# Study of Anti-nutritional Compounds, Antioxidant Activity and Fatty Acid Composition of *Moringa* (*Moringa oleifera* Lam.*)* Foliage

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**ABSTRACT—** ***The study was conducted to investigate the effects of cutting intervals on the anti-nutritional factors, antioxidant activity and fatty acid composition of Moringa (Moringa oleifera Lam.) foliage. An established Moringa plot dividing into 12 equal plots was subjected to 3 different maturity stages of harvesting at 4, 6 and 8 weeks in a completely randomized block design experiment. It was found that the level of total phenol and tannin (mg tannic acid equivalent/g dry weight) at 4 (51.86 and 34.90), 6 (43.89, and 27.96) and 8 (29.00 and 16.66) weeks of maturity decreased significantly. Similarly, with the increase of maturity the level of condensed tannin significantly decreased (0.23, 0.17 and 0.14 mg catechin equivalent/g dry weight, respectively). In the case of antioxidant activity, significantly higher DPPH inhibition activity was found after 4 weeks (60.1 %) compared to 6 and 8 weeks of maturity (56.0 and 53.4 %, respectively). However, the fatty acid composition of Moringa foliage was significantly affected with harvesting stage of maturity. It contained the highest level of α-linolenic acid followed by palmitic acid, linoleic acid, stearic acid and oleic acid, respectively (48.71, 21.65, 13.07, 5.89 and 4.63 % of the total identified fatty acids, respectively). The average level of poly unsaturated fatty acid (PUFA) (61.78 %) was about three times higher than the level of saturated fatty acids (31.24 %). In conclusion, harvesting of Moringa foliage at 8 weeks interval would have less anti-nutritional factors with more PUFA and antioxidant activities.***

**Keywords—**Moringa oliefera, anti-nutritional factors, antioxidant activity, fatty acid composition

## 1. INTRODUCTION

Over the last few years, the use of fodder trees and shrubs as feed resources has grown in interest for sustainable livestock production all over the world [1, 2]. Therefore, there is a need to investigate the suitability of available indigenous fodder trees to use as feed for livestock production. The International Institute for Tropical Agriculture (IITA) and the International Livestock Centre for Africa (ILCA) developed the cultivation of *Gliricidia sepium* and *Leucaena leucocephala* through alley farming and feed gardens for supplying fresh fodder for ruminants [3]. However, these species may have restrictions in terms of productivity, palatability, presence of toxic substances and adaptability [4]. Also, the lack of enthusiasm of smallholder farmers to apply these tree species as supplements for ruminants requires the search for other tree species. The limitations in nutritional exploitation of tree leaves are the presence of anti-nutritional and toxic factors [5].

*Moringa oliefera* is the most widely cultivated species of Moringaceae family in some Asian countries such as India, Pakistan, Bangladesh and Afghanistan [6]. It is reported that *Moringa oleifera* yielded significant amounts of fodder during the wet and dry season [7]. *Moringa* is reported to have high quality proteins for ruminants, which is easily digested and is influenced by the quality of its amino acids [8], with negligible amounts of anti-nutritional compounds [9]. Recent epidemiological and controlled-case studies reported that many anti-nutrients when present at low levels gives beneficial effects for the prevention of diseases like coronary diseases and cancers in human and animal models [10]. For this reason, anti-nutritional factors are often known as plant bioactives or non nutritive compounds.

Some scientists have placed attention on the nutritional and secondary metabolite properties of *Moringa* [11]. This could be due to the fact that it increases animal productivity as it has nutritional, therapeutic and prophylactic properties

[12]. It is reported that the leaves have immense nutritional value in terms of vitamins, minerals and amino acids [13]. It is also known that phenolic and flavonoid contents are directly linked to antioxidant properties [14]. Due to the presence of total phenols and flavonoids, the *Moringa* leaf may exhibit greater antioxidant properties. Nutrients and antioxidants play an important role in the animal's ability to overcome the detrimental effects of parasitism and diseases [13]. However, there are considerable variations in the nutritional values and antioxidant activity of *Moringa*, which depend on factors like season and agro-climatic location, genetic background, environment and cultivation methods [15]. Siddhuraju and Becker [14] also reported that antioxidant properties of *Moringa oleifera* leaves varied with agro-climatic locations. Also, the study on the effect of cutting interval on anti-nutritional factors, antioxidant activity and fatty acid composition of *Moringa oleifera* is scarce. Therefore, the study was conducted with the objectives to investigate the anti-nutritional factors, antioxidant activity and fatty acid composition of *Moringa* foliage at different cutting intervals.

## 2. MATERIALS AND METHODS

### 2.1 Location of Experimental Site

This study was conducted at the fodder plot of the Bangladesh Livestock Research Institute, Bangladesh during the rainy season from June to October, 2012 when the average rainfall, temperature and humidity were 280 mm, 28.8°C and 78%, respectively. The station was located at 23°42'0" North and 90°22'30" East at an altitude of 4 meters above the sea level. The Agro ecological zone belongs to the Madhupur Tract (Agro Ecological Zone 28) of Bangladesh, with Red-Brown Terrace strong acidic (pH 4.5–5.7) soils, with very little (< 1.5%) organic matter [16].

### 2.2 Preparation of Experimental Plots

A two years old plot of 201.67 $m^2$ containing 180 plants with an average height of about 2 meter was used for this study. First of all, fertilizer was applied at the rate of 500 kg goat manure $ha^{-1}$, 125 kg urea $ha^{-1}$, 100 kg Triple Super Phosphate (TSP) $ha^{-1}$, 50 kg Zinc Sulphate ($ZnSO_4$) and Borax 25 kg $ha^{-1}$. Then, after fifteen days of fertilizer application, all plants were cut uniformly at a height of 0.76 meter above the ground, and all foliage was removed. Weeding was done manually once a month. Pest and disease incidences were not experienced during the experiment. Newly grown leaves and twigs were used for sampling purposes according to the design of the experiment.

### 2.3 Layout of Plot and Design of the Experiment

The total experimental plot was divided into 12 sub-plots with an area of 16.8 $m^2$ containing a total of 15 plants per plot. Thus, the planting space was measured as 1.25 m × 0.75 m. Three cutting intervals of *Moringa* at 4, 6 and 8 weeks were arranged in a randomized complete block design with 4 replications.

### 2.4 Harvesting and Sampling of *Moringa* Foliage

The stems and leaves re-growths of plants (120 cm length) were hand harvested at 4, 6 and 8 weeks of maturity during the experimental period. Harvesting of samples was done from randomly selected five plants from each sub-plot in each treatment. Freshly harvested Moringa foliage (leaves and stems) was sun dried for 36 hours and then milled by passing through 1.0 mm screen of Willy Mill. These dried and milled samples were stored in air-tight plastic pack until laboratory analysis.

### 2.5 Extraction of Samples and Determination of Anti-Nutritional Compounds

Extract of *Moringa* foliage was extracted by taking one gram of sample in a 50 ml falcon tubes and adding 40 ml diethyl ether containing 1% acetic acid (v/v). The supernatant was discarded after 5 minutes and 20 ml of 70 % aqueous acetone was added and the falcon tubes were sealed with screw cap and kept in an electrical shaker for 2 hours. The tubes were centrifuged at 3000 rpm for 10 minutes. Finally, the supernatant was collected and kept in a refrigerator at 4 °C until further analysis**.**

The concentration of total phenols, tannins and non-tannin phenols were estimated according to the procedure of Makkar [17]. The concentrations of total phenols, tannins and non-tannin phenols were expressed as milligram (mg) tannic acid equivalent/g dry weight (DW). The concentration of condense tannin was quantified following to the method of Porter *et al*. [18]. The concentration was expressed as mg catechin equivalent/g DW. The amount of total flavonoids in the extracts was determined according to aluminum chloride assay according to Atanassova *et al*. [19]. The concentrations of flavonoids were expressed as mg rutin equivalent/g DW. For the determination of saponin, *Moringa* foliage samples were de-fatted according to the method of Sanbongi *et al*. [20]. Then, total saponin content was determined according to Makkar *et al*. [21] based on the vanillin-sulfuric acid colorimetric reaction. The concentration of saponin was expressed as mg diosgenin equivalent/g DW.

### 2.6 Antioxidant Activity (DPPH Free Radical Scavenging Activity)

The free radical scavenging activity of *Moringa* foliage extract was determined by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to method described by Shen *et al.*[22]. All determinations were conducted in three replications. The

DPPH activity activities of the tested samples were expressed as % of inhibition, and were calculated according to the following equation [23]:

Percent inhibition of DPPH activity = $[(A_0 - A_1) / A_0)] \times 100$ %, where $A_0$ was the absorbance value of the blank sample or control reaction and $A_1$ was the absorbance value of the test sample at a concentration of 125 microgram per mg (µg/mg).

### 2.7 Determination of Fatty Acid Composition

The fatty acid composition of *Moringa* foliage was determined by three major processes: total lipid extraction, preparation of fatty acid methyl esters (FAME) by transmethylation and quantification of FMAE using gas chromatography, respectively. The method described by Folch *et al*. [24] and modified by Ebrahimi *et al*. [25] was used for extraction of fatty acids from samples. Extracted fatty acids were transmethylated (to FAME) using 14% Methanolic Boron Trifloride (Sigma, St. Louis MO, USA) according to the method of AOAC (Association of Official Analytical Chemist) [26]. Finally, the prepared FAME was analysed using gas chromatography (GC; Agilent, 7890A) equipped with an automatic sampler, A 100 m × 0.25 mm ID × 0.2 µm film thickness capillary column was used to separate the methyl esters, which were detected with a flame ionized detector (FID). The injector and detector temperature was 250 and 270 °C respectively. High purity nitrogen was the carrier gas with a flow rate at 1.2 ml/min and split ratio of 1:20. The identification of individual FAMEs in the sample was achieved by matching the retention times with authentic standard mixture (Sigma chemicals). The fatty acid concentrations were expressed as percentage (%) of the total identified fatty acids.

### 2.8 Statistical Analysis

The data were subjected to one way ANOVA as completely randomized design using the GLM procedure of SAS [27]. Cutting intervals were used as treatment effect, with individual plot as the experimental unit. The differences between means were compared using Duncan's multiple range test [28].

## 3. RESULTS AND DISCUSSION

### 3.1 Anti-nutritional Compounds

The level of different anti-nutritional compounds in *Moringa* foliage at different cutting intervals is presented in Table 1. The content of total phenols and tannin was found to decrease significantly ($P<0.001$) with the increase of cutting intervals. The highest content of them was found in 4 weeks cutting foliage, whereas the lowest content was found after8 weeks of cutting foliage. A significant difference ($P<0.05$) in the concentration of condensed tannins was also found between foliage harvested a 4 and 8 weeks cutting intervals. However, the contents of non-tannin phenol, total flavonoids and total saponins did not differ significantly ($P>0.05$) with the increase of cutting interval from 4 to 8 weeks.

**Table 1.** Total phenols, tannins, non-tannin phenols, condensed tannins, total flavonoids, and total saponins in *Moringa* foliage at different cutting intervals (Mean ± SE; n=3)

| Anti-nutrients | Treatments | | | *P*-value |
|---|---|---|---|---|
| | 4 wks | 6wks | 8 wks | |
| Total phenol (mg tannic acid equivalent/ g DW) | 51.86 ± 0.34 [a] | 43.89 ± 0.70 [b] | 29.00 ± 0.41 [c] | <0.001 |
| Non-tannin phenol (mg tannic acid equivalent/ g DW) | 16.95 ± 0.49 [ns] | 15.93 ± 2.51 [ns] | 12.34 ± 1.58 [ns] | 0.22 |
| Tannin (mg tannic acid equivalent/ g DW) | 34.90 ± 0.70 [a] | 27.96 ± 1.94 [b] | 16.66 ± 1.18 [c] | <0.01 |
| Condensed tannins (mg catechin equivalent/ g DW) | 0.23± 0.02 [a] | 0.17 ± 0.01 [ab] | 0.14 ± 0.02 [b] | 0.05 |
| Total flavonoid (mg rutin equivalent / g DW) | 75.28 ± 3.94 | 73.84 ± 4.84 | 67.06 ± 0.97 | 0.31 |
| Total saponins (mg diosgenin equivalent/ g DW) | 13.97 ± 0.18 | 13.78 ± 0.42 | 13.48 ± 0.12 | 0.47 |

DW, dry weight; SE, standard error; n= number of observations; $P> 0.05$; ns, not significant.

In the present study, the total phenol content of *Moringa* foliage varied from 51.9 to 29.0 mg tannic acid equivalent/g DW. The values of total phenol at 6 and 8 week maturity are similar to the values reported in other studies [7, 29]. According to Makkar and Becker [29], these simple phenols do not produce any adverse effects when fed by ruminants at this concentration. The tannin content of total *Moringa* foliage in the present study was found to be 34.9, 28.0, and 16.7 mg tannic acid equivalent/g DW at 4, 6 and 8 weeks interval, respectively (Table 1). The range in tannin content values of *Moringa* foliage was similar to the findings of Makker and Becker [29], and Aye and Adegum [30]. It was noted that the polyphenolic compounds were higher at short cutting intervals than longer cutting intervals. However, it was previously reported that phenolic content increases with the increase of leaf age [31]. These variations may be influenced by heavy rainfall and high temperatures, season, and agro-climatic conditions at different locations.

The values of flavonoid in *Moringa* foliage were 75.28, 73.84 and 67.06 mg rutin equivalent/g DW at 4, 6 and 8 weeks interval, respectively. The values of total flavonoid compounds (TFC) of *Moringa* foliage found in this study were

similar to those of *Moringa* samples reported by Iqbal and Bhanger [32], particularly in samples from Nawabshah and Jamshoro from Pakistan. However, the total phenol compounds (TPC) of *Moringa* in this study was lower than that of moringa from Chakwal, Balakot and Chakwal in Pakistan [32]. These differences could be attributed to agro-climatic conditions and season, genetic variability, age, stages of leaf development, and post handling of samples.

It is recognized that phenol and flavonoid contents are directly associated with antioxidant properties [33]. It has been reported that polyphenols and flavonoids have protective effects against various degenerative diseases due to their properties of free radicals scavenging [34]. According to Rice-Evans *et al*. [35] the number of hydroxyl groups and the amount of conjugation are two important factors that determine the antioxidant potential of phenolic compounds. The better antioxidants are generally more conjugated and have numerous hydroxyl groups present (n=2 to 5), which enables the antioxidant to scavenge several radicals at once. The presence of these compounds in *Moringa* foliage is consequently a significant finding of the present study, which indicates that higher antioxidant activity of *Moringa* foliage may be correlated with phenolic and flavonoid contents (Figure 2 and 3, respectively).

Another group of anti-nutritional factors reported to occur in *Moringa* leaves are the saponins which may reduce protozoal activity in the rumen at higher concentration (2 to 4% of the dry matter of ruminant diet) [36]. The range of saponin values in *Moringa* foliage was from 12 to 14 mg diogenin equivalent/g DW. The saponin values of *Moringa* foliage found in this study were similar to those of *Moringa* leaf samples reported by Gupta *et al*. [37]. However, the saponin content of *Moringa* foliage in this study was much lower than that reported by Aye and Adegum [30]. Saponins include a large family of structurally related compounds, and consequently all saponins do not have the same impact on livestock [29].

### 3.2 Antioxidant Activity

The effects of *Moringa* foliage extracts on the DPPH radical scavenging activity are illustrated in Figure 1. The DPPH radical scavenging activity of *Moringa* foliage ethanol extract was markedly stronger ($P$<0.05) at early cutting interval of 4 weeks compared to 6 and 8 weeks.

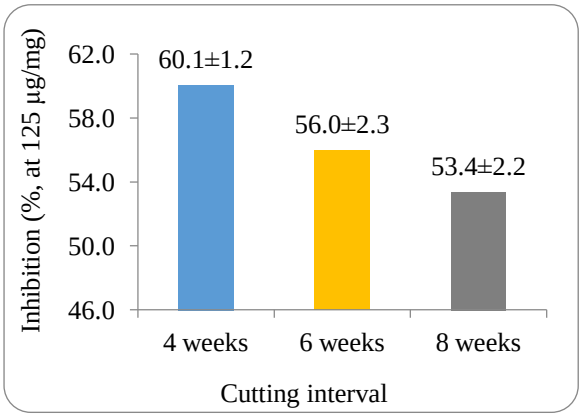


**Figure 1.** DPPH radical scavenging activity of *Moringa* foliage

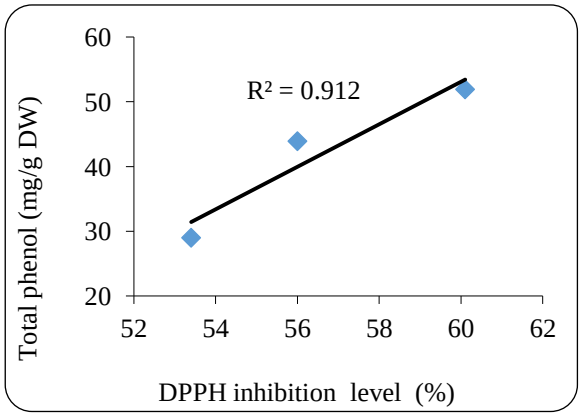


**Figure 2.** Relationship between total phenol and DPPH inhibition activity

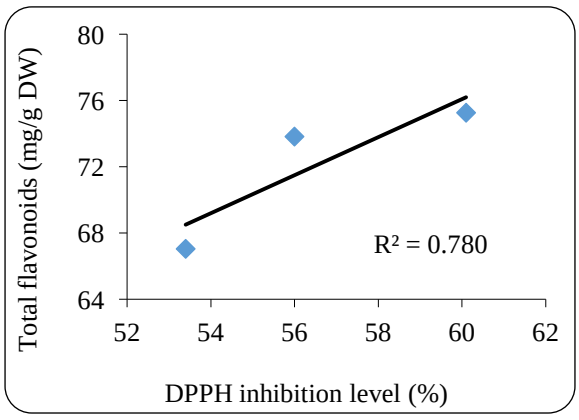


**Figure 3.** Relationship between flavonoids and DPPH inhibition

The percent inhibition of the DPPH free radical scavenging activity was 60.1, 56.0 and 53.4, respectively at 4, 6 and 8 weeks of *Moringa* foliage maturity. At 4 weeks cutting interval, *Moringa* foliage exhibited the strongest activity as a DPPH scavenger in the present study (Figure 1). The data suggests that the extract of *Moringa* foliage has an effective antioxidant activity against free radicals which might give significant protection against oxidative damage of living cells. Nouman *et al*. [7] found maximum antioxidant activities in *Moringa* leaf during the rainy and hot season, while the plants were harvested at shorter cutting intervals. Based on the results of the present study, it is apparent that there is a positive correlation between maturity stage of harvesting foliage and antioxidant activity.

The antioxidant activities of plant extracts are linked with the presence of phenols and flavonoids [38]. In the present study, a high correlation was found between the scavenging potency and total phenolic contents (Figure 2; $r^2$=0.91) and

flavonoid (Figure 3; $r^2$= 0.78) of the *Moringa* foliage extract. The results of this study suggest that *Moringa* foliage may have potential antioxidant activity due to their natural antioxidant compounds, such as phenolics and flavonoids. It has been reported that *Moringa* foliage could be used as a potential source of natural antioxidants due to their potent antioxidant activity [14].

### 3.3 Fatty Acid Composition

The fatty acid compositions of total foliage of *Moringa* at different cutting intervals are presented in Table 2. Nine fatty acids were identified in the dried *Moringa* foliage. There was no significant (*P*>0.05) difference between cutting intervals in terms of fatty acid values. In general α-lenolenic acid (C18:3n-3) was found to be present in higher amounts followed by palmatic acid (C16:0), lenoleic acid (C18:2n-6), stearic acid (C18:0) and oleic acid (C18:1n-9). Two polyunsaturated fatty acids were identified, namely α-linolenic and lenoleic acids.

The fatty acid profile of the *Moringa* foliage in the current study is similar to the findings of Moyo *et al*. [11]. The present study identified nine fatty acids from *Moringa* foliage, which was in agreement with the findings of Sena *et al*. [39]. The variation could be attributed to age of the leaves, fractions of leaf, twigs and stems, soil type and agro-climatic conditions. The amounts of total SFA, MUFA and PUFA that were obtained at different cutting intervals in the current study was consistent with the results of Sánchez-Machado *et al*. [40]. The α-Linoleic acid (C18:n-3) was found in higher amounts followed by palmitic acid (C16:0), which were similar to that findings by Sánchez-Machado *et al*. [40] and Amaglo *et al*. [41]. Two polyunsaturated fatty acids were identified, namely linoleic acid (C18:3n-3) and linolenic acid (C18:3n-3), while α-linoleic acid (C18:3n-3) recorded the highest value of 48.73%. Similar findings were observed by Sánchez-Machado *et al*. [40], and Moyo *et al*. [11]. In the present study, *Moringa* foliage at different cutting intervals showed significant amounts of α-linolenic acid (C18:3n-3) and linolenic acid (C18:2n-6), which are considered as essential fatty acids. The *Moringa* foliage contained more dietary polyunsaturated acids than saturated fatty acids. Hoffman and Wiklund [42] recommended diets with a higher content of PUFA and lower content of SFA would be desirable for promoting good health and prevent disease in humans. Wood *et al*. [43] suggested more consumption of α-linoleic acid (C18:3n-3), which encourages the endogenous synthesis of long chain omega-3 fatty acids in animals and humans.

**Table 2.** Effect of cutting interval on total fatty acid composition (%) of *Moringa oleifera* foliage

| Fatty acids (% of total identified fatty acids) | Cutting intervals (weeks) | | | P-value |
|---|---|---|---|---|
| | 4 | 6 | 8 | |
| Lauric (C12:0) | 0.60 ± 0.11 | 0.31 ± 0.02 | 0.50 ± 0.15 | 0.26 |
| Myristic acid (C14:0) | 2.19 ± 0.28 | 2.17 ± 0.10 | 1.88 ± 0.17 | 0.50 |
| Palmitic acid (C16:0) | 21.45 ± 2.68 | 22.40 ± 1.69 | 21.10 ± 0.44 | 0.13 |
| Palmitoleic acid (C16:1) | 2.23 ± 0.13 | 2.68 ± 0.36 | 2.11 ± 0.08 | 0.25 |
| Heptadecanoic acid (C17:0) | 0.96 ± 0.24 | 1.24 ± 0.06 | 1.30 ± 0.06 | 0.28 |
| Stearic acid (C18:0) | 6.08 ± 0.08 | 5.55 ± 1.14 | 6.05 ± 0.42 | 0.84 |
| Oleic acid (C18:1n-9) | 4.93 ± 0.33 | 4.86 ± 0.91 | 4.09 ± 0.34 | 0.57 |
| α-linoleic (C18:2n-6) | 13.32 ± 0.38 | 12.53 ± 0.57 | 13.35 ± 0.80 | 0.59 |
| α-linolenic (C18:3n-3) | 48.25 ± 1.92 | 48.28 ± 1.69 | 49.61 ± 0.44 | 0.78 |
| Total SFA | 31.27 ± 2.03 | 31.62 ± 1.09 | 30.84 ± 0.79 | 0.66 |
| Total MUFA | 7.15 ± 0.25 | 7.54 ± 0.60 | 6.20 ± 0.42 | 0.17 |
| Total n-6PUFA | 13.32 ± 0.38 | 12.53 ± 0.57 | 13.55 ± 0.80 | 0.59 |
| Total n-3PUFA | 48.25 ± 1.92 | 48.28 ± 1.69 | 49.61 ± 0.44 | 0.78 |
| Total PUFA | 61.58 ± 1.95 | 60.81 ± 1.37 | 62.96 ± 1.22 | 0.61 |
| n-6: n-3 | 0.28 ± 0.01 | 0.29 ± 0.003 | 0.27 ± 0.01 | 0.41 |

SFA, saturated fatty acid (C12:0+C14:0+C16:0+C18:0); MUFA, mono-unsaturated fatty acid (C16:1+C18:1n-9); n-6: n-3, ratio between total n-6PUFA and total n-3PUFA; *P*> 0.05, not significant.

Polyunsaturated fatty acids are important for human and animal health. They are of attention because they are precursors of long chain n-3 PUFA such as ecosopantanoic acid (EPA) and Docosahexaenoic (DHA) in eicosanoids biosynthesis, which are observed as important bio-regulators of many cellular processes [44]. They are associated with the improvement and functionality of the immune system. The composition of fatty acids in the animal's body is linked to the presence of some of their precursors in the diets, in view of the fact that some of the fatty acids are absorbed in the body without bio-hydrogenation [45].

## 4. CONCLUSION

*Moringa* foliage contained negligible amounts of saponins and condensed tannins, and appreciable quantity of total phenol and flavonoid compounds that may be attributed to its antioxidant activity, and subsequently *Moringa* foliage exhibited high free radical scavenging activity. *Moringa* foliage had more poly-unsaturated fatty acids (PUFA) compared to saturated fatty acids (SFA) irrespective of stage of maturity.

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