Abstract

*Moringa oleifera* is a highly valued plant with a profile of important minerals and is a good source of protein, vitamins, β-carotene, amino acids and various phenolics. In the present study, methanolic extract of leaves and pods were analysed for phytoconstituents, antioxidant properties, nutrient, mineral and vitamin. The results of the antioxidant activities were moderate in comparison to the standard antioxidant and the leaf extract was superior to the pod in terms of antioxidant potentials. The IC_{50} values for DPPH radical scavenging activity were 150, 240 and 14 μg/ml for leaf, pod and standard respectively. Pod was a good source of carbohydrate, lipid, protein and amino acids as indicated by the results. The moisture, ash and crude lipid of leaves were 80.02%, 6.85% and 1.83% respectively; those of the pod were 74.06%, 7.18% and 2.32% respectively. The mineral composition unravels a high concentration of iron and calcium followed by sodium, potassium and magnesium. The present results revealed that, the leaves and pod contain an appreciable amount of nutrients and can be included indiets to supplement our daily nutrient requirements.

Key Words: *Moringa oleifera*- Bhagya KDM 01, antioxidant, DPPH, phytoconstituents, IC_{50}

Introduction

*Moringa oleifera* is the most widely cultivated species of a monogeneric family, the Moringaceae, which is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is also known as the horseradish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nebeday, saijhan, sajna or Ben oil tree. It is a perennial softwood tree and for centuries has been advocated for traditional medicinal and industrial uses. All parts of the *Moringa* tree are edible and have long been consumed by humans (1). The uses for *Moringa* in plenty as described by Fuglie (2): alley cropping (biomass production), animal forage (leaves and treated seed-cake), biogas (from leaves), domestic cleaning agent (crushed leaves), blue dye (wood), fencing (living trees), fertilizer (seed-cake), foliar nutrient (juice expressed from the leaves), green manure (from leaves), gum (from tree trunks), honey- and sugar cane juice-clarifier (powdered seeds), honey (flower nectar), medicine (all plant parts), ornamental plantings, bio pesticide (soil incorporation of leaves to prevent seedling damping off), pulp (wood), rope (bark), tannin for tanning hides (bark and gum), water purification (powdered seeds). *Moringa* seed oil (yield 30-40% by weight), also known as Ben oil, is a sweet non-sticking, non-drying oil
that resists rancidity. The plant has been advocated as an outstanding indigenous source of highly digestible protein, Ca, Fe, Vitamin C and carotenoids suitable for utilization in many of the so-called “developing” regions of the world where undernourishment is a major concern (1).

Recently, the pharmaceutical industries are facing many challenges favoring the use of plant natural products over the current chemo-clinical drugs available for the treatment of different diseases [3]. Developing cheaper, effective, new plant-based drugs with better bioactive potential and the fewest possible side effects is needed. Hence, attention has been directed toward biologically active extracts and compounds from plant species to fight against microbial diseases (4-9), as well as against degenerative diseases caused by free radicals (10).

A comparative study on antioxidant potentials in sprouts vs. seeds revealed that sprouts have higher antioxidant capacity compared to seed extracts (11). Plant derived drug serve as a prototype to develop more effective and less toxic medicines (4).

In the present study we reported phytoconstituents, nutrient composition and antioxidant properties in leaves and pods of Moringa oleifera - Bhagya KDM 01 variety.

**Material and Methods**

**Collection and Extraction of plant material:** The plant material was collected from breeders plot, washed and rinsed with water to remove all the dirt and unwanted particles and then macerated into small particles. For phytochemical screening and antioxidant studies, they were weighed and mixed with methanol and incubated for a week time. After one-week incubation the mixture was filtered using Whatman No. 1 filter paper and evaporated at room temperature. The dry material left off after evaporation was used for further studies. For nutrient analysis, extraction was followed with requisite modifications. The samples were dried at room temperature to remove residual moisture, then placed in paper envelope and oven-dried at 55°C for 24 hours (12). The dried samples were ground into powder using pestle and mortar and sieved through 20-mesh-sieve. The resulting powder was used for the nutrients analysis.

**Preliminary phytochemical screening:** The methanol extract of M. oleifera was screened for the presence of various phytoconstituents viz. steroids, alkaloids, glycosides, flavonoids, carbohydrates, amino acids, prote ins and phenolic compounds as described by Kokate et al. (13).

**Antioxidant activity**

**Total phenolic content estimation:** The total phenolic content was estimated according to the method of Makkar et al 1997 (14). The aliquots of the extracts were made up to the volume of 1ml with distilled water. Then 0.5ml of Folin-Ciocalteu reagent and 2.5ml of sodium carbonate solution (20%) were added. After mixing, solution was incubated at 90°C for one minute and the absorbance was recorded at 725nm against the reagent blank. Catechol is used to prepare the standard curve and total phenolic content of the extracts were expressed as catechol equivalent in µg/mg of extract.

**Total antioxidant capacity:** The total antioxidant capacity was measured by spectrophotometric method as described by Prieto et al. (15). A concentration ranging from 50-250 μg/ml of plant extract was added in an eppendorf tube with 1ml of reagent solution (0.6M H₂SO₄, 28mM sodium phosphate, 4mM ammonium molybdate mixture). The tubes were incubated for 90min at 95°C. The mixture was cooled to room temperature and the absorbance was read at 725nm against the reagent blank. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicates and values are expressed as equivalents of ascorbic acid in μg/mg of extract.

**DPPH radical scavenging assay:** The free radical scavenging activity of the extracts, based on the scavenging activity of the stable DPPH free radical, was determined by the method of Raghavendra et al.
described by Wong et al. [16]. Different concentrations ranging from 50-250 μg/mL of plant extract was added to 4 mL of a 0.004% methanol solution of DPPH. After 30 minutes of incubation at room temperature, the absorbance was recorded at 517 nm. A control reading was obtained using methanol instead of the extract. Ascorbic acid was used as the standard control and the percentage inhibition was calculated from the optical density of the treated and control samples using the following formula. The inhibition curves were prepared and IC<sub>50</sub> values were obtained.

\[
\% \text{ of inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100
\]

Where, \(A_0\) is the absorbance of the control (without test samples) and \(A_1\) is the absorbance of test samples.

Reducing power assay: The reducing power of the extracts was evaluated according to Oyaizu [17]. 1 mL solution of the extract (50-250 μg/mL) was mixed with equal volume of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide and placed in water bath at 50°C for 20 min. Then it was cooled rapidly and 1 mL of 10% trichloroacetic acid was added and vortexed, centrifuged at 800 g for 10 min and its 1.5 mL supernatant was mixed with equal volume of distilled water and 1 mL of 0.1% ferric chloride and left for 10 minutes incubation and absorbance was read at 700 nm. The reducing property of test sample was standardized against quercetin.

Nutrient analysis: The moisture, fiber, ash and crude fats, of the samples were determined by proximate analyses. Moisture was determined by oven dehydration method at 105°C up to the constant weight. Crude fat was determined by ether extraction method using soxhlet apparatus. Crude fiber was determined by acid digestion and alkali digestion method. Ash content was determined in muffle furnace at 550°C for 6 h. The protein was determined by Lowery’s method and total sugar was estimated by anthrone method and total amino acid was determined as described by Sadhashivam Manikham (18).

Determination of Minerals and Vitamins: For minerals determination 0.5 g of each sample was wet digested with HNO<sub>3</sub>: HClO<sub>4</sub> (2:1) for 2-3 h on heating mantle (19). Digested samples were filtered through 0.45 μm pore size Millipore filter and volume was made to 100 mL with distilled water. Concentration of Ca, Mg, Mn, Fe, and Zn, was determined on Atomic Absorption Spectrophotometer (Perkin Elmer AA Analyst 700) equipped with standard hollow cathode lamps as radiation source and air acetylene flames, while Na, K, and P concentration was determined on Flame Photometer. Vitamin C was determined in fresh samples by dichlorophenol Indophenol dye reduction method (20) and vitamin A and β-carotene was determined as described by Harold et al. (21).

Statistical analysis: All the experiments were carried out in triplicates. The result were pooled and expressed as mean ± standard error (SE). Base of the regression lines plotted for % inhibition versus concentration, inhibition concentration (IC 50) was calculated. The data were evaluated by one-way ANOVA and Microsoft office Excel 2007 software.

Results

Table 1. Results of qualitative phytochemical analysis of methanol leaves and pod extracts

<table>
<thead>
<tr>
<th>Tests</th>
<th>Leaves</th>
<th>Pods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amino Acids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Flavanoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins &amp; Polyphenols</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Preliminary phytochemical screening: Phytochemicals are in the strictest sense of the word chemicals produced by plants (1). The phytochemical examination revealed the Antioxidant properties in Moringa...
presence of sterols, alkaloids, tannins, saponins, flavonoids, phenols etc. in the methanol extract of the leaf and pod of Moringa the results are tabulated in table 1.

**Total phenolic content estimation:** The total phenolic content of the three leaf extracts were compared with standard curve of catechol ($y = 0.005x - 0.011$, $R^2=0.987$) and the results were expressed as the number of equivalents of catechol ($\mu$g/mg of extract). Among the two methanolic extracts, leaf extract of *M.oleifera* showed prominent total phenolic activity (54$\mu$g of catechol/mg of extract) when compared to pod extract (27.93$\mu$g of catechol/mg of extract). The results were presented in Fig 1.

**Total antioxidant capacity:** Both the leaf and pod methanol extracts showed a very potent total antioxidant capacity. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid ($\mu$g/ml of extract) ($y = 0.003x + 0.029$, $R^2=0.991$). The ascorbic acid equivalence of methanolic extract was shown in fig.2. Among the two extracts, total antioxidant capacity was found to be highest in leaves (55$\mu$g of ascorbic acid/mg of extract) followed by pod (22 $\mu$g of ascorbic acid/mg of extract).

**DPPH radical scavenging assay:** The comparison of the antioxidant property by DPPH
radical scavenging assay of the leaf and pod with that of the reference standard is depicted in Figure 3. The assay is based on the ability of an antioxidant present in the sample to decolorize DPPH free radical by virtue of their scavenging activities. The DPPH radical contains an odd electron that is responsible for the absorbance at 517 nm and also for the visible deep purple colour (19). The IC$_{50}$ value revealed the more effectiveness of the methanolic leaf extract (150µg·mL$^{-1}$) than the pod extract (240µg·mL$^{-1}$) comparable with the ascorbic acid (14µg·mL$^{-1}$). Both extracts exhibited a significant dose-dependent inhibition of DPPH activity.

**Reducing power assay:** The reducing power of a compound is related to its electron transfer ability and may serve as a significant indicator of its potential antioxidant activity [22]. The reducing power of the leaf and pod extract of *M. oleifera* increased in a dose dependant manner. The leaf extract was found to be remarkable than pod extract, which increased gradually with a rise in the concentration. The result of reducing power activity was shown in fig. 4.

**Nutrient analysis:** The nutrient profile of the leaves and pod of *Moringa* is given in Table 2.

![IC$_{50}$ values of DPPH radical scavenging activity of methanol extract of leaves and pods of *Moringa oleifera*](image)

![Reducing power activity](image)

**Fig. 3.** IC$_{50}$ values of DPPH radical scavenging activity of methanol extract of leaves and pods of *Moringa oleifera*

**Fig. 4.** Reducing power assay of methanol extract of leaves and pods of *Moringa oleifera*
values for moisture content showed leaves having highest value (80.02%) than in the pod (74.06%). The ash content of leaves (6.85%) was lower than that of the pod (7.18%). The high ash content is a reflection of the mineral contents preserved in the food materials. The results therefore suggest a high deposit of mineral elements in the pod. Crude fat content of leaves (1.83%) were lower when compared to that of the pod (2.32%). The high concentration of fiber were accounted in pod (21.96%) rather in leaves (18.43%) and this makes it a more favorable vegetable since high fiber content of foods help in digestion and constipation. Nitrogen containing substances are found in fruits in different combinations: proteins, aminoacids, amides, amines, nitrates, etc. Among nitrogen-containing substances proteins and amino acids are most important (23). Pod had a higher protein (70 mg-g) and amino acid (40 mg-g) content than leaves (65 mg-g and 30 mg-g respectively) and this makes pod a good source of protein and amino acid when compared leaves. Carbohydrates are the source of energy required for various activities. In the present study the estimated sugar content in pod (130 mg-g) stood to be higher than that for leaves (104 mg-g).

**Mineral and Vitamin analysis:** The mineral profile estimated in the present study is shown in Table 2. The results unravel that leaf is rich source of all these minerals than the pod that promotes wellbeing in humans. Of the two samples leaves had a very high iron and calcium content with the pod sample having relatively lower. The high value of iron in these leaves makes them an additional source of iron. Calcium helps to build up strong bones and teeth so their consumption can add to daily calcium requirements of each individual. Sodium, potassium and phosphorus are important for

<table>
<thead>
<tr>
<th>Sl no</th>
<th>Parameters</th>
<th>Leaves</th>
<th>Pods</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Moisture (%)</td>
<td>80.02±0.01</td>
<td>74.06±0.01</td>
</tr>
<tr>
<td>02</td>
<td>Ash (%)</td>
<td>6.85±0.23</td>
<td>7.18±0.19</td>
</tr>
<tr>
<td>03</td>
<td>Crude fat (%)</td>
<td>1.83±0.17</td>
<td>2.32±0.16</td>
</tr>
<tr>
<td>04</td>
<td>Crude fiber (%)</td>
<td>18.43±0.28</td>
<td>21.96±0.23</td>
</tr>
<tr>
<td>05</td>
<td>Sugar (mg-g)</td>
<td>104.43±0.61</td>
<td>130.57±0.68</td>
</tr>
<tr>
<td>06</td>
<td>Protein (mg-g)</td>
<td>65.71±0.32</td>
<td>70.64±0.21</td>
</tr>
<tr>
<td>07</td>
<td>Total amino acid (mg-g)</td>
<td>30.42±0.12</td>
<td>40.54±0.16</td>
</tr>
<tr>
<td>08</td>
<td>β-Carotene (mg-g)</td>
<td>0.32±0.02</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td>09</td>
<td>Calcium (mg-g)</td>
<td>3.41±0.07</td>
<td>0.74±0.07</td>
</tr>
<tr>
<td>10</td>
<td>Potassium (mg-g)</td>
<td>2.21±0.03</td>
<td>3.28±0.01</td>
</tr>
<tr>
<td>11</td>
<td>Sodium (mg-g)</td>
<td>2.43±0.02</td>
<td>3.40±0.03</td>
</tr>
<tr>
<td>12</td>
<td>Phosphorus (mg-g)</td>
<td>3.21±0.04</td>
<td>2.16±0.03</td>
</tr>
<tr>
<td>13</td>
<td>Magnesium (mg-g)</td>
<td>0.50±0.01</td>
<td>0.49±0.01</td>
</tr>
<tr>
<td>14</td>
<td>Iron (µg-100g)</td>
<td>31.35±0.23</td>
<td>10.68±0.21</td>
</tr>
<tr>
<td>15</td>
<td>Copper (µg-100g)</td>
<td>7.30±0.08</td>
<td>5.73±0.07</td>
</tr>
<tr>
<td>16</td>
<td>Zinc (µg-100g)</td>
<td>21.24±0.37</td>
<td>18.53±0.41</td>
</tr>
<tr>
<td>17</td>
<td>Manganese (µg-100g)</td>
<td>56.72±0.31</td>
<td>48.52±0.27</td>
</tr>
<tr>
<td>18</td>
<td>Vit C Content (mg-g)</td>
<td>0.75±0.03</td>
<td>0.69±0.03</td>
</tr>
<tr>
<td>19</td>
<td>Vit A Content (IU-100g)</td>
<td>5.51±0.05</td>
<td>1.96±0.04</td>
</tr>
</tbody>
</table>
chemical reaction within the cells and regulate the transfer of nutrients to the cells. Sodium works in conjunction with potassium for extracellular fluid balances (24). Magnesium, manganese, copper and zinc was yet another mineral assessed (Table 4) which is important for any biochemical process in an organism, promotes balancing of minerals and it is necessary for normal job of muscles, and nervous (24) system, activity of hormones, manufacture of energy maintenance of health of reproductive system, immune system and regulation of an intimate rhythm and arterial pressure together with calcium (25).

Results of vitamins C, A and \( \beta \)-Carotene are also shown in Table 4. Maximum concentration of these constituents was found in leaf than in pod implicating that leaves are good source than the pod. The concentration of Vitamin C, A and \( \alpha \)-Carotene in leaf were 0.75mg/g, 5.51IU/100g and 0.32mg/g respectively and in the pod their concentrations were 0.69mg/g, 1.96IU/100g and 0.28mg/g respectively. Vitamin C (Ascorbic acid) is watersoluble vitamin required in high amount, as its loss is frequent from body. It participates in reversible oxidation-reduction system. Vitamin C prevents scurvy disease and also aids in the formation of folic acid derivatives, which are essential for DNA synthesis. Similarly, Vitamin A is necessary for a variety of functions such as vision, proper growth and differentiation, reproduction and maintenance of epithelial cells.

Discussion

Plants are very rich source of essential biochemical and nutrients such as carbohydrates, carotene, vitamins, calcium, iron, ascorbic acid, and palpable concentrations of trace minerals (26). A diet providing 1-2% of its caloric energy as fat is said to be sufficient to human beings, as excess fat consumption yields to certain cardiovascular disorders such as atherosclerosis, cancer and aging (27) and the fibre RDA nutritional purposes, considering to the amount and values for children, adults, pregnant and breast-feeding mothers are 19-25%, 21-38%, 28% and 29% respectively (28). The high value of carbohydrate and protein suggest its nutritional quality and this may be a veritable tool been used as source of body nourishment (29).

It is estimated that 70 biological trace elements are needed by all living things for the normal function of their metabolism, reproductive and immune system[30]. There has been a great emphasizing on the important roles of mineral and trace elements to human health and wellbeing. The presence of phytochemicals, in addition to vitamins and provitamins, have crucial nutritional importance in the prevention of chronic diseases, such as cancer, cardiovascular disease, and diabetes (31,32). Phytoconstituents like flavonoids, steroids and tannins are found to have analgesic and anti-inflammatory effects (33). Children, women of reproductive age and pregnant women are most vulnerable to micronutrient deficiency and anemia (34). Hence, they need food with high content of these minerals and vitamins.

Total antioxidant capacity by phosphomolybdenum method is based on the reduction of Mo VI to Mo V by the sample analyte and the subsequent formation of green P/Mo V complex at acidic pH. The phosphomolybdenum method is quantitative since the total antioxidant activity is expressed as the number of equivalents of ascorbic acid (15). A comparable study on the antioxidant capacity by the phosphomolybdenum method was carried out by several researchers. [35-38]. On the basis of results of three assays viz, DPPH, reducing power and total antioxidant capacity, the methanolic extract of leaf was found to be superior to the pod.

DPPH has long been recognized as a convenient reagent to quantify antioxidants in complex biological systems and has been widely used for this purpose. DPPH, a commercially available radical serves as the oxidizing radical to be reduced by the antioxidant and as the indicator for the reaction. The comparison of the results among the methanolic extract of leaf and pod have recorded moderate antioxidant...
activities. Similar results were obtained by several investigators by using the DPPH assay to study antioxidant capacity of the medicinal plants for their relative antioxidant property. (39-42).

The reducing ability of a compound generally depends on the presence of reductants, which have been exhibited antioxidative potential by breaking the free radical chain (Fe$^{3+}$ was transformed to Fe$^{2+}$), by donating a hydrogen atom. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reductive ability was measured in terms of Fe$^{3+}$ to Fe$^{2+}$ transformation in the presence of different concentrations of the methanolic extract of leaf and pod. Potent antioxidant capacity in terms of reducing capacity therefore can be attributed to reductones in the extracts because reducing properties are generally associated with the presence of reductones. The results were in conformity with observations of several investigations. (37, 43-45).

Conclusion

The leaf and pod methanol extracts of *M. oleifera*-Bhagya KDM 01 variety showed a moderate antioxidant activity in terms of DPPH, reducing power and total antioxidant capacity and yet these can be used as an easily accessible source of natural antioxidant however, the phytocons-tituents responsible for the antioxidant activity are not much clear. The nutritional analysis shown that pod is a good source of carbohydrate, lipid, protein and amino acids. Leaves are good sources of minerals and vitamin A and C. The results suggest that the leaves and pod if consumed insuffi cient amount could contribute greatly towards meeting human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition. From the result, the leaves and pods are recommended for continuous use for nutritional purposes, considering to the amount and diversity of nutrients they contain. The data confined in the present report is in the proximity with the earlier studies conducted in other varieties of *Moringa*. Chemical analysis alone, however, should not be the exclusive criteria for judging the nutritional significance of a plant part. Thus, it becomes necessary to consider other aspects such as presence of antinutritional/toxicological factors and biological evaluation of nutrient content.

Competing interests: The authors have no conflict of interests to declare.

References


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Antioxidant properties in Moringa


