 3-4 drops of NaOH was added and then copper reagent was added drop-wise and was vortexed well. A violet purple colour indicated presence of protein.

2.3.3 Fat

2-3 drops of sample extract were dropped on filter paper. Appearance of oily patch on filter paper indicates presence of fat.

2.3.4 Alkaloids

To the equal volume of sample extract, wagner's reagent was added and placed into a clean test tube. Then it was observed for some time. A brown precipitate indicated presence of alkaloids.

2.3.5 Tannins

0.5 g of each powdered sample was boiled with 5ml of distilled water for 10 min. The solution was filtered and then 1 % $FeCl_3$ was added when the filtrate was hot. A blue green colour or brown colour precipitation showed the presence of tannins.

2.3.6 Terpenoids

4-5 ml aqueous extract of each sample was mixed with 2 ml of chloroform and concentrated H_2SO_4 in a test tube. Appearance of reddish brown color showed presence of terpenoids.

2.3.7 Flavonoids

To 3-4 ml of aqueous extracts 2 % (w/v) NaOH was added and a yellowish color was develpoed. Discolouration occurs after adding of concentrated H_2SO_4.

2.3.8 Glycosides

To the extract, 2 ml of cold glacial acetic acid was added containing 1drop of $FeCl_3$ solution and 1 ml concentrated H_2SO_4. Formation of brown ring confirmed the presence of glycosides.

2.3.9 Saponins

1g of powdered sample was taken in a conical flask and 10 ml of distilled water was added and boiled for 10 min. The solution was filtered and to 2.5ml of filtrate, 10 ml of distilled water was added and vortexed. Then, 3 drops of olive oil was mixed and again vortexed that indicated the presence of saponins.

2.4 Quantitative analysis

2.4.1 Determination of total phenols

Total polyphenol content in the sample extract were determined using Gallic acid as standard. The extract solution was prepared using 70 % (v/v) ethanol. The concentration of sample was 50 mg/ml. 1ml of this extract solution was mixed with 10 %, 1ml of Folin-Ciocalteau (FC) and with an aqueous solution of 7.5 %, 0.8 ml Sodium Carbonate (Na_2CO3). Then volume was made up to 10 ml using distilled water. After 30 min of incubation in dark at room temperature, the absorbance of the reaction compound at 765 nm was measured in spectrophotometer. The overall phenol content was expressed as mg of Gallic acid equivalents (GAE/g of dry weight of the sample) [9].

2.4.2 Determination of total flavonoids

Total flavonoid content in the sample extract was determined using Quercetin as standard. The extract solution was prepared using 70 % ethanol. The concentration of sample was 50 mg/ml. 1 ml of this extract was mixed with 0.15 ml of a 5 % Sodium Nitrate ($NaNo_2$). After 6 min, 0.15 ml of 10 % Aluminium Chloride solution ($AlCl_3$) was added. Again after 6 min, 2 ml of 4 % Sodium Hydroxide

(NaOH) was added and volume was made up to 10 ml. The solutions were mixed well and kept in dark for 15 min at room temperature and the absorbance of reaction compound at 510 nm was measured in spectrophotometer (Cystonic UV vis 118). The overall flavonoid content was expressed as mg of Quercetin equivalents (QE/g of dry weight of the sample) [10].

All the statistical analyses were done by using the Microsoft Office Excel 2007. All the experiments were carried in triplicate.

3. Results and Discussion

3.1 Nutrients and phytochemistry

The ethanolic extracts of the *Moringa sp*. contained protein, carbohydrate and fat in leaf and stem whereas bark did not contain fat. The molisch's test showed the presence of carbohydrate for the ethanolic extract of the samples. So, the present study revealed that the different parts of *Moringa* contained all basic nutrients and may support its potential use for nutritional purposes.

The ethanolic extracts of leaf and stem of *Moringa* contained alkaloid which implies that the consumers could determine the phytochemistry of their *Moringa* preparations by choosing the method of extraction that would be preferred for Phytochemical screening (Table 1). Tannins were present in all the parts of the tree and could be consumed. Flavonoids were present in leaf and stem. Glycosides and saponins were absent in the samples.

3.2 Moisture content

Table 2 shows moisture content (gm %) of *Moringa* (leaves stem and bark). Result showed that moisture content was maximum in case of leaves (74.90 gm %) whereas, bark had the lowest amount of moisture (56.64 gm %).

3.3 Polyphenol content

Phenolic compounds are widely distributed in plants, which have gained great attention, due to their antioxidant activities and free radical scavenging abilities which potentially have beneficial implications for human health [11].

In this present study the total phenolic content was determined in comparison with standard gallic acid and the results were expressed in terms of mg Gallic Acid Equivalent (GAE)/ g for dry weight.

From Fig 1, it was shown that the polyphenol content (mg GAE/ g) of leaves, stem and bark of *Moringa* sp. varied significantly ($P<0.001$). The amount of polyphenol content (mg GAE/ g) in

leaves, stem and bark were 31.54, 20.46 and 27.9 (mg GAE/ g) respectively that revealed the polyphenolic content was maximum in leaves and minimum in Stem.

Methanolic extract have shown higher scavenging activities than hexane that is the reason for using *Moringa* sp. traditionally to treat many oxidative related diseases [13].

3.4 Flavonoid content

Flavonoids including flavones, flavonols and condensed tannins are secondary plant metabolites. The antioxidant activity of which depends on the presence of free OH group. Plant flavonoids have antioxidant activity in vitro and also act as in vivo. Flavonoids are water soluble polyphenolic molecule. Flavonoid constituents are considered to be the most vital antioxidant components of plant and significant correlation between concentration of plant flavonoids and total antioxidant capacity has been reported [12].

The present study showed the flavonoid content (mg QA/ g) of leaves, stem and bark varied significantly ($P<0.001$). The amount of flavonoid content in leaves, stem & bark were 25.86, 7.14 and 15.59 (mg QA/g) respectively (Fig 2) that revealed the flavonoid content was maximum in leaves and minimum in stem.

4. Conclusion

The present study deals with the evaluation and comparison of nutrients, phytochemicals and antioxidant contents of different parts of *M. oleifera* tree.

Protein, fat and carbohydrate were found in *M. oleifera*. Also various phytochemicals like tannins, terpenoids, alkaloids, flavonoids, glycosides and saponins were found in *M. oleifera*. There were no variation in the distribution of the protein, terpenoids and tannins. However there were variation in the distribution of cabohytrates, fat, flavonoids and alkaloids.

Polyphenols and flavonoids represent the antioxidant content of the samples in this present study. From the study it has been concluded that the polyphenol and flavonoids content is found to be more in leaves. Thus, it may be concluded that this is the reason it is used traditionally to treat many oxidative related diseases.

By doing further study it can be proved that *Moringa* can be consumed to prevent malnutrition mainly in subtropical areas as its is most common in that area. *Moringa* is often thought to be most nutritionally complete of all food supplements, containing many important nutrients. Thus, it can also prove the validity of traditional utility of *Moringa* in various folklores. *M. oleifera* leaf has countless uses as a supplement for maintaining good health and for preventing diseases like

anti-cancer, anti- diabetic, anti-oxidant, reduces excess fat from the body & many other health related problems.

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Table legends

Table 1. Nutrients and phytochemical groups of ethanolic extract of various parts of *Moringa* sp.

Table 2. Determination of moisture content (g %) of different parts of *M. oleifera*.

Figure legends

Fig 1. Graphical representation of total polyphenol content of different parts of *Moringa* sp.

Fig 2. Graphical representation of flavonoids content of different parts of *Moringa* sp.

TABLE 1. NUTRIENTS AND PHYTOCHEMICAL GROUPS OF ETHANOLIC EXTRACT OF VARIOUS PARTS OF *Moringa* sp.

Phyto-constituents	Aqueous extracts		
	leaves	stem	bark
Carbohydrates (Molisch's test)	+	+	+
(Fehling's test)	+	-	+
(Barfoed test)	+	-	-
	+	+	+
(Biuret test)			
	+	+	-
Alkaloids	+	+	-
Tannins	+	+	+
Terpenoids	+	+	+
Flavonoids	+	+	-
Glycosides	-	-	-
Saponins	-	-	-

TABLE 2. DETERMINATION OF MOISTURE CONTENT (G %) OF DIFFERENT PARTS OF *M. oleifera*:-

Samples	Moisture content (g %)
Leaves	74.9%
Stem	61.28%
Bark	56.64%

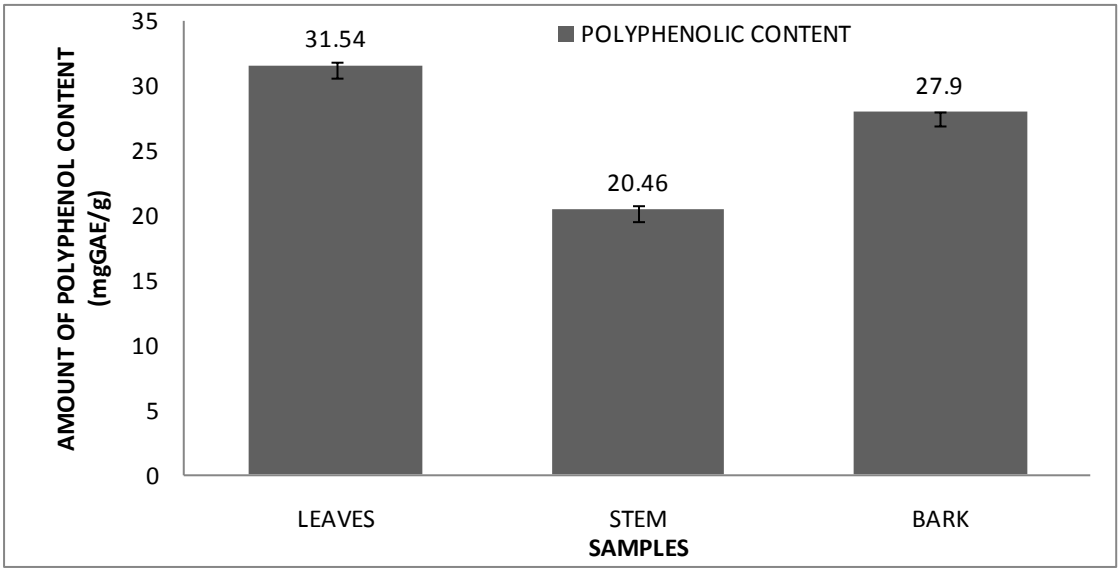

Fig 1. Graphical representation of total polyphenol content of different parts of *Moringa* sp.

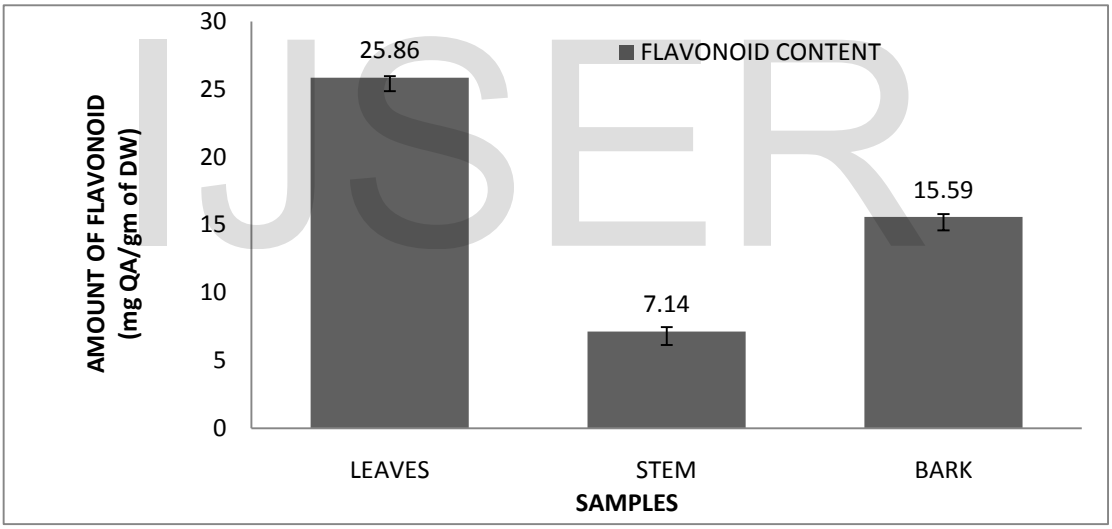

Fig 2. Graphical representation of total flavonoids content of different parts of *Moringa* sp.