

## Proximate and Phytochemical Composition of *Moringa oleifera* Leaves at Three Stages of Maturation

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**Abstracts:** The aim of the study is to determine the proximate and anti-nutrient composition of dried leaves of *Moringa oleifera* harvested at different stages of the plants maturity. The leaves were harvested at three period of time viz: 10th (early stage), 15th (Mid stage) and 20th (late stage) week after pruning. The result of the proximate analysis revealed that the early stage has the highest carbohydrate content (55.14%). Mid stage recorded the highest moisture (6.3%) while the late stage has highest protein content (28.08%), crude fiber (10.11%), ash (9.25%), fat (2.5) and pH (6.27). The Phytochemicals detected in both aqueous and methanolic extracts of each of the different stage of leaves maturation were the same except for tannin which was present in aqueous extract but was not detected in methanolic extracts. The detected antinutrients are alkaloids, tannins, phenolics, saponins, flavonoids, steroids while phylobatanin and triperthenes were not. All these results indicate that the leaves harvested at the late stage had a higher percentage of imbedded nutrients than others, it was therefore concluded that harvesting at that stage is recommended. The presence of some phytochemicals like saponins and flavonoids explained the medicinal action of the plant encountered in its therapeutic uses.

**Key words:** Aqueous, extracts, flavonoids, methanolic, nutrition, saponins

### INTRODUCTION

Increased vegetable utilization and consumption are critical to alleviate world-wide incidence of nutritional deficiencies. Our survey and investigations on some plants indicated that *Moringa oleifera* is one of the promising plants which could contribute to increased intake of some essential nutrients and health-promoting phytochemicals. Phytochemicals are present in virtually all of the fruits, vegetables, legumes (beans and peas), and grains we eat, so it is quite easy for most people to include them in their diet. *Moringa oleifera*, or the horseradish tree, is a pan-tropical species that is known by such regional names as benzolive, drumstick tree, kelor, marango, saijhan, and sajna (Fahey, 2005). It is the most widely cultivated species of a monogeneric family, the Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. Today it has become naturalized in many locations in the tropics and is widely cultivated in Africa, Ceylon, Thailand, Burma, Singapore, West Indies, Sri Lanka, India, Mexico, Malabar, Malaysia and the Philippines (Fahey, 2005). It is one of the newly discovered vegetable which is gaining wide acceptance in Nigeria (Anonymous, 2006). It is widely grown and cultivated in the northern part of Nigeria where it is locally called Zogeli (among the Hausa speaking people). *M. oleifera* can be grown in a variety

of soil conditions preferring well-drained sandy or loamy soil that is slightly alkaline (Abdul, 2007; Anjorin *et al.*, 2010). It is considered one of the world's most useful trees, as almost every part of the tree can be used for food, or has some other beneficial properties. The leaves, especially young shoots, are eaten as greens, in salads, in vegetable curries, and as pickles. The leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value. The leaves are considered to offer great potential for those who are nutritionally at risk and may be regarded as a protein and calcium supplement (Rajangam *et al.*, 2001). It is a general belief that the stage of maturity of plant affects the concentration of nutrients of leaves, thus it is very important to choose a suitable stage of harvesting (Yu *et al.*, 2004). In order to contribute to the growing body of knowledge on this subject, the present study analyzed the proximate and phytochemical constituents of three different stages of maturity of *Moringa Oleifera* tree harvested after ten (10)-Early stage, fifteen (15)-Mid stage and twenty (20)-Late stage weeks of pruning.

### MATERIALS AND METHODS

**Collection and preparation of samples:** The research was conducted between September and December, 2010.

The leaves of *Moringa oleifera* were harvested from our institute's premises in Kano, Kano State, Nigeria. The leaves were harvested at three period of time viz: 10th, 15th and 20th week after pruning. Each leaf was destalked, washed and air-dried at average room temperature of 75% and 31°C. Continuous turning of the leaves was done to avert fungal growth for two week to constant weight. They were kept away from high temperatures and direct sun light to avoid destroying active compounds.

The dried leaves were ground to fine powder using a Warring 240v 4L blender, Thomas scientific, Swedesborn, U.K.

The aqueous extract of each sample was prepared by soaking 100 g of dried powdered samples in 500 mL of distilled water for 12 h. The filtrate was used for phytochemical screening. The methanolic extracts of the leaves of each *Moringa oleifera* was prepared using the same method for the aqueous extract, but methanol was the solvent used instead of distilled water. Proximate analyses were carried out on the dried samples of leaves of *Moringa oleifera*.

**Proximate analysis:** Proximate analyses were carried out according to the procedure of Association of Official Analytical Chemist (AOAC, 1990).

**Phytochemical screening:** Phytochemical screening procedures carried out were adapted from the previous work on plant analysis (Odebiyi and Sofowora, 1979). This analysis determines the biologically active non-nutritive compounds that contribute to the flavor, colour and other characteristics of plant parts. Examples of these are alkaloids, tannins, hydroxyanthraquinones, phenolics steroids, saponins, flavonoids and phlobatannins.

**Reagents and apparatus:** All the reagents and chemicals used were of analytical grade obtained from BDH and Sigma-Aldrich. Distilled water and acid washed glassware were used throughout the analysis.

## RESULTS AND DISCUSSION

The results (Table 1) obtained from proximate analysis of the *Moringa* leaves establishes that they can be ranked as carbohydrate rich leaves due to their relatively high content when compared with the other components of the leaves.

Moisture content ranges from 6.0-6.3%. No significant difference was recorded. The low moisture content of the leaves would hinder the growth of micro-organisms and the storage life would be high (Adeyeye and Ayejuyo, 1994). The ash content is relatively low (5.75-9.25%) especially at the early stage, this indicates that at this stage some minerals are not yet fully

established and which may not be available to man. There was no significant increase ( $p>0.05$ ) between the mid and late stage but with a corresponding decrease in the early stage of maturity. The ash content of the leaves were lower than that of some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* (20.05%), *Acalypha marginata* (15.68%) but they compare favorably with some other vegetables such as *Occimum gratissimum* (8.00%) and *Hibiscus esculentus* (8.00%) (Akindahunsi and Salawu, 2005). The crude protein content of the leaves is 27.61, 23.70 and 28.08% for early stage, mid stage and late stage of maturity respectively. They compared favourably with *Amaranthus caudatus* (20.59%) cassava leaves (*Manihot utilisima*), 24.88%, *Piper guineenses* 29.78% and *Talinum triangulare* 31.00% (Etuk *et al.*, 1998; Akindahunsi and Salawu, 2005).

The moderate amount of fat at each stage of maturity indicates that the vegetable is not a source of lipid accumulation which can cause arteriosclerosis, aging (Antia *et al.*, 2006). It is very low when compared to *Talinum triangulare* (5.90%), *Baseila alba* (8.71%), *Amaranthus hybridus* (4.80%), *Calchorus africanum* (4.20%), *Acalypha racemosa* (6.30%) (Ifon and Bassir, 1979; Akindahunsi and Salawu, 2005). The high protein and low fat characteristic of *Moringa* leaves has been previously reported by Makkar and Becker (1996). These results are in agreement with the values reported by Oduro *et al.* (2008) and Fuglie (2001).

There was no significant difference in the crude fiber content of the three stages of maturation. *Moringa Oleifera* has higher crude fiber content than *Cnidioscolus chayamansa* (0.92%), *Solanium nodiflorum* (0.78%) *Senecio bialfrae* (0.92%) but compare with *Acalypha hispada* (10.25%), *Acalypha racemosa* (7.20%) and *Acalypha marginata* (11.50%).

The carbohydrate content is very high with no significant difference between the Early stage and Mid stage with a significant decrease in Late stage, this shows that as the plant grow older, the carbohydrate content reduces and the protein content increase.

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties, they are found generally in plants (Phytochemicals.info). The Phytochemical screening in Table 2 and 3 showed that all leaves contain phenolics, Tannins, Alkaloids, Saponins, Flavonoids, Steroid and does not contain phylobatanin, and triperthenes. The findings in this study agree with earlier studies which also found that, not all phytochemicals are present in all plant parts and that those present differ according to the type of the extracting solvent used (Tijjani *et al.*, 2009; Ayinde *et al.*, 2007). Generally, It was also observed that at all the stages of maturity for both methanolic and aqueous extract, they are either positive or negative equally except for tannins

Table 1: Proximate composition of different stages of maturity of *Moringa oleifera* leaves

Stage of maturation	Moisture (%)	Protein (%)	CHO (%)	Crude fibre (%)	Ash (%)	pH	Fat (%)
Early stage	6.0±0.03 <sup>a</sup>	27.61±1.91 <sup>ab</sup>	55.14±0.22 <sup>c</sup>	9.80±1.45 <sup>a</sup>	5.75±0.21 <sup>a</sup>	6.01	1.5±1.31 <sup>a</sup>
Mid stage	6.3±2.01 <sup>a</sup>	23.7±0.12 <sup>b</sup>	53.53±1.21 <sup>b</sup>	8.20±0.01 <sup>b</sup>	8.00±0.02 <sup>b</sup>	6.12	2.0±1.32 <sup>a</sup>
Late stage	6.1±1.79 <sup>a</sup>	28.08±2.75 <sup>a</sup>	47.09±0.06 <sup>a</sup>	10.11±0.27 <sup>a</sup>	9.25±0.06 <sup>b</sup>	6.27	2.5±1.21 <sup>a</sup>

Each value is a mean of four different determination ± Standard deviation

Table 2: Phytochemical constituents of aqueous extracts of different stages of maturity of *Moringa oleifera* leaves

Stage of maturation	Alkaloids	Tannins	Phenolics	Saponins	Flavonoids	Steroids	Phylobatanin	Tripertenes
Early stage	+	+	+	+	+	+	-	-
Mid stage	+	+	+	+	+	+	-	-
Late stage	+	+	+	+	+	+	-	-

+: Detected; -: Not Detected

Table 3: Phytochemical constituents of methanolic extracts of leaves of different stages of maturity of *Moringa oleifera* leaves

Stage of maturation	Alkaloids	Tannins	Phenolics	Saponins	Flavonoids	Steroids e	Phylobatanin	Tripertenes
Early stage	+	-	+	+	+	+	-	-
Mid stage	+	-	+	+	+	+	-	-
Late stage	+	-	+	+	+	+	-	-

+: Detected; -: Not Detected

which was positive for aqueous extract but was negative for methanolic extract.

Flavonoids are widely distributed in plants fulfilling many functions. They have been shown to have anti-fungal activity *in vitro* (Galeotti *et al.*, 2008). The potent antioxidant activity of flavonoids reveals their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals, this may be the most important function of flavonoids (Alan and Miller, 1996).

The presence of Flavonoids at all stages of maturity of *Moringa oleifera* may be responsible for the medicinal qualities accorded the leaves. They also induce mechanisms that may kill cancer cells and inhibit tumor invasion (Williams *et al.*, 2004).

Saponins were also detected in *Moringa oleifera* leaves and they have been shown to possess both beneficial (cholesterol lowering) and deleterious (cytotoxic; permeabilization of the intestine) properties (Price *et al.*, 1987, Oakenful and Sidhu, 1989). Although some saponins have been shown to be highly toxic under experimental conditions, acute poisoning is relatively rare both in animals and man (Osagie, 1988). Studies have illustrated the beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. Due to its ability to form froth, soap is being produced locally from it for bathing.

*M. oleifera* leaves also contain alkaloids which are nitrogen-containing naturally occurring compound, commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms (Kasolo *et al.*, 2010). This could be responsible for their much acclaimed medicinal values though the exact mode of action is poorly understood.

The presence of tannins in the leaves also agreed with the works of Kasolo *et al.* (2010) who also discovered

traces of tannins in the leaves grown in Uganda. Tannins are polyphenols that are obtained from various parts of different plants belonging to multiple species. Tannins can also be effective in curbing hemorrhages as well as restrict bare swellings. While tannins are proved haemostatic, they are also beneficial when applied on mucosal coating in mouth. Hence, herbs possessing tannins are widely used as mouthwashes, eyewashes, snuff and even as vaginal douches and also treat rectal disorders (Elvin-lewis *et al.*, 1977). When applied internally, tannins affect the walls of the stomach and other digestive parts. They sour the mucus secretions and contract or squeeze the membranes in such a manner that secretions from the cells are restricted. Long-term and/or excessive use of herbs/vegetables containing high concentrations of tannins is not recommended (Reed, 1995). Steroid's presence in moringa leaves is of great importance as they are of interest in pharmacy due to their relationship with such compounds as sex hormones. Steroids increase protein synthesis, promoting growth of muscles and bones. They reduce the recovery time needed between training sessions and enable athletes to train more intensively for longer periods. Phenolic was present both in aqueous and methanolic extract of the leaves. Phenols are strong antioxidants which prevent oxidative damage to biomolecules such as DNA, lipids and proteins which play a role in chronic diseases such as cancer and cardiovascular disease. Plant phenols may interfere with all stages of the cancer process, potentially resulting in a reduction of cancer risk (Hollman, 2001). In general, the presence of these Phytochemicals could account for the much touted medicinal properties of these leaves in various disease conditions such as atherosclerosis, arthritis, diabetes nausea, asthma, skin antiseptic, diarrhea, dysentery, colitis and cancer.

## CONCLUSION

The present study has provided some comparative biochemical information on the proximate and phytochemistry of three different stages of maturity of moringa leaves grown under Nigeria (Kano) climatic condition. There are indications that all the three are good sources of nutrients and Phytochemicals at varying degrees. Although, the leaves of all the stages have varying percentages of the nutritional composition tested for, the late stage recorded the highest. Therefore, the choice depends on the individual as it is a potential leaf source of food that is suitable for fortification of foods and their use as nutritional supplements is highly promising.

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## REFERENCES

- Abdul, D.A.S., 2007. Economic Importance of *Moringa oleifera* in Tafa Local Government Area of Niger State. NDE Project. Federal College of Forestry Mechanization, Kaduna, Nigeria, pp: 34.
- Adeyeye, E.I. and O.O. Ayejuyo, 1994. Chemical composition of *Cola accuminata* and *Garcinia kola* seeds grown in Nigeria. Int. J. Food Sci. Nutr., 45: 223-230.
- Alan, L. and N.D. Miller, 1996. Antioxidant flavonoids, structure, function and clinical usage. Alt. Med. Rev., 1: 103-111.
- Akindahunsi, A.A. and S.O. Salawu, 2005. Phytochemical Screening and antinutrient composition of selected tropical green leafy vegetables. Afr. J. Biotech., 4: 497-501.
- Anjorin, T.S., P. Ikokoh and S. Okolo, 2010. Mineral composition of *Moringa oleifera* leaves, pods and seeds from two regions in Abuja, Nigeria. Int. J. Agric. Biol., 12: 431-434.
- Anonymous, 2006. *Moringa oleifera*, Medicinal and Socio-Economic uses. International Course on Economic Botany, National Herbarium Leiden, the Netherlands.
- Antia, B.S., E.J. Akpan, P.A. Okon and I.U. Umoren, 2006. Nutritive and anti-nutritive evaluation of sweet potatoes (*Ipomoea batatas*) leaves. Pak. J. Nutr., 5: 166-168.
- AOAC, 1990. Official Methods of Analysis. 15th Edn., Association of Official Analytical Chemists, Washington D.C.
- Ayinde BA, D.N. Onwukaeme and E.K.I. Omogbai, 2007. Isolation and characterization of two phenolic compounds from the stem bark of *Musanga cecropioides* R. Brown (*Moraceae*). Acta Pol. Pharm., 64: 183-185.
- Elvin-Lewis, P., F.L. Memory and H. Walter, 1977. Medical Botany: Plants Affecting Man's Health. Wiley, New York. ISBN: 0-471-53320-3.
- Etuk, E.U., M.N. Bassey, U.O. Umoh and E.G. Inyang, 1998. Comparative nutritional studies on three local varieties of *Heinsia crinita*. Plant Var. Seeds, 11: 151-158.
- Fahey, J.W., 2005. *Moringa oleifera*: A review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1, Trees Life J., 1: 5.
- Fuglie, L., 2001. The Miracle Tree: The Multiple Attributes of Moringa, Dakar, Senegal.
- Galeotti, F., E. Barile, P. Curir, M. Dolci and V. Lanzotti, 2008. Flavonoids from carnation (*Dianthus caryophyllus*) and their antifungal activity. Phytochem. Lett., 1: 44.
- Hollman, P.C., 2001. Evidence for health benefits of plant phenols: Local or systemic effects?. J. Sci. Food Agric., 81: 842-852.
- Ifon, E.T. and O. Bassir, 1979. The nutritive value of some Nigerian leafy green vegetables Part 2, Distribution of protein, carbohydrate. Fat. J. Food. Chem., 5: 231-235.
- Kasolo, J.N., S. Gabriel, L.O. Bimenya, O. Joseph and J.W. Ogwal-Okeng, 2010. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. J. Med. Plants Res., 4(9): 753-757.
- Makkar, H.P.S. and K. Becker, 1996. Nutritional value and antinutritional components of whole and ethanol extracted *Moringa oleifera* leaves. Ani. Feed Sci. Technol., 63(1-4): 21-24.
- Oakenful, D. and G.S. Sidhu, 1989. Saponins. In: Cheeke, P.R. (Ed.), Toxicants of Plant Origin. Academic Press, New York, 2: 78-113.
- Osagie, A.U., 1988. Anti-nutritional Factors. In: Osagie, A.U. and O.U. Eka, (Eds.), Nutritional Quality of Plant Foods Post Harvest Research Unit. Department of Biochemistry, University of Benin, Benin City, Nigeria, pp: 221-224.
- Odebiyi, O.O. and E.A. Sofowora, 1979. Phytochemical screening of Nigerian Medicinal plants 2nd OAU/STRC Inter-African Symposium on Traditional Pharmacopoeia and African Medicinal Plants (Lagos) No., 115: 216-220.
- Oduro, W., O. Ellis and D. Owusu, 2008. Nutritional potential of two leafy vegetables: *Moringa oleifera* and *Ipomoea batatas* leaves. Sci. Res. Essay, 3(2): 57-60.
- Phytochemicals, 2011. Retrieved from: <http://www.phytochemicals.info/>, (Assessed on: February 10, 2011).

- Price, K.R., I.T. Johnson and G.R. Fenwick, 1987. The chemical and biological significance of saponins in foods and feedstuffs. *CRC Crit. Rev. Food Sci. Nutr.*, 26: 27-135.
- Rajangam, J., R.S. Azahakia Manavalan, T. Thangaraj, A. Vijayakumar and N. Muthukrishan, 2001. Status of production and utilisation of Moringa in Southern India. In: Lowell, J.F. (Ed.), *The Miracle Tree/ The Multiple Attributes of Moringa*. CTA. USA.
- Reed, J.D., 1995. Nutritional toxicology of tannins and related polyphenols in forage legumes. *J. Anim. Sci.*, 73(5): 1516-1528.
- Tijjani, M., I. Bello, A. Aluyu, T. Olurische, S. Maidawa, J. Habila and E. Balogun, 2009. Phytochemical and antibacterial studies of root extract of *Cochlospermum tinctorium*. *Res. J. Med. Plants.*, 3: 16-22.
- Williams, R.J., J.P. Spencer and C. Rice-Evans, 2004. Flavonoids: antioxidants or signalling molecules? *Free Radical Biol. Med.*, 36(7): 838-849.
- Yu, P., D.A. Christensen and J.J. McKinnon, 2004. *In situ* rumen degradation kinetics of timothy and alfalfa as affected by cultivar and stage of maturity. *Can. J. Anim. Sci.*, 84: 255-263.