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National Animal Production Research Institute
Ahmadu Bello University
P.M.B 1096, Shika-Zaria,
Kaduna State, Nigeria.
Email:japr@napri.gov.ng Url:journal.napri.gov.ng

PROXIMATE COMPOSITION AND SOME BIOACTTIVE COMPONENTS OF *Moringa oleifera* SEEDS AT THREE STAGES OF MATURITY

***Odafe, S. G. and Imasuen, J. A.**
Dept. of Animal Science, Faculty of Agriculture, University of Benin, Benin-City Nigeria
**Corresponding Author: +234(0)81303319; shalomeodafe@gmail.com; gideon.odafe-shalome@uniben.ed*

ABSTRACT

Moringa oleifera seeds were evaluated for proximate and phytochemical components at three maturity stages of plant growth. Alkaloid content varied significantly (P<0.05) between the three stages. Alkaloid content (g/100gdm) increased from 0.61 in 4thyear to 0.81 in the 6th year and then decreased significantly (P<0.05) to 0.41 in the 7thyear of growth. Oxalate content (mg/100 g) decreased non-significantly (P>0.05) from 3.69 in the 4th year to the low concentration of 3.0, in the 6thyear of growth and then increased significantly (P<0.05) to 3.38 in the 7thyear. Saponin decreased from 4.15 in the 4th year to 2.05 in the 6th year of growth and then decreased further to 1.74 g/100gdm, in the 7thyear. The decrease was significant (P<0.05) between the 4th and 6thyear, but not significant (P>0.05) between the 6th and 7th year of plant maturity. Tannin content (mg/100g) increased significantly (P<0.05) from 1.79 in the 4th year to the highest level of 13.71, in the 6th year and then decreased significantly to 5.12 in the 7th year of plant growth. Mean values of proximate composition (g/100g) ranged from 32.80-43.33 crude protein, 3.46-6.31Ash, and 10.46-17.42 carbohydrate. Crude fibre values were 6.36-8.20, lipid: 21.03-30.36, and moisture: 9.15-9.37. Crude fibre, ash, protein and moisture levels were significantly (P<0.05) higher at the 6th than at 4th and 7th year of maturity. It is recommended that moringa seeds be exploited as supplements in nutrition and health.

Keywords: Moringa seeds, Proximate composition, Alkaloids, Saponins tannins, Oxalates

INTRODUCTION

Moringa (*M. oleifera)* plant is widely cultivated in Nigeria, where it is known by various tribal names -Zogallagandi (Hausa), Ewe-igbale (Yoruba) and Okwe-oyibo (Igbo) - Keay (1989). It is a high potential economic plant with variable functional properties; the seeds and leaves are useful in traditional medicine and food (Ram, 1994; Makkar and Becker,1997; Muzquiz,2004; Ferreira *et.al*,2008; Leon *et.al*,2008; Bukar *et.al*, 2010; and Oluduro, 2012). There are also indications that the concentration of phytochemicals in plants tend to vary within and between plant species and between different parts of same plant (Hyder *et al*, 2002; Springer *et al.*, 2002; Kowalski and Wolski, 2006). Earlier reports indicated that pods and seeds of *M. oleifera* contain insignificant amounts of tannins whereas saponins and alkaloids were present in biologically significant, but non-toxic levels in the leaves and stem (Makkar and Becker, 1997).

Nutritional studies, reported by Igwilo *et al* (2010), suggested that experimental feed containing 35.7% moringa seed, and administered to rats for 21 days did not support growth, but rather there was decline in body weight. That was despite high protein and fat contents of the seed. This implies that either the seed protein is not digestible or the endogenous toxins - non-nutrient chemical substances in plants, hindered digestive processes and likely interfered with normal metabolism in the animal subjects. The balance between the beneficial

and hazardous effects of the secondary metabolites thus depends on their concentrations, chemical structure, time of exposure and interaction with other dietary components.

This study was conducted to ascertain the levels of four phytochemicals in the seeds of *M. oleifera.* Each of the four - oxalate, tannins, saponins and alkaloids - represent one major class of non-nutrient components of plants, which have known biological properties, tonicity values and varying degrees of mammalian toxicity. The objectives were to determine by qualitative and quantitative analyses the chemical composition of seeds, compare the nutrient and non-nutrient components in the seed, as the plant matured through three stages of growth.

MATERIALS AND METHODS

Sourcing and preparation of samples

Naturally dried, high quality pods of *M. oleifera* were harvested from trees cultivated in botanical gardens that are located in the different areas of Benin-City, which lies between latitude 6˚00′ and 7˚30′N and longitude 5˚.00′ and 3˚00′E, in the rain forest region of Nigeria (daily temperature:22-34˚C; relative humidity:79-90%; annual rainfall: 2000mm; soils: lateritic). The trees were randomly selected with the help of the Garden Curators who identified the trees and ascertained their ages.

Pods that were harvested were grouped into sets according to the age of trees - 4^{th}, 6^{th} and 7^{th} year of maturity. Each set of samples was processed separately and labelled accordingly. The pods were split with ease by hand, to remove the winged and coated seeds, which were spread on trays and left to stand for 24 hours. Thereafter seed coat was removed by gentle abrasion using laboratory mortar and pestle. Broken seed coat and wings were separated from clean seeds by winnowing and the clean seeds milled into flour using a Eurosonic electronic blender, model ES-1731F. Seed flour was sieved through 250μm mesh; to separate seed coat particles; residues were discarded while the cleaned seed flour obtained were transferred separately into airtight plastic containers, sealed and labelled and then kept in cold storage until required for analyses.

Proximate Analyses

Proximate analyses of seed flour were performed using standard procedures as outlined by AOAC (2005). The constituents determined were moisture, ash, crude fibre, crude protein and carbohydrate. The samples (5g each) were oven-dried at 105^0C until constant weight was achieved, and then moisture content was computed using standard equation which expresses weight of dried sample in relation to initial weight (5g) of fresh sample (AOAC, 2005). Oil was extracted from 2g of sample with petroleum ether using the Soxhlet system; while ash was estimated by incinerating 2g of sample in a Uniscope muffle furnace, model SM 9080, set at 550^oC for 3 hours. The micro-Kjedahl method was used for crude protein determination; while crude fibre was obtained by subjecting defatted samples through a process involving boiling under reflux with a solution containing H_2SO_4, filtering and incinerating the final residue. Total soluble carbohydrates - nitrogen free extract (NFE) was determined by subtracting from 100 the summation of values of the other components.

Phytochemical Analyses

Oxalate

The procedure described by Day and Underwood (1986) was followed to determine the oxalate content. 75ml of 1.5N H_2SO_4 was added to 1g of the sample. The solution was placed on magnetic stirrer for about one hour and then filtered using Whatman No.43. A portion (25ml) of the sample filtrate (extract) was collected and heated to 80 – 90^oC against 0.1N $KMnO_4$ solution until a faint pink colour appeared and persisted for 30 seconds. The oxalate content was obtained as follows: 1ml of

0.1 $NKMO_4$ = 0.9004 mg/g. % oxalate = Titre value Titre value × 0.9004 mg/g.

Saponin

The Saponin content was determined by the methods of Obadoni and Ochuko (2001). Portions (20g each) of milled seed sample were measured into a conical flask and 100cm^3 of 20% aqueous ethanol was added. The samples were heated in a water bath for 4hr with continuous stirring at 55°C. Each mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml by heating over water bath at about 90°C. The concentrate was transferred into a 250ml separator funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated; then 60ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponins content was thus calculated as percentage.

Alkaloids

Alkaloid was determined by the Harborne (1998) method. A measure of each sample (5g) was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added and allowed to stand for 4hr; then filtered and the extract was concentrated in a water bath to one-quarter of the original volume. Concentrated NH_3OH (ammonium hydroxide) was added to the extract drop wise, until the alkaloid precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The alkaloid was dried to remove residual solvent, and then weighed.

Tannin

Tannin was determined by the Van-Burden and Robinson (1981) method. A measure (500mg) of each sample was weighed into a 50ml plastic bottle and filled to mark with distilled water; shaken for 1hr on a Contech mechanical shaker, model CS 10; and then filtered into a 50ml volumetric flask and made up to mark. Then 5ml of the filtrate was transferred by pipette into a test tube, and mixed with 2ml of 0.1M FeCl3 in 0.1N HCl and 0.008M potassium ferricyanide. The absorbance was measured at 120nm, within 10 min, using an SPC spectrophotometer, model 260.

Statistical analysis

Variance analysis was performed on the data generated using GenStat 12th edition at 5% probability level. Mean separation and tests of significance were achieved using Duncan Multiple Range Test (DMRT) of the same statistical software (Duncan, 1955).

The statistical linear model for this experiment was: $Y_{ij} = \mu + \alpha_i + \varepsilon_{ij}$

(where: Y_{ij}=Individual observation, μ=Overall mean, α_i=Effect of treatment, ε_{ij}=Error term).

RESULTS

Proximate composition of *M. oleifera* seeds at different ages

The results of proximate analyses of the moringa seeds are presented in Table 1. The levels of crude protein, ash, carbohydrates, crude fibre, lipid and moisture in the seeds were significantly different ($p<0.05$) between the three sets (age groups) of moringa plants. Crude protein content increased significantly ($p<0.05$) with maturity from the 4th year up to the 6th year and then decreased non-significantly ($p>0.05$) in the 7th year. Ash and crude fibre contents also increased from the 4th year to 6th year and decreased in the seeds of 7th year plants (Table 1).

Carbohydrate content of the seeds measured was 17.41% in the 4th year plants and progressively decreased as the plants advanced in age. Seeds from the 4th year group recorded the highest

ether extract content (30.36%), which reduced significantly (P<0.05) in the 6th year (21.02%) and then increased significantly (P<0.05) in 7th year (27.09%). The level of moisture was not significantly (P>0.05) different between seeds of the different age groups.

Table 1: Proximate composition of *M. oleifera* seeds (g/100gDM)

	Age of Maturation			
Proximate composition	**4th year**	**6th year**	**7th year**	**SEM**
Ash	3.46[c]	6.32[a]	4.60[b]	0.01
Carbohydrates	17.42[a]	11.21[b]	10.46[c]	0.041
Crude fibre	6.79[b]	8.20[a]	6.36[c]	0.03
Ether extract	30.36[a]	21.03[c]	27.09[b]	0.03
Moisture	9.15[a]	9.37[a]	9.15[a]	0.01
Crude protein	32.801[c]	43.903[a]	42.332[b]	0.004

[abc]Means with different superscript along rows are significantly different (P<0.05).

Phytochemicals in *M. oleifera* seeds at different ages of maturity

Alkaloid content of *M. oleifera* seeds varied significantly (P<0.05) between the different stages of growth (Table 2). Seeds harvested from the 6thyear plants had the highest concentrations of alkaloid; which was significantly (P<0.05) higher than the levels in the 4thyear, which was highly significantly (P<0.01) higher than the level in seeds of the 7thyear plants.

Oxalate content (mg/100 g) of *Moringa* seeds in 7thyear plants was significantly (P<0.05) higher than the level in seeds harvested from 6thyear plants; but not significantly (P>0.05) different from the level in the seeds from 4thyear plants.

Saponin concentrations (g/100 g) in seeds decreased with age of plant. The decrease was significant (P<0.05) between the 4th and 6thyear, but not significant (P>0.05) between the 6th and 7th year of plant maturity.

Tannin content (mg/100g) was lowest in the seeds of 4th year plants (1.80). The seeds from 6th year plants recorded the highest concentration (13.71) which was significantly higher than the concentrations in seeds of 4th year (p<0.05) and seeds in the 7th year (p<0.01) plants (Table 2).

Table 2: Phytochemical components of different ages of *Moringa* seeds

	Age of Maturation			Dd
Phytochemicals	**(I) 4th year**	**(II) 6th year**	**(III) 7th year**	**SEM**
Alkaloid (g/100g)	0.61[b]	0.81[a]	0.41[c]	0.001
Oxalate (mg/100 g)	3.69[ab]	3.00[b]	3.89[a]	0.223
Saponin (g/100g)	4.15[a]	2.05[b]	1.74[b]	0.091
Tannin (mg/100 g)	1.80[c]	13.71[a]	5.12[b]	0.011

[abc] Means with different superscripts in the same row differ significantly (P<0.05)

DISCUSSION

Proximate composition of *M. oleifera* seeds at different stages of growth

The results of this study indicate that the proximate composition of the seeds of moringa plant grown in Benin metropolis, have similar values with those reported elsewhere by previous workers (Anwar et al, 2005; Anwar and Rashid, 2007; Rahman et al, 2009; Compaore et al, 2011; Barakat and Ghazal, 2016; Oladeji *et al,* 2017). Secondly, our findings evidently confirm the general assertion that *Moringa* seed is, potentially, a rich source of nutrients. At the three stages of plant growth examined, the seeds contained high levels of crude protein (CP); and this qualifies it as a classical protein source, i.e. a biological or agricultural product that contains no less than 20%CP (Olomu, 2011). The CP content (g/100gdm) of moringa seeds, at 4th, 6th and 7th year of growth was 32.80, 43.90 and 42.33 respectively. This implies that the seeds can be harvested and exploited nutritionally, and for its protein content at any of these stages of growth. According to the result of this study however, the best time to harvest the pods is when the plant is in the 6thyear, when CP content is about 44%.

Moringa is a non-legume; barring experimental and laboratory variations, the mean CP values measured in the seeds, at the three different stages of growth are higher than the values of CP for some non-leguminous feeding stuff, including cotton seed, sunflower seed, sesame seed and canola seed (Olomu, 2011). Conversely, these results indicate that moringa seeds also compare favorably with legumes such as soybean, groundnut, bambara nut, which are conventional protein sources having CP values that are higher than 20% as reported by Egedege (2012).

Of interest is the fact that like soybean, moringa plant produces seed that is evidently rich in oil and high in protein. This study recorded 21.03 - 30.36% oil content; corroborating reports that *Moringa* is potentially an oilseed (Anwar *et al*, 2005; Anwar and Rashid, 2007; Rahman *et al*, 2009; Igwilo *et al*, 2010; Latif *et al*., 2011; Compaore et al, 2011; Bamishaiye *et al*,2011; Pandey *et al*, 2011; Ogunsina *et al*, 2014; Barakat and Ghazal, 2016; Oladeji *et al*, 2017). The highest level of oil was recovered for the seeds when the plant was 4 years old. Hence this report is suggesting that seeds for oil extraction should be harvested when the plant is at 4th year of growth. In parallel, the prospects are very high, that defatted moringa seeds could provide an economical source of protein, as an alternative or as protein supplement in traditional diets. Studies on the characteristic and properties of moringa seed oil and proteins would therefore be a worthwhile venture in further research.

The moringa seed may not be considered a carbohydrate source; because in the older plants we observed that the carbohydrate content of the seeds reduced significantly ($p<0.05$) while protein content increased. The ash content of moringa seed was relatively low (3.46-.6.31 %) especially at the early stage of maturity. This indicates that at this stage, some minerals are not yet fully established and hence may not be available for utilization. Thus, we recommend that when seeds are harvested for use as feeding stuff at the 4th year of growth, mineral supplements should be included in the dietary formulations for animals. Also in this study, moisture content measured in the seeds was notably low, i.e. below 10%, implying low water activity in the seeds. This could be of advantage to farmers and processors of moringa seeds; because it means that inexpensive and minimal drying and packaging processes would be required to secure a long period of storage and preservation of the seeds.

Phytochemical components of *M. oleifera* seeds at different stages of growth

This study was aimed at ascertaining the presence and concentration of four phytochemicals in *M. oleifera* seeds harvested at three stages of plant growth. Some phytochemicals may elicit very harmful biological responses, while some are widely applied as nutraceuticals or pharmacologically-active agents. The phyto-compounds are not established nutrients but nevertheless could potentially serve as vegetable drug for maintaining organs and systems in human body; and hence seem to contribute significantly to protection against degenerative health conditions (Dreosti, 2009; Egedege, 2012; Oladeji *et al*, 2017).

It is generally considered that the concentrations of phytochemicals increase with plant maturity. Hence this study presented results that are at variance from some earlier published results but also similar to others. The four phyto-compounds - alkaloids, oxalate, saponin and tannin - assayed in this study, were present in moringa seeds at the three different stages of maturity (4th, 6th and 7th year); and at levels that are significantly lower than the mean values previously reported for each of these compounds in moringa seeds by Kawo *et al* (2009).

Alkaloids are known to impart bitter taste to most foods (Ijeh *et al*., 2010). Low doses of some alkaloids tend to mediate important pharmacological activities; whereas very high doses of alkaloids may present toxic or fatal effects (Simee, 2011). The alkaloids present in the moringa seeds examined in this study occurred at levels that are comparable to the amount (0.85%) found in African bread-fruit which is a popular and well consumed commodity, by local communities within and around Benin metropolis, according to reports by Ijeh *et al* (2010). This implies that the level of alkaloid in moringa seeds can as well be tolerated by humans. However, the types and characteristics of the alkaloids in these plants require determination.

The findings of this study also corroborate the assertions that young plants contain lower amounts of oxalate (Singh and Singh, 1992 Achakzal *et al*, 2009), along with higher amounts of alkaloids (AchakzaI *et al*, 2009; Dung, 2012) than the older plants. We observed that the younger (4th and 6th year) moringa plants contained significantly ($P<0.05$) higher levels of alkaloids and lower levels of oxalate in their seeds, than the older (7th year) plants. Oxalate content of *Moringa* seeds in 7th year plants was significantly ($P<0.05$) higher than the level in seeds harvested from 6th year plants; but not significantly ($P>0.05$) different from level in seeds from the young 4th year plants.

Tannin content was independent of age; seeds from plants in the 6th year of maturity contained the highest level of tannin compared to the 4th year seeds, which had the lowest level. The mean tannin value of 13.71 mg/100g we found in this study was recorded for the 6th year seeds. That value is lower than the total acceptable

tannin daily intake for man, which has been pegged at 560 mg/100g (Gemede and Ratta, 2014). Thus the level of tannin in *M. oleifera* seed indicates that up to 100 g of moringa seeds can be consumed without any deleterious effects attributable to tannin.

Whereas there have been the assertions that older plant parts contain higher levels of tannin, while young parts show mixed lower trend in tannin contents (Singh and Singh, 1992: Achakzal *et al*., 2009). This study has shown that there is no relationship between age of plants and tannin levels in structural parts, such as the seed; just as Reed (1995) observed that tannin concentration was variable between different chronological stages in the same tree; while there was also variation between young plants, premature plants and mature plants in their tannin content. These observations are not inadmissible, because the relative differences in the maturity age of plants considered for study coupled with the fact that tannins are polyphenols with variable characteristics, determined by species differences, ecological and bioclimatic factors (Cordenunsi *et al*, 2002; Egedege, 2012) would always pose a variation in results obtained.

The concentration of saponin in moringa seeds, as revealed by the results of this study, indicate a tendency to decrease as the plant matures into the late years. A significant ($P<0.05$) decrease was recorded for saponin levels as the plant grew progressively from the 4^{th} year to the 7^{th} year. Thus, unlike tannin and oxalates, which increased with age, maturity dependent decreases in saponin level are characteristics of moringa seeds as the plant advances in age. This indicates that moringa seed and its derived products would create no significant hazard for the consumer; hence this study suggests further characterization of the phytochemicals and determination of their critical toxicity levels by incorporating moringa seeds at graded levels in trial diets for different species of animals.

CONCLUSION AND RECOMMENDATIONS

Alkaloids in *M. oleifera* seeds increased with age during the 4^{th} to 6^{th} years of plant maturity, and later declined at an older age. The concentration of oxalate increased with age of maturity; oxalate content was higher at the 7^{th} year than at earlier years. Saponin content of seeds decreased progressively with age of plant. Tannin content was independent of age of plant. Seeds from plants in the 6^{th}year of maturity contained the highest level of tannin, compared to seeds in the 4^{th} year, which had the lowest concentration.

Proximate analyses showed high nutrient density during the 6^{th} year of growth. Seeds can serve as potential protein source, CP (32.80-43.33%); and as oilseed, EE (21.03-30.36%). The carbohydrate content (10.46-17.42%) of seeds was high at the 4^{th} year of maturity and progressively reduced as the tree matured into late age. Ash, crude fibre, and moisture levels were significantly ($P<0.05$) higher at the 6^{th} than at earlier and later years of maturity. The low moisture content (9.15-9.37%) imply possible long period of storage of the seeds.

This study recommends that moringa seeds be exploited as supplements in nutrition and health. Seeds for oil should be harvested in the 4^{th} year; seeds for protein are prime in the 6^{th} year. Hence studies to characterize the seed oil and protein and to determine their properties and economics would be necessary. The characterization of each bioactive compound and bioassay trials to determine their properties and interactions in animal systems is also recommended.

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