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# Nutrient Content, Mineral Content and Antioxidant Activity of *Amaranthus viridis* and *Moringa oleifera* Leaves

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## ABSTRACT

This study discusses the nutrient composition and the nutraceutical importance of green leaves and wild gathered foods in an area with surplus food production in Kanpur. The study presents a nutrition composition and antioxidant activity of *Amaranthus viridis* whole herb and *Moringa oleifera* leaves for their nutraceutical value. Levels of some nutrients in *Moringa oliefera* leaves and *Amaranthus viridis* whole herb were determined using standard analytical methods. In *M. oleifera* leaves, crude protein was 20.51%, crude fiber 19.25%, crude fat 2.63%, ash content 5.13%, moisture content 71.73%, carbohydrate content 43.78% and the calorific value 430.41 kcal. For *A. viridis* crude protein was 2.11%, crude fiber 1.93%, crude fat 0.47%, ash content 1.85%, moisture content 87.90%, carbohydrate content 7.67% and the calorific value 43.35 kcal. The elemental analysis of the leaves in mg/100 g dry matter (DM) reveals the calcium and iron content of *M. oleifera* 2007.67 and 26.34, respectively. Leaves of *A. viridis* contained Calcium 330 mg/100 g, Fe 18.2, Mg 1842, P 52, K 3460, Na 108, Zn 10, Cu 300, Mn 8, Se 1.98 and Cr 0.92 mg. The antioxidant activity, $IC_{50}$ μg $mL^{-1}$ (DPPH method) of *M. oliefera* leaves and *A. viridis* herb was found to be 49.86 and 28.92, respectively. The study concludes that selected plant samples are an important source of proteins, crude fiber, carbohydrates, energy and minerals. The plants contain an appreciable amount of nutrients and can be included in diets to supplement our daily nutrient needs and to fight against many of the diseases as nutraceuticals.

**Key words:** Nutraceutical, chemical analysis, antioxidant activity, DPPH method

## INTRODUCTION

Herbs not only provide us chemicals of medicinal value but also provide us nutrition and trace elements. Minerals and trace elements are chemical elements required by our bodies for numerous biological and physiological processes that are necessary for the maintenance of health. Vegetables are important sources of protective foods, which are highly beneficial for the maintenance of good health and prevention of diseases (Sheela *et al*., 2004). Much effort has been concentrated on seeds while leafy vegetables have to large extent been ignored. They are known as potential sources of minerals and vitamins (Ifon and Basir, 1979). The 30 to 40% of today's conventional drugs used in the medicinal and curative properties of various plants are employed in herbal supplement botanicals, nutraceuticals and drug (Schulz *et al*., 2001). The nutritive value of plant plays great role in plant and human being, so material extracted from the natural plant through chemical or biotechnology Method (Chapman, 1967). Natural products with antioxidant activity may be used

to help the human body to reduce oxidative damage. Many herbs, fruits and vegetables have been investigated for their antioxidant activities in the last years (Dimitrios, 2006). Dietary sources have been recognized as safe and effective antioxidants in terms of their efficiency and non-toxicity (Block *et al*., 1992). *Moringa oleifera* commonly known as (family: Moringaceae) horse radish tree or drumstick tree is both nutritional and medicinal with some useful minerals, vitamins, amino acids, etc. (Ramachandran *et al*., 1980). A native of the sub-Himalayan regions of North West India *Moringa oleifera* is indigenous to many countries in Africa, Arabia, South East Asia, the Pacific, Caribbean Islands and South America (Nadkarni, 1976). Almost all the parts of this plant root, bark, gum, leaf, fruit (pods), flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal, hematological and hepatorenal disorders (Morimitsu *et al*., 2000). The flowers and roots are used in folk remedies, for tumors, the seeds for abdominal tumors, leaves applied as poultice to sores, rubbed on temples for headaches and are said to have purgative properties (Anwar *et al*., 2007). *Moringa oleifera* is called as Miracle Vegetable" because it is both a medicinal and a functional food (Verma *et al*., 1976).

*Amaranthus viridis* L. (Amaranthaceae), commonly called 'Choulai' in Hindi, has been used in Indian and Nepalese traditional system to reduce labor pain and act an antipyretic (Kirtikar and Basu, 1987). The Negritos of the Philippines apply the bruised leaves directly to eczema, psoriasis and rashes etc. (Quisumbing, 1951). Other traditional uses range from an anti-inflammatory agent of the urinary tract, venereal diseases, vermifuge, diuretic, antirheumatic, antiulcer, analgesic, antiemetic, laxative, improvement of appetite, antileprotic, treatment of respiratory and eye problems, treatment of asthma (Anonymous, 1988; Arshad and Khan, 2000). Furthermore, the plant possesses antiproliferative and antifungal properties as well as ribosome inactivating protein, β-carotene (Kaur *et al*., 2006; Sena *et al*., 1998) and antiviral activities (Obi *et al*., 2006). In addition the whole plant possesses analgesic and antipyretic properties and is used for the treatment of pain and fever, respectively in traditional systems of medicine (Yusuf *et al*., 1994). The fact that green leafy vegetables play important role in fighting diseases it is not consumed frequently. Based on this, the present study attempts to reveal the nutritional composition of *Amaranthus viridis* and *M. oliefera* and their suitability as nutraceuticals.

## MATERIALS AND METHODS

The leaves of *M. oliefera* were collected from university Campus and *A. viridis* herb was purchased from local market in the month of November, 2010. The authentication of plants was done from National Botanical Research Institute, Lucknow, India (NBRI-SOP-202). The plants and leaves were washed with de-ionized water and disinfected with 0.1% $HgCl_2$ for five minutes and shade dried. The washed and dried material was ground to fine powder using grinder. Moisture, protein, fat, fiber and ash analysis were conducted on ground fresh leaves. All analyses were conducted in duplicate and results were based on fresh weight per 100 g of sample. The dried samples were stored in a dark cupboard in capped bottles in desiccators and used within 1 month after harvesting.

## CHEMICAL ANALYSIS

For the chemical analysis, aliquots were made from 0.5 g of fresh weight from each sample analyzed. Two replicates were made from separate aliquots. Energy was calculated (kcal/100 g fresh weight) as described by the WHO (1985) by multiplying the values obtained for protein, carbohydrates and fat by 4.00, 3.75 and 9.00, respectively; the results are

expressed in kcal. Moisture, ash, crude protein, fat and dietary fiber were analyzed by the reported methods. Moisture was determined using the drying oven method, by drying a representative 5 g sample in an oven at 105°C for 3 h. Ash content was determined by the incineration of a sample (4 g) in a muffle furnace at 600°C for 6 h until the ash turned white. Crude protein was estimated by the Kjeldahl method. Total protein calculated by multiplying the evaluated nitrogen by 6.25. Fat was determined by petroleum ether extraction in a soxhlet apparatus. A representative 3 g of sample was extracted for 6 h. Dietary fiber was analyzed by an enzymatic gravimetric method. Carbohydrates (g/100 g) were estimated by using a difference method of Anonymous (1990), by subtracting the sum of the percent of protein, moisture, fat and ash from 100. Mineral elements (calcium, copper, iron, magnesium, manganese, zinc, sodium, selenium, chromium and phosphorus) determined in homogenized samples. Three replicate aliquots (approximately 0.5 g) from each of the homogenized plant specimens were weighed and 3 mL concentrated nitric acid, 1 mL concentrated hydrogen peroxide were added. Each vessel was closed with cover and kept in microwave oven for digestion. The digested contents from the vessels were transferred into 50 mL flasks and the volume was made up using double deionized water (FAO, 1985). Concentrations were determined with spectrometer. Samples of respective mineral solutions were quantified against standard solutions of known concentration that were analyzed.

## ANTIOXIDANT ASSAY

**Qualitative analysis:** The plants extract were applied on a TLC plate as a spot (100 μg $mL^{-1}$) for chromatographic separation of the extract using the mobile phase Methanol: Chloroform (95:5, v/v) and were allowed to develop the chromatogram for 30 min. After completion of the chromatogram the whole plates were sprayed with DPPH (0.15 % w/v) solution using an atomizer. The color changes (yellowish color development on pinkish background on the TLC plate) were noted as an indicator of the presence of antioxidant substances.

**Quantitative analysis:** The free radical scavenging capacity of the extracts was determined using DPPH method (Braca *et al*., 2001; Viturro *et al*., 1999). DPPH solution (0.004% w/v) was prepared in 95% methanol. Extract of the plants were mixed with 95 % methanol to prepare the stock solution (5 mg $mL^{-1}$). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and extracts were added to test tube so that the final volume was 3 mL and after 10 min, the absorbance was read at 515 nm using a spectrophotometer (Shimadzu- Pharmaspec-1700 UV-visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (5 mg $mL^{-1}$). Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol served as blank. The % Scavenging of the DPPH free radical was measured by using the following equation:

$$\% \text{ Scavenging activity} = \frac{\text{Abs. of control} - \text{Abs. of test sample}}{\text{Abs. of control}} \times 100$$

## RESULTS

**Chemical composition:** *A. viridis* yielded 43.35 kcal/100 g and *M. oliefera* 430.41 kcal/100 g. Moisture content in *A. viridis* and *M. oliefera* was 87.90 and 71.73±0.02%, respectively. Crude protein content ranged from 2.11% in *A. viridis* and 20.51±0.01% in *M. oliefera*. Crude fat content ranged from 0.47% in *A. viridis* and 2.63±0.03 % in *M. oliefera*. Crude fiber content ranged from

Table 1: Nutritional composition of *A. viridis* and *M. oliefera*

| Sample | Energy (kcal) | Moisture (%) | Protein (%) | Fat (%) | Fibre (%) | Ash (%) | Carbohydrate (%) |
|---|---|---|---|---|---|---|---|
| *A. viridis* | 43.35 | 87.90 | 2.11 | 0.47 | 1.93 | 1.85 | 7.67 |
| *M. oliefera* | 430.41 | 71.73 | 20.51 | 2.63 | 19.25 | 5.13 | 43.78 |

Table 2: Mineral content of *A. viridis* and *M. oliefera*

| Sample | Ca | P | K | Na | Mn | Cu | Zn | Mg | Iron | Cr | Se |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *A. viridis* (mg/100 g) | 330 | 52 | 3460 | 108.0 | 8 | 300 | 10 | 1842 | 18.2 | 0.92 | 1.98 |
| *M. oliefera* (mg/100 g) | 2007.67 | 123.7 | 1732 | 129.3 | 7.68 | 0.825 | 2.59 | 1896.2 | 26.34 | 0.42 | 2.87 |

Table 3: Comparison of antioxidant activity

| Samples | $IC_{50}$ (μg $mL^{-1}$) |
|---|---|
| *A. viridis* methanol extract | 28.92±0.03 |
| *M. oleifera* methanol extract | 49.86±0.02 |
| Ascorbic acid standard | 56.44±0.01 |

1.93 in *A. viridis* and 19.25±0.05% in *M. oliefera*. Ash content ranged from 1.85 in *A. viridis* and 5.13±0.03% in *M. oleifera*. The carbohydrate content of samples ranged from 7.67 in *A. viridis* and 43.78±0.02% in *M. oleifera* (Table 1).

**Mineral content:** Mean values for mineral content of nutritional importance are shown in Table 2.

**Antioxidant activity:** A comparison of the antioxidant activity ($IC_{50}$) of the methanol extracts of *A. viridis* and *M. oliefera* along with Ascorbic acid is shown in Table 3. High levels of antioxidant activity were noticed in *M. oliefera* (49.86 μg $mL^{-1}$) but lesser level in *A. viridis* (28.92 μg $mL^{-1}$).

## DISCUSSION

The ash content of *M. oleifera* leaves was higher than that of the *A. viridis*. The high ash content of the *M. oleifera* leaves is a reflection of the mineral contents preserved in the food materials. The results therefore, suggest a high deposit of mineral elements in the leaves. The ash was subjected to acid digestion and analyzed for mineral content (Table 2).

Crude fat content of *A. viridis* (0.47%) were lower when compared to that of the Moringa leaf (2.63%). A diet including *M. oleifera* should be more palatable than that with *A. viridis* because dietary fats function to increase food palatability by absorbing and retaining flavours (Lindsay, 1996). 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 (Davidson *et al*., 1975).

The protein content of *M. oliefera* (20.51%) was quite high as compared to *A. viridis* (2.11%). This makes the *M. oliefera* leaves to be a good source of proteins. The crude fiber content of *M. oliefera* leaves was higher (19.25%) than that of *A. viridis* (1.93) and this makes it a more favorable vegetable since high fiber content of foods help in digestion and prevention of colon cancer (Saldanha, 1995). Non-starchy vegetables are the richest sources of dietary fiber and are employed in the treatment of diseases such as obesity, diabetes and gastrointestinal disorders (Agostoni *et al*., 1995).

The caloric value obtained shows that *A. viridis* is having the lower value (43.35 kcal) while *M. oliefera* (430.41 kcal) shows higher calorific value. The calorific value of the plants make them good source of energy. In addition the lower calorific value of *A. viridis* makes it good in the diet of the obese. Minerals are important in the diet because they serve as cofactors for many physiologic and metabolic functions. The biological effects of the trace elements in living system strongly depend upon their concentration and thus should be carefully controlled especially when herbs and drugs are used in human (Jacob, 1994). Cr is implicated in maintenance of blood sugar, prevention of arteriosclerosis and control of cholesterol levels. Human studies suggest that chromium picolinate enhances insulin sensitivity, glucose removal and may improve lipid ratios in obese and type 2 diabetics (Cefalu *et al*., 2002). It is also suggested that Cr has a potential beneficial antioxidant effect in patients with type 2 diabetes when combined with Zn and Cu supplementation (Anderson *et al*., 2001). Mn is a component of several enzymes including manganese-specific glycosyltransferase and phosphoenol pyruvate carboxykinase and essential for normal bone structure. Mn deficiency can manifest as transient dermatitis, hypocholesterolemia.

Cu is universally important cofactor for many hundreds of enzymes. It functions as a cofactor and activator of numerous enzymes which are involved in development and maintenance of the cardiovascular system. A Cu deficiency can result in a decrease in the tinsel strength of arterial walls, leading to aneurysm formation and skeletal maldevelopment (Tilson, 1982).

Selenium is a trace mineral that is a component of glutathione peroxidase. Reduced glutathione plays many roles, the most important of which is as an antioxidant, protecting cells and tissues against harmful reduced oxygen metabolites (Rotruck *et al*., 1973).

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva *et al*., 2002).

The $IC_{50}$ value of *M. oliefera* methanol extract 49.86 μg $mL^{-1}$, as opposed to that of ascorbic acid ($IC_{50}$ 56.44 μg $mL^{-1}$) which is a well-known antioxidant.

The high protein content of *M. oleifera* leaves with a fairly high concentration of calcium, Iron and *A. viridis* with fairly high concentration of potassium, copper and iron; make it a potential nutraceutical that is suitable for fortification of foods. These plant organs might be explored as a viable supplement and a ready source of dietary minerals in human food to fight various diseases.

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