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ORIGINAL RESEARCH

Nutrients Composition, Phenolic Content and Antioxidant Activity of Green and Yellow Moringa (*Moringa oleifera*) leaves

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Abstract

This work aimed at investigating the impact of the change in color of *Moringa oleifera* leaves on its nutritional value, phytochemical composition and antioxidant activity. The green and yellow moringa leaves (fresh) were dried in an oven at 45 °C for 48 h and blended. The powders of each plant material were divided into two parts, one for the determination of the proximate composition and mineral content, and the other for the determination of the phenolic content and antioxidant activity. Results showed that the change in color of *Moringa oleifera* leaves does not affect its proximate composition, phenolic content, metal chelation activity and ferric reducing antioxidant power. The change in the color of the leaves from green to increases their radical scavenging activity and iron content (61.41 to 96.68%). However, it significantly reduces the potassium (3144 to 131.20%), sodium (141.70 to 15.90%), calcium (2346.80 to 1576%) and Magnesium (657.50 to 471.40%) content of the leaves. Green *Moringa oleifera* leaves can be recommended for their good nutritional value (mineral element) compared to yellow leaves.

Practical Applications

The knowledge of the nutrient and phytochemical composition as well as the antioxidant activity of green *Moringa oleifera* leaves can help in food formulation and supplementation in order to reduce poor nutrition. Its leaves can serve as natural preservative in foods as well as traditional medicine in order to reduce the damages caused by free radicals in the body.

Keywords: *Moringa oleifera*, yellow and green leaves, nutrient content, phenolic content, antioxidant activity.

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1. Introduction

Moringa oleifera, generally known as *miracle plant* is the most cultivated plant from the Moringaceae family. It is mostly produced for its nutritional and medicinal properties. The plant is friendly with tropical, subtropical and semi-arid climate, where it primarily grows (Clement *et al.*, 2017). It is a fast growing plant of great economic importance for the food and medical industries (Anwar *et al.*, 2007). The plant is becoming increasingly more appreciated around the world, as almost every part of the tree can be used (Biel *et al.*, 2017). All plant parts (pods, seeds, leaves, barks, roots, flowers) are used for medicinal purposes. The roots are shredded and used as condiment, the leaves can be eaten fresh, cooked, or stored as dried powder for months without refrigeration and loss of nutritional value (Mishra *et al.*, 2012), the seeds are used for oil extraction that can be consumed or used for water purification (Ghebremichaela *et al.*, 2005).

The nutritional composition of moringa leaves showed that they are indigenous sources of digestible proteins (containing all essential amino acids), vitamins, mineral and carbohydrates that are necessary for human beings of all ages (Fahey *et al.*, 2001). Their seeds are rich in unsaturated oil which is good in the prevention of coronary diseases. Apart from that, moringa plant is a powerful medicinal plant that has been used for centuries to cure or prevent several diseases. The plant is used to prevent or treat gastrointestinal problems, headache, inflammation, hypertension, ulcers, anemia, fever, eye infection, bronchitis, poor nutrition, skin infections, cancers, diabetes etc (Clement *et al.*, 2017; Geleta *et al.*, 2016). These medicinal properties are generally attributed to the rich phytochemical composition of the moringa plant. Moringa has been found to be a good source of polyphones and antioxidants (FAO/WHO, 1993). Phytochemicals such as vanillin, phenolic acids flavonoids, carotenoids, ascorbates, tocopherols, beta-sistosterol, kaempferol, moringine, omega fatty acids have been reported in its flowers, roots, fruits, seeds and pods (Clement *et al.*, 2017).

Generally, the appearance of yellow leaves is observed in some moringa garden or farms, leading to important economic losses in the industry. Even with that, people still used them as medicine or food supplement without reservation. It has been reported that the yellow color of *Moringa oleifera* leaves can sometime be an indication of nutrient or environmental problems. It can also be due to Chlorosis of the plant. In this case, leaves lack the essential green pigment chlorophyll. Possible causes include poor drainage, damaged roots, compacted soil, high soil pH, and nutrient deficiencies. It can also be the consequence of the attack of the plant by fungi such as *Cercospora* spp and *Septoria lycopersici* (Armelle de Saint Sauveur & Broin, 2010). Such a change in color can affects the composition and activity of the leaves. Several studies have been conducted on the nutritional composition and medicinal properties of moringa leaves. However, little or no information on the impact of the changes in color of moringa leaves on their nutrient and phytochemical composition as well as the antioxidant activity. The objective of this study was therefore to evaluate the variation in nutrient, phytochemicals and antioxidant activity of green and yellow moringa leaves.

2. Material and methods

2.1. Material

All the chemicals and reagents used were of analytical reagent grade. The fresh yellow and green Moringa leaves were harvested in April 2017 at the Catholic University Institute of Buea garden, located at Molyko, Buea, South West Region, Cameroon.

Green *Moringa oleifera* leaves

Yellow *Moringa oleifera* leaves

2.2. Methods

2.2.1. Proximate composition of Moringa leaves

Moisture, fat, ash and protein content of green and yellow *Moringa oleifera* leaves were determined using standard analytical methods described by AOAC (1990) procedures. Moisture content was determined by drying the leaves in an oven at 103 °C to constant weight according to the AOAC procedures 925.40. Ash content was determined by incineration of the leaves at 550 °C according to the AOAC procedures 942.05. Nitrogen (N) content was determined using the micro-Kjeldahl method, according to AOAC procedures 984.13. The protein content was calculated as N x 6.25. Lipid content was determined using Soxhlet apparatus with hexane, following the AOAC 963.15 methodology. The total percentage carbohydrate content was determined by the difference method as reported by Onyeike *et al.* (2015). This method involved adding the total values of crude protein, crude fat, moisture and ash constituents of the sample and subtracting it from 100. All samples were analysed in triplicate.

2.2.2. Mineral content of Moringa leaves

For the determination of minerals, yellow and green leaves were ashed at 550 °C and dissolved with 10 mL of 20% HCl in a beaker and then filtered into a 100 mL standard flask to determine the mineral content. Calcium (Ca), magnesium (Mg), sodium (Na), potassium (K) and iron (Fe) were determined by atomic absorption spectrometer (Varian 220FS Spectra AA, Les Ulis, France). Phosphorus (P) was determined colorimetrically using the vanado molybdate, according to AOAC procedure 965.17 (AOAC, 1990). Mineral contents of the samples were determined from calibration curves of standards minerals. All samples were analyzed in triplicate.

2.2.3. Changes in color on the phenolic content and antioxidant activity of Moringa leaves

2.2.3.1. Extraction of polyphenols

Polyphenols were extracted from plant materials using the maceration method, as described by Womeni *et al.* (2016). The fresh Moringa leaves (Yellow and Green) were dried in an electric air-dried oven at 45 °C for 48 hours. The dried leaves were ground in a blender machine (Moulinex) and sieved (Diameter of pore: 1mm). About 20 g of each powder was extracted into 200 mL of Methanol. The mixture was regularly subjected to shaking during the extraction. After 48

hours of maceration, the mixture was filtered with a Wathman N°1 filter paper. The obtained filtrates were subjected to rotatory evaporation at 45 °C under reduced pressure for the removal of the solvent, and the solvent residues was removed by drying the extract at 45 °C until the extract became solid and the weight constant. The dried extracts were stored at 4 °C for further analysis.

2.2.3.2. Total phenolic content

The total phenolic content of yellow and green moringa leave extracts extract was determined using the Folin-Ciocalteu colorimetric method, as described by Gao *et al.* (2000). In a test tube of 5 mL volume, 20 µl of a 2 mg/mL extract solution was added, followed by the Folin–Ciocalteu reagent (0.2 mL) and distilled water (2 mL). After 3 min incubation of the solution mixture at room temperature, 1 mL of 20% sodium carbonate solution was added and the mixture re-incubated for 20 min under the same conditions. The absorbance of the resulting blue-coloured solution was measured at 765 nm using a spectrophotometer. The total phenolic content of the extract was calculated from the gallic acid standard curve, and expressed as milligrams equivalents gallic acid per gram of extract.

2.2.3.3. DPPH Radical scavenging activity

The radical scavenging activity of moringa leave extracts was determined using the 2,2-diphenyl-1-picryl hydrazyl (DPPH) method, as described by Womeni *et al.* (2016). 4.5 mL of 0.002% alcoholic solution of DPPH was added to 0.5 mL of different concentrations (125, 250, 500, 1000 and 2000 µg/mL) of samples and standard solutions separately, in order to have final concentrations of products of 25-200 µg/mL. The samples were kept at room temperature in the dark and after 30 min; the absorbance of the resulting solution was measured at 517 nm. The absorbance of the samples, control and blank were measured in comparison with methanol. Synthetic antioxidant, butylated hydroxytoluene (BHT), which is a recognized powerful radical scavenger, was used as the positive control. The following formula was used for the calculation of the radical scavenging activity:

$$AA\% = [(Abscontrol – Abssample) \times 100/Abscontrol]$$

2.2.3.4. Ferric reducing antioxidant power

The antioxidant potential of moringa leave extracts was also evaluated by its ability to reduce iron (III) to iron (II) following the method of Oyaizu (1986). An aliquot of 0.5 mL plant essential oil (125, 250, 500, 1000 and 2000 µg/mL) was mixed with 1 mL phosphate buffer (0.2 M, pH 6.6) and 1 mL of 1% aqueous $K_3Fe(CN)_6$ solution, well shaken and incubated at 50 °C for 30 min. After incubation, 1 mL of 10% TCA solution was added to stop the reaction and the mixture was centrifuged at 3000 rpm for 10 min. 1.5 mL of supernatant, 1.5 mL of distilled water and 0.1 mL of 0.1% $FeCl_3$ solution were mixed and incubated for 10 min and absorbance read at 700 nm on spectrophotometer. The blank sample, containing all the reagents but no essential oil was prepared under the same conditions. Catechin, a recognized powerful ferric reducer compound, was used as positive control to compare the reducing power of the essential oil. Higher absorbance indicates higher reducing power.

2.2.3.5. Metal chelation activity

The antioxidant potential of moringa leave extracts was also evaluated by its ferrous ion chelating activity (Benzie & Szeto, 1999). In test tubes containing 160 µl of sample solution (1000 µg/mL), 160 µl of aqueous solution of 1, 10-phenanthroline (0.25 %) and 400 µl of methanolic $FeCl_2$ (0.1 %) were added. After 10 min incubation at room temperature, 880 µl of distilled water was added and the absorbance was measured at 510 nm. The metal chelating efficiency of the oil was compared to that of catechin (positive control). The inhibition percentage (IP) of the formation of the complex Fe^{2+}-phenanthroline was calculated using the following formula:

$$IP\% = Abs\ sample \times 100/Abs\ control$$

2.2.4. Statistical analysis

Results (Mean ±Standard deviation) obtained in the present study were subjected to one-way analysis of variance (ANOVA) with Dunnett and Student-Newman- Keuls test using Graphpad-InStat version 3.05, to evaluate the statistical significance of the data. A probability value at $p< 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Results

3.1.1. Changes in the proximate composition of Moringa leaves

The moisture, protein, lipid, ash and carbohydrate content of yellow and green moringa leaves powders are presented in table 1. Generally, no significant change ($p>0.05$) was registered between the proximate composition of green and yellow moringa leaves.

3.1.2. Changes in the mineral content of Moringa leaves

Table 2 presents the mineral content of yellow and green moringa leaves. Generally a significant decrease ($p<0.001$) in phosphorus, potassium, sodium, calcium and magnesium was registered in the yellow leaves compared to the green ones. However, the iron content of yellow leaves was higher ($p<0.001$) than that of the green leaves.

3.1.3. Total phenolic content

Figure 1 shows the phenolic content of green and yellow moringa leave extracts. No significant difference ($p>0.05$) was recorded between the phenolic content of both extracts.

Table 1: Proximate composition green and yellow moringa leaves

	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)	Carbohydrate (%)
Green leaves	9.86±0.12[a]	16.00±1.54[a]	16.33±0.32[a]	35.81±1.67[a]	22.00±2.53[a]
Yellow leaves	10.56±1.14[a]	12.00±2.21[a]	16.57±0.29[a]	33.94±0.77[a]	26.93±1.11[a]

Data are presented as Mean ± Standard deviation. Values for the same parameter with different superscripts are significantly different ($p<0.05$)

Table 2: Changes in mineral content of green and yellow moringa leaves

	P (mg/100g)	K (mg/100 g)	Na (mg/100 g)	Fe (mg/100 g)	Ca (mg/100 g)	Mg (mg/100 g)
Green leaves	64.82±0.33[a]	3144.00±8.22[a]	141.70±2.32[a]	61.41±1.10[a]	2346.80±12.23[a]	657.50±7.44[a]
Yellow leaves	0.83±0.03[b]	131.20±2.31[b]	15.90±1.75[b]	96.68±0.56[b]	1576.00±1.54[b]	471.40±13.55[b]

[a-b]Data are presented as Mean± Standard deviation. Values for the same parameter with different superscripts are significantly different (p<0.05)

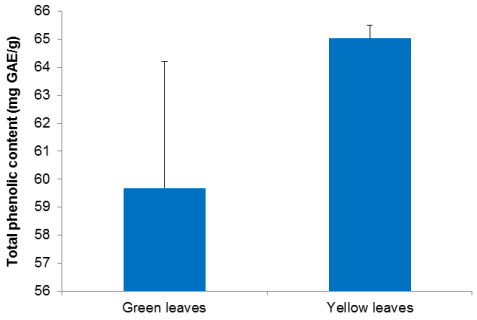


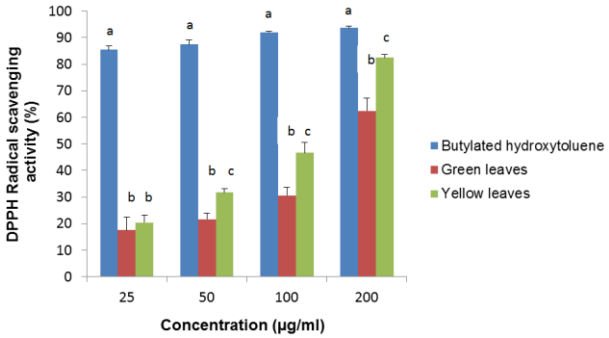
Figure 1: Total phenolic content of green and yellow *moringa oleifera* leaves

3.1.4. Changes in radical scavenging activity

The ability of green and yellow moringa leaves extracts to scavenge the DPPH radical is presented in figure 2. The yellow leaves exhibited significantly higher ($p<0.05$) radical scavenging activity compared to the green leaves and this at concentrations 50, 100 and 200 µg/mL. At concentration 25 µg/mL, the activity of both plants was similar. However, the radical scavenging activity of both extracts was significantly lower ($p<0.001$) than that of butylated hydroxytoluene at all concentrations.

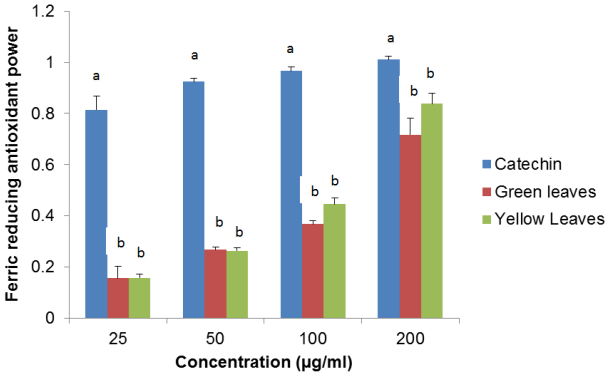
3.1.5. Ferric reducing antioxidant power

Figure 3 shows the ferric reducing antioxidant power of green and yellow moringa leaves. No significant difference ($p>0.05$) was registered between the activity of green and yellow leaves extracts. However, the activity of both plant samples was significantly lower ($p<0.001$) compared to that of Catechin.



[a-c]Data are presented as Mean± Standard deviation. Values for the same parameter with different superscripts are significantly different (p<0.05)

Figure 2: Radical scavenging activity of green and yellow *Moringa oleifera* leaves

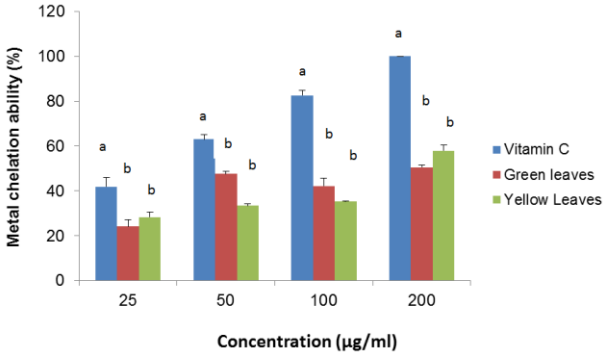


[a-b]Data are presented as Mean± Standard deviation. Values for the same parameter with different superscripts are significantly different (p<0.05)

Figure 3: Ferric reducing antioxidant power of green and yellow *moringa oleifera* leaves

3.1.6. Metal chelation ability

The chelation ability of green and yellow moringa leaves extracts is shown in figure 4. At concentration 25, 100 and 200 µg/mL, no significant difference ($p>0.05$) was observed between the metal chelation activity of green and yellow moringa leaves, while at concentrations 50 µg/mL, the activity of green leaves extract was significantly higher ($p>0.05$) than that of yellow leaves. Generally, the activity of both extracts was significantly lower ($p<0.001$) compared to that of vitamin C.



[a-b]Data are presented as Mean± Standard deviation. Values for the same parameter with different superscripts are significantly different ($p<0.05$)

Figure 4: Metal chelation activity of green and yellow *moringa oleifera* leaves

3.2. Discussion

The analysis of the proximate composition of green and yellow *Moringa oleifera* leaves showed no difference in their moisture, protein, lipid, ash and carbohydrate content. This suggests that, the change in color of these leaves does not affect their proximate composition. The moisture content of both leaves varied between 9.86-10.56% which was significantly higher than that reported by Verma & Nigam (2014), Chinwe *et al.* (2015) and Castillo-Lopez *et al.*, 2017) who respectively obtained moisture content of 6.43%, 6.12% and 3.79-3.88% with the same part of the plant. However, the lipid content obtained in this study (16.33 and 16.57% for green and yellow leaves respectively) was similar to that reported in by Verma & Nigam (2014) in moringa leaves harvested in Jabalpur (16.07%), but significantly higher than that obtained by Chinwe *et al.* (2015) with the same leaves in Ecuador (9.22%). The protein, ash and carbohydrate content recorded in this work varied between 12-16%, 33.94-35.81% and 22-26% respectively. The amount of protein was significantly lower than that reported by Verma &Nigam (2014) and Chinwe *et al.* (2015) who respectively obtained 34.93% and 24.31%. However, it was significantly higher than that reported by Amaybe (2016) which is 10.71%. The ash content reported in this study was significantly higher compared to that obtained by these same authors. Their ash content varied between 7.29-12.23%. As far as the carbohydrate content is concerned, the concentration obtained in this work was significantly lower than that reported by Chinwe *et al.* (2015), Amaybe (2016) and Verma & Nigam (2014) who respectively obtained 55.97, 57.61 and 29.92%. The variations observed with the data obtained from the literature can be attributed to climatic factors, location, harvesting period, nutrition of the plant etc (Kim & Choe 2004).

Results of the mineral composition showed that the amount of phosphorus, potassium, sodium, calcium and magnesium in green moringa leaves is significantly higher than that of yellow leaves, and that the concentration of iron in yellow leaves is higher than that of green ones. The significant decrease in K, Na,

Ca and Mg in the yellow leaves compared to green ones can be attributed to the attack of the leaves by fungi (*Cercospora* spp and *Septoria lycopersici*). Also, research has shown that, the presence of the arbuscular mycorrhizal fungi in the plant rhizosphere triggers the production of Abscisic Acide (ABA) and Strigolactones hormones which reduces the mineral content in leaves by pushing or draining out minerals, water, sugar, gases from the leaves resulting to degradation of the chlorophyll content (Abdallah *et al.*, 2010).This could equally contribute to the significant decrease in mineral content in the yellow leaves. It can also be the consequence of poor drainage, damaged roots, compacted roots, high soil pH, and nutrient deficiencies (Armelle de Saint Sauveur & Broin, 2010). Certain nutrients such as phosphorus, potassium and magnesium are mobile in plants and these nutrients can move from yellow leaves to the younger and green leaves when the plants run low of the mobile nutrients thus contributing to nutrient use efficiency and continuous growth of the plant (Avice & Etienne, 2014). Leaf mineral content decreases in some plant species, as a result of remobilization and hence a significant drop in chlorophyll content and chlorosis initiation (Fischer, 2007). White (2012) reported that an immobile nutrient like iron, is essentially stuck in yellow leaves reason while a high Iron content observed in the yellow than green leaves. The amount of potassium (3144 mg/100 g), sodium (141.70 mg/100 g), iron (61.41mg/100 g) calcium (2346.80 mg/100 g) and magnesium (657.50 mg/100 g) obtained in this study with green leaves was significantly higher than that reported by Amaybe (2016) with the leaves of the same plant in Ethiopia who respectively obtained 1845, 8.13, 19.37, 2016.50 and 322.5 mg/100 g respectively for K, Na, Fe, Ca and Mg. However, the phosphorus content (64.82 mg/100 g) obtained with the green leaves was significantly lower than that reported by Prachi & Bhogaonkar (2017) who obtained a value of 112 mg/100 g. The determination of the total phenolic content of green and yellow moringa leaves showed that this parameter is not affected by the change in color of the plant material. The phenolic content of both leaves obtained in this study varied between 59-66 mg GAE/g. This value was lower than that reported by Castillo-Lopez *et al.* (2017) who obtained total phenolic content of 76.63 and 71.08 mg GAE/g in the methanolic extracts of long and short moringa leaves pods respectively.

Results of the antioxidant activity generally showed that the methanolic extracts of green and yellow *Moringa oleifera* leaves have interesting antioxidant activity. Similar results were previously reported by other authors with the leaves of the same plant (Fitriana *et al.*, 2016 ; Castillo-Lopez *et al.*, 2017 ; Wright *et al.*, 2017). However, their activity was lower than that of the synthetic antioxidants tested. The purity of the synthetic antioxidants can explain the difference in the activity observed. The data of the radical scavenging activity showed that the yellow leaves are more active than green ones. At concentration 200 µg/mL, the activity of yellow and green moringa leaves was within 60-85%, which is not far from the values reported by Fitriana *et al.* (2016) and Castillo-Lopez *et al.* (2017) who respectively obtained activities of 85 and 87.92% in the methanolic extracts of the leaves of the same part of plant. The highest activity registered in yellow leaves compared to green ones can be attributed to the presence of some radical scavenging molecules which are free when the

chlorophyll is destroyed. As far as the ferric reducing antioxidant power and the metal chelation ability are concerned, no difference was observed in their activities at all concentrations. This can be explained by the fact that the antioxidants with such mechanism of actions are not affected by the change in color which might be related to the loss of the chlorophyll. The interesting ferric reducing antioxidant power and the metal chelation ability obtained in this study with *Moringa oleifera* leaves extracts is in agreement with the results of Vanisha *et al*. (2013) and Vijayarajan & Rajasekara (2016).

4. Conclusion

This study was conducted in order to evaluate the impact of the changes in color on the nutrient composition, phenolic content and antioxidant activity of green and yellow *Moringa oleifera* leaves. Results showed that the change from green to yellow of *Moringa oleifera* leaves significantly reduces its amount of K, Na, Ca and Mg; but increases its iron content. The proximate composition, phenolic content, metal chelation activity and ferric reducing antioxidant power of this plant material are not affected by its change in color (green to yellow). However, the change in color significantly increases the radical scavenging activity of moringa leaves. The use of green leaves only, as they contain good amount of mineral elements compared to the yellow leaves can be recommended.

Conflict of interest

The authors declare that they do not have any conflict of interest.

Ethics

This Study does not involve Human or Animal Testing.

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