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**PROVENANCE EFFECT ON THE NUTRIENT
COMPOSITION OF MORINGA OLEIFERA LEAVES**

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ABSTRACT

Background: Moringa oleifera is a tree species to which a variety of benefits have been attributed and many of these benefits have through research been given scientific basis.

Purpose: The study aims to ascertain the effect of provenance on the nutrient composition of M. oleifera leaves.

Methods: The proximate composition of the M. oleifera leaves was determined using AOAC official method of analysis.

Results: The results of the analysis revealed the presence of high crude protein (26.43%) in Lafia compared to those obtained from Jos and Alkaleri. The results showed that M. oleifera leaves obtained from Jos has significantly higher ($P>0.05$) amount of ash, crude fiber, moisture content and lower fat content ($P<0.05$). Moringa leaves obtained from Lafia had a significantly higher amount of crude protein compared to the other locations assessed. Although M. oleifera leaves obtained from Alkaleri had a significantly higher ($P<0.05$) amount of nitrogen free extract compared to the other locations assessed.

Conclusion: The results showed that provenance have an effect on the nutrient composition of M. oleifera leaves and therefore, further studies need to be done to determine the effect of provenance sources other than those evaluated on the nutrient composition of M.oleifera leaves.

Keywords: *Moringa oleifera, Provenance, Proximate analysis, Nutrient composition.*

INTRODUCTION

In recent years, interest has grown in the utilization of Multipurpose Trees. These are trees that are deliberately grown and managed for more than one product. They may supply food in the form of fruits, nuts or leave that can be used as a vegetable and fodder while at these time supplying firewood, adding nitrogen to the soil, or supply some other combination of multiple outputs (Iqbal *et al.*, 2006). Nigeria is a net importer of food. The urban and rural areas are vulnerable to chronic food shortages, unbalanced nutrient, erratic food supply, poor quality food, high food cost and even total lack of food (Isaac, 2010). This phenomenon cuts across all age groups and categories of individuals on the rural and urban areas. There is high level of malnutrition among children in the rural and urban Nigeria. The figures differ with geopolitical zones, with 56 percent reported in rural area of the South West and 84.3 percent in three rural communities in the Northern part of Nigeria (Isaac, 2010). The problem of nutrition in Nigeria has not been adequately and critically analyzed, despite various approaches at assessing the challenges. At present, *M. oleifera* is a nutritious plant with high amount of natural diseases preventing nutrients (Isaac, 2010). A large number of studies have been done on the nutritional qualities of *M. oleifera*, however there is need to investigate whether provenance has effect on the nutrient composition of *M.oleifera* leaves (Ayerza, 2011).The findings of this study would contribute towards further realization of the tremendous potentials of this species.

MATERIALS AND METHODS

The leaves of *M.oleifera* were obtained from three (3) provenance sources: Alkaleri, Jos and Lafia. Alkaleri, is located in Bauchi State in the Sudan Savannah Zone, situated at 10. 7761° N, 9. 9992° E. It has an average rainfall of 1000mm a year which occurs between May and October with the relative humidity of about 53% it has an average temperature of 30°C. Jos is located in Plateau State in the Northern Guinea Savannah Zone. It is bounded in the north by Bassa local government, to the east by Bauchi state and the South by Riyom and Barkin-ladi local government areas (Binbol *et al.*, 2016). The climate of Jos Plateau is dominantly influenced by its relatively high altitude and position along the Inter Tropical Convergence Zone (ITCZ) and has an average height of about 1250 m above mean sea level. It has a mean of minimum and maximum temperature 16-26EC (Guntul *et al.*, 2007).It is controlled by 2 wind systems that affect the Nigerian climate, the moist South-westerly winds during the rainy season and the dry North Easterlies during the dry season. The South-westerly winds are responsible for much of the rains occurring between April and October, while the North Easterlies are responsible for the dry season lasting from November to Marc (Guntul *et al.*, 2007). Lafia is the capital city of Nasarawa State of Nigeria. It has a geographical extent of latitude 08° 33' N and longitude 08° 32' E. This is categorized to be within the north-central geopolitical zone of the country. Ecologically, it is also known to have the southern guinea savanna vegetation having an annual precipitation range of 1000 to 1500mm and mean annual temperature range of 24° C to 33°. This type of vegetation comprises mainly few trees, abundant woody shrubs and grasses. The soil of Lafia is predominantly sandy loam. Lafia, Nigeria is known to have two main seasons which are the wet and dry seasons. Wet season occurs between May to September while the dry season falls between October and April (Gbenga & Rahmad, 2020).

The materials used for this study include: Silica dish, Oven, Sulfuric acid, Kjeldahl flask, Distilled water (100cm³), 40% Sodium Hydroxide Solution, 10.0cm³ of 2% boric acid, Esther extract (oil), Soxhlet extractor, Reflex condenser, Small flask, Cotton wool, Thimble, 150 cm³ of petroleum ether, Electrothermal heating mantle, 500ml of glacial acetic acid, 450m³ of water, 50ml concentration of nitric acid, 20gm trichloacetic acid, water-jacket condenser, Wathman filter paper, Methylated spirit.

Sample Collection

Fresh sample of leaves from healthy plants were obtained from *Moringa* tree in the three (3) provenance sources: Alkali in Bauchi State, Jos in Plateau State and Lafia in Nassarawa State. The leaves were identified by a plant Taxonomist at Forest Herbarium Jos (FHJ), Federal College of Forestry Jos.

Preparation of Sample

The leaves were washed using distilled water to remove impurities, air dried in the laboratory for two weeks and pulverized into powder using mortar and pestle. The powder was then used for the analysis.

PROXIMATE ANALYSIS OF SAMPLE

The methods of the Association of Official Analytical Chemists (AOAC, 2005 and 2007) were used for determination of moisture content, crude fiber, protein, fat and nitrogen free extract (NFE). The Proximate values were reported in Percentages.

Moisture Content: The water content of *M.oleifera* leaves powder was determined by weighing 2-5 grams into a silica dish, which had been previously dried and weighed. The dish and sample were placed inside Kjeldahl flask in hot air oven for 24 hours at 60°C-70°C, as drying at high temperature may result in losses of heat labile and volatile component. Finally, it was dried to a constant weight, cooled for ten minutes in a desiccator before weighing. The dried portion was used for the determination of protein, ash, fat and crude fiber. Moisture content (%) = $100 \frac{(B-A)}{(C-A)}$

(B-A)

Where:

A = Weight of crucible (g)

B = Weight of crucible + wet sample (g)

C = Weight of crucible + dry sample (g)

Nitrogen Determination: The Nitrogen of Protein and other components were converted to Ammonium sulphate using acid digestion with boiling Sulphuric acid. Two and half grams (2.5g) of *Moringa* leaf powder was placed in a Kjeldahl flask and about 200milligram and catalyst mixture were added. Ten cubic centimeters (10cm³) of concentrated Sulphuric acid was added to the content of the flask. It was heated for one minute and frothing ceased and the heat was increased to digest for three (3) hours. It was allowed to cool with distilled water (100cm³). Ten (10.0cm³) aliquot of the diluted solution were distilled by pipette into the distillation chamber of macro Kjeldahl distillation apparatus. Ten (10cm³) of 40% sodium hydroxide solution was added

and steamed. It was distilled into 10.0cm³ of 2% of boric acid containing mixed indicator (note, colour changed from red-green) it was titrated with standard 0.01 N or 0.02 N hydrochloric acid to grey end point.

$$\text{Percentage (\%) N} = \frac{(a-b \times 0.01 \times 14.0657) \times 100}{D \times e}$$

- a = Titer value for the sample
 b = Titer value for the blank
 c = Volume to which digestion is made up with distilled water
 d = Aliquot taken for distillation
 e = Weigh of dried sample (my)

To convert to percentage crude protein, was multiplied by necessary conversation factor (6.25).

Ash Determination: The residue of *Moringa* leaves used in the moisture content determination was charred in the muffle furnace between 500°C-600°C until the ash turned to grey and was allowed to cool and then weighed.

$$\% \text{ Ash} = 100 \frac{B - A}{C - A} \times 100$$

Where:

- A = Weight of Crucible
 B = Weight of Crucible + Sample
 C = Weight of Crucible + Ash

Fat Determination (Ether-extract): The soxhlet was fitted with a reflux condenser, and a small flask which was dried in an oven and weighed. Two (2) grams of *Moringa* leaves powder was weighed and transferred to a "flat free extraction thimble" and plugged lightly with the aid of cotton wool. The thimble was then placed in an extractor and 150vm³ of petroleum ether (B.P 60-80°C) was added into a flask and siphoned over once. More ether was added into kjedahl flask until the barrel of the 100ml extractor was half filled, then the condenser was replaced and the joints were tighten and replaced on the water bath electrothermal heating mantle. Source of heat was adjusted and the ether was watched over until it siphoned. The flask was detached and the barrel content of the extractor was siphoned into the ether stock bottle. The thimble was removed and dried in an oven. The condenser and the flask were replaced and the ether was distilled continuously until the flask was completely dried and detached. The exterior which contains the oil was cleaned and dried in an oven. The extracted residue for the "fiber" determination was kept.

$$\text{Ether extracts} = \frac{\text{Weight of oil} \times 100}{\text{Weight of material}}$$

Crude Fiber Determination: Acid method for crude fiber determination was used according to (AOAC, 2005 and 2007). This is an alternative procedure to the more conventional method of Crude Fiber Determination. Five hundred (500) ml glacial acetic acid, 450ml of water and 50ml of concentrated nitric acid were mixed. Twenty (20) grams of trichloroacetic acid was dissolved in the mixture of *Moringa* leaves powder and one (1) gram of defatted was weighed out into 250 ml of conical flask. One hundred (100) of trichloroacetic acid was added into the flask. It was then refluxed for exactly 40 minutes counted from the time heating commenced. A three (3) feet long air condenser was used to process the loss liquid. The flask was disconnected and allowed to dry and weighed. It was washed ten (10) times with hot distilled water and methylated spirit or absolutely ethanol. The filter paper containing the residue was dried in an oven at 105° overnight, thereafter it was then transferred to a desiccator and weighed after cooling. Crucible ash was weighed together with the filter paper containing the fiber. It was allowed to ash overnight at 500° cooled and weighed. Then the percentage of the Crude Fiber was calculated.

$$\text{Crude Fiber Content (\%)} = 100 \frac{A-B}{C}$$

Where:

- A = Weight of Crucible
- B = Weight of Crucible with dry residue (g)
- C = Weight of Crucible with Ash (g)
- D = Weight of Ash (g)
- E = Weight of sample Digested (g)

Nitrogen Free Extract Determination: The nitrogen free extract of *Moringa* leaves powder was determined by adding the value of the moisture content (%), crude protein (%), crude fat (%), crude fiber content (%) and ash content (%) respectively by subtracting hundred 100.

$$\text{Nitrogen Free Extract (\%)} = 100 - (A+B+C+D+E)$$

Where:

- A = Moisture Content (%)
- B = Crude Protein (%)
- C = Crude Fat (%)
- D = Crude Fiber Content (%)
- E = Ash Content (%)

RESULTS

The results in table I show that the crude protein composition of *Moringa oleifera* leaves obtained from Lafia was significantly higher ($P < 0.05$) than in those obtained from Alkaleri and Jos

Table 1: Crude Protein composition (%) of *Moringa oleifera* leaves obtained from provenance sources

Location	Jos	Lafia	Alkaleri
1	24.63	26.06	21.26
2	22.69	26.83	21.15
3	23.91	26.41	22.01
Mean	23.74	26.43	21.47

LSD_{0.05}^{1.06}

The results in Table 2 show that the Ash composition of *Moringa oleifera* leaves obtained from Jos was significantly higher ($P < 0.05$) than those obtained from Alkaleri and Lafia.

Table 2: Ash composition (%) of *Moringa oleifera* leaves obtained from provenance sources

Location	Jos	Lafia	Alkaleri
1	13.19	9.87	9.74
2	13.31	9.7	9.16
3	13.55	9.53	9.45
Mean	13.35	9.0	9.45

LSD_{0.05}^{1.06}

The results in Table 3 show that the Crude Fibre composition of *Moringa oleifera* leaves obtained from Jos was higher but not significantly ($P > 0.05$) than those obtained from Lafia and Alkaleri.

Table 3: Crude Fibre composition (%) of *Moringa oleifera* leaves obtained from 3 provenance sources.

Location	Jos	Lafia	Alkaleri
1	14.33	14.25	13.8
2	14.2	13.91	14.3
3	13.94	14.01	13.95
Mean	14.16	14.06	14.02

The results in Table 4 show that the Fat composition of *Moringa oleifera* obtained from Jos was significantly lower ($P<0.05$) than those obtained from lafia and Alkaleri.

Table 4: Fat composition (%) of *Moringa oleifera* leaves obtained from 3 provenance sources

Location	Jos	Lafia	Alkaleri
1	5.98	7.69	8.33
2	4.96	8.91	8.23
3	6.91	8.18	8.17
Mean	5.95	8.26	8.24

The results in Table 5 show that the Moisture content of *Moringa oleifera* leaves obtained from Jos was significantly higher ($P<0.05$) than those obtained from Alkaleri and Lafia.

Table 5: Moisture Content (%) of *M. oleifera* leaves obtained from 3 provenance sources.

Location	Jos	Lafia	Alkaleri
1	3.67	3.55	2.84
2	3.5	3.4	3.04
3	3.6	3.49	2.91
Mean	3.59	3.48	2.93

LSD_{0.05}^{0.14}

The results in Table 6 show that Nitrogen Free Extract composition of *M. oleifera* leaves obtained from Alkaleri was significantly higher ($P<0.050$) than those obtained from Jos and Lafia.

Table 6: Nitrogen Free Extract (NFE) composition (%) of *Moringa oleifera* leaves obtained from 3 provenance sources.

Location	Jos	Lafia	Alkaleri
1	46.87	42.13	46.87
2	44.84	40.65	47.16
3	41.69	41.87	46.42
Mean	44.47	41.55	46.82

LSD_{0.05}^{2.5}

DISCUSSION

Crude protein levels of *Moringa oleifera* leaves from the three provenance source ranges between (26.83 %), (26.41 %) and (26.06 %). This is similar to the findings of Fuglie (2001) that suggested *Moringa oleifera* containing high amount of proteins. The crude protein of *Moringa oleifera* leaves from Lafia was higher than that of Jos and Alkaleri though there is no significant differences ($P > 0.05$). These variations may be due to the periodic higher temperatures in the Guinea Savanna at certain times of the year. Temperatures in Guinea Savanna rise beyond the actual climatic requirements of the *Moringa oleifera* plant (25 - 35°C) to a maximum of about 42°C in March. Although increasing temperatures activates enzymatic activities, higher temperatures beyond the plant requirement causes many cell proteins that function as enzymes or structural components to become unfolded or misfolded, there by leading to loss of proper structure and activity (Taiz & Zeiger, 2002). This agrees with the findings of Modi (2007) who reported that cool environmental conditions are associated with high total protein in leafy vegetables while hot temperatures had a significant decrease in leaf protein content. Apart from the higher temperatures in the savanna, the long periods of drought during the dry season could be a contributing factor.

The ash content of *Moringa* leaves obtained from Jos has significantly higher ($P < 0.05$) with a value of (13.35 %) than those obtained from Lafia and Alkaleri with a value of (9.0 %) and (9.45 %) respectively. Thus, the ash content of the dried leaf powder is considered to be a measure of the mineral content. The results indicate that the dried *Moringa* leaves have high deposits of mineral elements, which is in agreement with the findings of Moyo (2011) who worked on nutritional and functional properties of *Moringa oleifera*. Therefore, the dry matter content value reported by William Jasper Asante (2014) was higher (30.92%) than the value obtained in this study. The high dry matter content of savanna leaves makes them more favourable for animal fodder. William Jasper Asante (2014) indicated that the higher the dry matter of fodder, the higher its digestibility (the proportion of a feed an animal can use to satisfy its nutritional requirements). He further reported that digestibility is positively related to the energy content and protein of animal fodder.

Nuhu (2010) also indicated that *Moringa oleifera* leaf meal could be used to improve daily weight gain, dry matter and crude protein digestibility of rabbits. In the current situations where natural range lands are getting extinct due to rapid encroachment, *Moringa oleifera* can be incorporated into agroforestry practices to provide fodder to increase animal production in Jos. The Crude Fiber Content of *Moringa* leaves obtained from Jos is higher than those obtained from Alkaleri and Lafia. Our findings are not in agreement with Biel (2017) .Who reported that crude fibre was not found in the extracted leaves of *moringa oleifera*. They work on nutritional quality and safety of *Moringa oleifera* leaves as an alternative source of protein and minerals. However, the crude fiber value reported in our study is higher than (14.16 %) the value reported by Salma Sultana 2020 (6.00–9.60%) who worked on nutritional and functional properties of *Moringa oleifera*.

The Fat Content of *Moringa* obtained from Jos is significantly low ($P > 0.05$) with a value of (5.95 %) than those obtained from Lafia and Alkaleri with a value of (8.26%) and (8.17 %) respectively. Therefore, the findings of this study is similar to findings of Salma (2020) who reported that *Moringa* leaves contain a low fat content (4.03-9.51%) which is desirable. Moreover, *Moringa* contains more dietary polyunsaturated fatty acids than saturated fatty acids. A higher content of polyunsaturated fatty and a lower amount of saturated fatty acids is desirable (Hoffman *et al.*, 2016). As such, the inclusion of polyunsaturated fatty in the diet is recommended, as they can

prevent the occurrence of diseases, thereby promoting good health. Therefore, low level of fat shows that Moringa is not a source of lipids that could cause arteriosclerosis; hence, a leaf extract would be suitable for individuals suffering from or prone to cardiovascular diseases.

The Moisture content of Moringa oleifera leaves obtained from Jos was significantly higher ($P < 0.05$) with a value of (3.59 %) than those obtained from Alkalari and Lafia. This findings is in agreement with the findings of William Jasper Asante, (2014) who recorded high moisture content ranging from 67 to 75% respectively. This shows that high moisture content recorded by William *et al.*, (2014) is significantly higher (67 to 75%) than the value obtained in this study (3.59 %). The low moisture values recorded in Moringa oleifera leaves from the Alkalari and Lafia may be associated with hot and dry winds of the harmattan during sample collection (December) which comes with high rates of evapotranspiration. Modi (2007) reported that transpiration rates are at their peak in hot dry/windy environment. The effects of annual harmattans are more severe in Lafia and Alkalari (savanna) than the plateau's montane grasslands of Jos. During this period, atmospheric humidity becomes very low in Alkalari and Lafia. However, with plant leaves relative humidity between cells approach 100%, therefore when stomata opens, water vapour inside the leaf moves out forming a bubble of higher humidity around the plant. The difference in relative humidity around the stomata and adjacent air regulates transpiration rates and pulls water up through the xylem tissues (Taiz & Zeiger, 2002).

The Nitrogen Free Extract (NFE) in the samples from Alkalari is higher (46.82 %) than that of Jos and Lafia this could be due to higher temperatures. Although photosynthesis and respiration are inhibited at higher temperatures, photosynthetic rates drop before the respiration (Modi, 2007). At any temperature above the plant temperature compensation point, photosynthesis cannot replace the carbon used as a substrate for respiration. As a result, carbohydrate reserves decline, and also lead to loss of sweetness as well. Taiz and Zeiger (2002) reported imbalances between photosynthesis and respiration and associated it to the deleterious effects of high temperatures on plant development. The sugar content of Moringa oleifera leaves from Jos and Lafia were (44.47 %) and (41.55 %) respectively.

CONCLUSION

This study investigated the effect of provenance on the nutrient composition of *M.oleifera* leaves obtained from three (3) provenance sources. The results showed that provenance have an effect on the Nutrient Composition of *M.oleifera* leaves. Therefore, further studies need to be done to determine the effect of provenance sources other than those evaluated on the nutrient composition of *M.oleifera* leaves.

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