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Effect of tree age on nutritional, anti-nutritional and proximate composition of *Moringa stenopetala* leaves in South west Ethiopia

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Moringa stenopetala **is one of the indigenous agro-forestry tree species cultivated in multi-storey inter-cropping system in the southern dry land areas of Ethiopia. It is an economically important tree species in which most parts of the plant are used for different purposes. Leaves of the plant collected from age classes of the tree are edible after processing. Despite its wider usage in the southern parts of the country, little is known about the nutritional composition of the leaves at different age classes of the tree. We investigated the effect of tree age on nutritional, ant-nutritional and proximate composition of** ***M. stenopetala*** **leaves and determined the moisture content, ash content, crude fiber, crude protein, fat, tannin content, phytate content, iron, calcium, phosphorus and potassium in the leaves. Authentic representative** ***M. stenopetala*** **leave samples were collected from 3, 4, 5, 6 and 7 years old tree in** ***Derashe*** **area, southern part of Ethiopia. The collected leaf samples were dried and subjected to physico-chemical analysis following standard methods of analysis. The laboratory results were analyzed using SAS Statistical software. The analysis indicated a significant difference in the main effect of all nutrition and anti- nutritional parameters between the five classes at 1% significant level. However, there was no significant difference in the cationic composition of iron and zinc among age classes of the tree. This study suggests that plant age has an influence on nutrition, anti-nutritional composition and most of the proximate elements.** ***M. stenopetala*** **trees that grew up well and reached age of five had better nutritional, anti-nutritional and proximate composition implying that leaf collection should to be done on trees that are established and grown well and reached at least five years of age.**

Key words: Moringa stenopetala, nutritional, anti-nutritional, proximate composition.

INTRODUCTION

The Eastern Africa region is predominantly a dry land with about 60% of the total land area classified as arid or semi-arid (Funk et al., 2008). The rural economy is dominated by subsistence agriculture or pastoralists that

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are dependent on the available natural resources. Drought poses the greatest challenge to the livelihoods ofcommunities in this region, a problem that is compounded by over-reliance on traditional farming/ pastoral activities, which cannot cope with adversity brought about by drought. Moringa stenopetala is widely cultivated in Southern Ethiopia especially in Gamu-Gofa, Sidama, Konso and the adjoining provinces Abuye et al. (2003). *Moringa* is a drought tolerant fast growing indigenous tree mainly planted and maintained for its nutritional value (Assefa et al., 2015). Literature shows that Moringa leaves are rich sources of minerals and amino acids (Anjorin et al., 2010; Wakil and Kazeem, 2012).

Related to the above mentioned points, the country gives great attention to the most important local survival strategies and intensifies the agricultural system to overcome the reaped climatic shocks that hamper agricultural production leading to food shortage (Guinad and Lemessa, 2000). Strategies to overcome climatic shocks and reduce agricultural production include strengthening the households' resources base (Erhabor and Emokaro, 2007; Ibrahim et al., 2009) and diversifying alternate food sources (Ghosh et al., 2011). This study focused on determining the appropriate age of *Moringa stenopetala* for better nutritional composition. We investigated impact of different age classes on nutritional value of *M. stenopetala* leaves.

MATERIALS AND METHODS

Site description

The plant leaves for the experiment were collected from the *Derashe woreda* of the South Nations and Nationalities Peoples Region (SNNPR). *Derashe woreda* is one the best potential sites for growing and production of *M. stenopetala* trees. It is located in the Great Rift Valley Region at 630 Km South west of Addis Ababa in Ethiopia, at 7°4°N latitude and 38°31°E longitude. The *Woreda* has an altitudinal range from 1300 up to 2545 m above sea level and average annual rain fall of 952.1 mm. The mean temperature ranges between 12 and 27°C.

Sample preparation

M. stenopetala tree ages of 3, 4, 5, 6 and 7 were randomly selected from farmers' field/garden. The fresh leaf samples were collected in the morning by hand from the crowns of each selected tree in four directions (North, South, West and East) to capture possible variations in the leaf characteristics. Composite leaf samples per tree were taken for further analysis. The sample leaves were transported to the Wood Technology Research Center in Addis Ababa in plastic bags. The leaves were spread on thin canvas sheet placed in a greenhouse and dried in the open air until the leaves were dried uniformly. The dried leaves were hand-crushed and further grounded using mortar and pestle to 2 mm size. The crushed leave samples were then put in the paper bag and kept at room temperature until the laboratory analysis was carried out.

Nutritional and proximate composition analysis

Analysis on the nutritional and proximate composition of *M. stenopetala* leaves was done in the nutritional laboratories of Ethiopian Health Institute. The nutritional aspects/characteristics of the *Moringa* leaves included moisture content, ash content, crude fiber, nitrogen content, crude protein and mineral contents.

Determination of moisture content

Three replicates of ground Moringa leaves that weigh accurately 2 g from each tree age was weighed and dried at 105°C for 5 h using Oven (Memert Germany ALC-210.4 model). Oven dry weight was recorded after allowing the samples to cool in a desiccator before reweighing. Moisture content was expressed as a percentage of the weight loss from the original weight (Yebeyen et al., 2009; Nilsen SS 2010.

Determination of ash

Three replicates of grounded *Moringa* leaves that weigh accurately 2 grams from each tree age were first heated on a burner in air to remove its smoke. Then each leaf sample was burned in a furnace at 550°C for 1.30 h. The ash content was expressed as a percentage ratio of the weight of the ash to the oven dry weight (Nielsen SS 2010).

Determination of total nitrogen and crude protein

Exactly 0.5 g of each Moringa leave sample, in three replicate for each tree age, was weighed and transferred into 2300 Kjeldahl digestion tube plus one Kjeldahl tablet, copper sulfate-potassium sulfate catalyst. Then 10 ml of concentrated, nitrogen free and sulfuric acid was added. The tube was then mounted in the digestion heating system which was previously set to 240°C and capped with an aerated manifold. The solution was then heated at the above temperature until a clear pale yellowish-green color was observed. This indicates the completion of the digestion. The tubes were then allowed to cool at room temperature. Their content was quantitatively transferred to Kjeldahl distillation apparatus followed by addition of distilled water and 30% (w/v) sodium hydroxide. Steam distillation was then started and the released ammonia was absorbed in 25 ml of 2% boric acid. Back titration of the generated borate was then carried out versus 0.02 M Hydrochloric acid using methyl red as an indicator. Blank titration was carried out in the same way. The percentage of nitrogen content was then calculated (AOAC, 1990).The protein content was calculated using the nitrogen conversion factor of 6.25 as proposed by Greenfield and Southgate, 2003).

Determination of mineral content

Ash from a sample of Moringa leaves was dissolved in concentrated sulfuric acid. Then, the solution was used for the determination of the minerals studied except for phosphorus which was determined by an atomic absorption spectrometer. Appropriate standard solution was prepared for each metal and used by atomic absorption spectrometer (model AAA-6800) to prepare the graph for the determination of the amount of each metal from the test solution. Wet ash method was used for the determination of phosphorus in the sample leaves. The ash was dissolved in vanadomolybdic acid reagent in which phosphate reacts to form a yellow molybdovanadophosphoric acid. Finally, the amount of phosphorus was determined using CECL 1021 model a UV–VIS spectrometer at 400 nm in 1 cm cells, and expressed as % Na_3PO_4 (AOAC, 2005).

Table 1. Analyses of Variance for characterization of *M. stenopetala* leave with different age for nutritional and proximate composition analysis.

Source of variation	DF	Average Mean									
		MC	AC	CF	CP	Fat	Fe	Zn	Ca	P	K
Age	4	8.98***	11.22***	35.74***	32.29***	0.38***	18.58^{ns}	1.44ns	282.03***	352.05***	1710.46***
CV		2.13	5.73	0.53	0.48	4.27	22.13	20.43	7.89	2.56	0
R^2		0.98	0.91	0.99	0.99	0.88	0.45	0.08	0.55	0.99	1

***: Significant at p = 0.001; **: Significant at p = 0.01.

Phytate determination

This was determined using the method described by Latta and Eskin (1980). A 0.5 gm of dried *Moringa stenopetala* sample leaves was extracted with 10 ml of 0.2N HCl for 1 h at an ambient temperature. 2 ml of wade reagent solution was added into 3 ml of the supernatant extraction solution; the solution was homogenized and finally centrifuged for 10 min at 3000 rpm. The clear supernatant solution was taken to measure the absorbance at 500 nm using UV-VIS spectrophotometer (CECL 1021 model mad in England). The amount of Phytic acid was calculated using Phytic acid standard curve and result was expressed as Phytic acid in mg/100 gm (Burns et al., 2001).

Tannin determination

This was determined using the method described by Burns et al. (2001).One gram of dried M. stenopetala leaf sample was extracted with 10 ml of 1% HCl in methanol using mechanical shaker for 24 h at room temperature and centrifuged for 5 min. 1 ml of the clear supernatant solution was taken and mixed with 5 ml of Vanillin HCl reagent and stood for 20 min until the reaction was completed. The absorbance of the clear supernatant solution at 500 nm was measured using UV-VIS spectrophotometer (CECL 102 model mad in England). The amount of Tannin was calculated using SPSS plot's slope; and intercept of the standard curve is as given in the mathematical equation (Latta and Eskin, 1980).

$$Tannin\ in\frac{mg}{g} = (As - Ab) - \frac{Intercept}{Slope * d * w}$$

Where: As = Sample absorbance, Ab = Blank absorbance, d = Density of solution (0.791 g/ml) and w = Weight of sample in gram.

Data analysis

The nutritional value, photochemical and anti-nutrient analysis data were subjected to one way of variance analysis (ANOVA) statistical method using Generalized Linear Models Procedure (GLM) (Gomez and Gomez, 1995). A total of five age treatments with three replications and 12 test parameters were designed in the experiment. Statistical analysis of data was carried out using SAS Version 9. Means that exhibited significant differences were compared using Least Significant Difference (LSD) at (P <0.001) level.

RESULTS AND DISCUSSION

Nutritional and anti-nutritional composition parameters

The analysis of variance showed that the effect of all nutrition and anti- nutritional composition parameters differed among the five tree ages at 1% significant level. Exception to this was for the cationic composition of iron and zinc that did not show any difference between tree ages (Table 1).

Moisture content

The overall mean moisture content for the *M. stenopetala* leaves across the age in the experiment was 8.99%.Trees at the age of four had significantly higher moisture content (10.60%), followed by trees at the age of three (9.88%). The lower moisture content of leaves was obtained at trees with the age of five (7.79%) and six (8.07%) (Table 3). In this study, the moisture contents of matured tree of 6 and 7 years *M. stenopetala* leaves were compared (Abinet et al., 2011). Almost similar values were observed. Similar to the situation, we have noted that maximum moisture content (9.88, 10.6 and 7.79%) was found in the study on tree of 3; 4 and 5 of *M. stenopetala* leaves compared to the previous results (6.43 to 6.83%) obtained by Stevens et al. (2015) on proximate and anti-nutritional composition on different accession of *Moringa oleifera* leaves across Nigeria.

The variation of moisture content in different studies was predictable as tree age variation, leaves collection period (harvesting time) and different system of cultivation managements used.

Ash content

The overall mean ash content for the *M. stenopetala* samples across the age in the experiment was 11.23%. Ash content of *M. stenopetala* leaves from tree of 4 years showed significantly higher ash content (14.33%) than all other tree ages (Table 2). No significant difference was observed in ash content among tree of 3, 6 and 7 years. The ash content of this study compares with those previously analyzed (3.91 to 4.74%) by Steven et al. (2015) and similarly studied by Aberra et al. (2015) in which the value ranged from 7.10 to 8.03%; it was maximum but similar to Abuye et al. (2003)'s study, which was reported to be 9.1 to 14.2%.

Table 2. Main effects of different age of *M. stenopetala* leaves for nutritional and proximate composition.

Source of variation	Mean square							
	MC	**AC**	**CF**	**CP**	**Fat**	**Ca**	**P**	**K**
3	9.88^{b}	10.53^{bc}	32.84^{e}	31.53^{d}	0.36^{b}	315.19^{a}	304.61^{d}	1713.15^{c}
4	10.6^{a}	14.33^{a}	34.57^{d}	32.54^{b}	0.42^{a}	262.21^{b}	85.19^{e}	1874^{b}
5	7.79^{d}	11.22^{b}	35.31^{c}	34.40^{a}	0.44^{a}	263.33^{b}	467.24^{a}	1878.12^{a}
6	8.07^{d}	10.07^{bc}	35.36^{a}	31.83c	0.35^{b}	292.94^{ab}	456.12^{b}	1420.40^{e}
7	8.59^{c}	9.99^{bc}	37.62^{b}	31.16^{e}	0.35^{b}	276.52^{ab}	447.11^{c}	1666.67^{d}
Mean	8.99	11.23	32.29	32.29	0.38	282.04	352.05	1710.47

Crude fiber

The crude fiber content ranged from 35.36% (age = 6) to 32.84% (age = 3) with the overall mean crude fiber content of 32.29%. These crude fiber contents of *M. stenopetala* leaves were higher than the crude fiber reported by Aberra et al. (2015), ranging from 16.7, 17.2 and 18.7%.

Fat

The fat content of *M. stenopetala* leaves ranged from 0.35 to 0.44% with the overall mean fat content of 0.38%. Significantly higher fat content was observed at tree age of 5 (0.44%) and 4 (0.42%) than the other tree age (Table 2). The fat content of *M. stenopetala* leaves at *Derashe* differ from other studies reported by Morlu et al. (2017) (5.8% for *Moringa avalifolia* and 6.61% for *M. oleifera*), This variation might be attributed to the difference in latitude, longitude, annual rainfall, humidity and soil type (Dechasa et al., 1995).

Crude protein

The crude protein content of *M. stenopetala* leaves ranged from 31.16 to 34.4% with the overall mean crude protein content of 32.29%. Significantly higher crude protein content was observed at tree age of 5 (34.4%) and 4 (32.54%) than the other tree age (Table 2). Statistically lower crude protein was recorded on leaves at tree age of 3 (31.53%) and tree age of 7 (31.16%), which is 10.40% lower crude protein content than the maximum (Table 2). The amount of protein found in this study is comparable with the previous study by Morlu et al. (2017), which ranged from 23.21 to 24.75% on different accretion of *M. oleifera* across Nigeria.

Proximate composition

A significant variation between tree ages was observed for Ca, P and K but not for Iron and Zinc (Table 1). The amount of phosphorus, potassium, and calcium in the *M. stenopetala* leaves ranged from 85.19 mg/100 g to 467.24 mg/100 g; 1420.40 mg/100 g to 1878.12 mg/100 g and 263.33 mg/100 g to 315.19 mg/100 g, respectively (Table 2). Significantly higher values of cationic composition phosphorus (467.24 mg/100 g) and potassium (1878.12 mg/100 g) were recorded on leaves from tree age of 5. A higher cationic composition of calcium was recorded on leaves from tree age of 3 (315.19 mg/100 g). The observed value of the different minerals in the present study differs from another study (Morlu et al., 2017).These variations might be attributed to differences in the varieties used by these researchers. Different ecotypes and varieties of *M. stenopetala* are found in Ethiopia (Eyassu et al., 2014).

Anti-nutritional composition

The results of this study showed a significant difference in values of phytate and tannin among tree ages (Table 3). *M. stenopetala* leaves from tree age of 6 contain more tannin (403.94 mg/100 g) and phytate (725.60 mg/100 g). The lower tannin (163.89 mg/100 g) and phytate (544.32 mg/100 g) content was found in leaves from tree age of 5 (Table 4). The finding of the present study is comparable with another report by Ijarotimi et al. (2013) that reported the tannin content of 347.67 mg/100 g. The tannin content reported by Ijarotimi et al. (2013) is actually 52.86% higher than the lowest value we found

Table 3. Analyses of Variance for characterization of *M. stenopetala* leave with different age for photochemical and anti-nutrient analysis.

Source of variation	DF	Average mean	
		Taannin	Phytate
Age	4	249.53***	650.45*
Cv		6.46	9.54
R^2		0.98	0.64

Table 4. Main effects of different age of *M. stenopetala* leaves for photochemical and anti-nutrient analysis.

Source of variation	Mean square	
	Tannin (Latta and Eskin, 1980)	Phytate (Burns et al., 2001)
3	244.04^{c}	708.79^{ab}
4	171.36^{d}	676.27^{ab}
5	163.89^{e}	544.32^{c}
6	403.94^{a}	725.60^{a}
7	264.44^{b}	597.29^{bc}
Mean	249.53	650.45

from leaves of 5 years old tree. Similar anti-nutritional factors in plant foods were also reported by Habtamu and Negussie (2014). The phytate content obtained in this study was higher that of the study by Abinet et al. (2011), who reported 378.44 mg/100 g of *M. stenopetala* leave samples collected from different parts of Ethiopia. The variation might be due to the differences in geographical location and soil type between the study areas.

Conclusion

This study has clearly indicated that the nutritional, anti-nutritional and proximate composition of *M. stenopetala* leaves showed variation among the tree ages. The nutrient composition of *M. stenopetala* leaves in most cases was found higher in trees of 4 and 5 years compared to those of 3, 6 and 7, while anti-nutritional compositions, which are tannin and phytate of *M. stenopetala* leaves in most cases was found least for trees of 5 years compared to tree of 3, 4, 6 and 7 years; proximate composition has an adequate amount on trees of 3 and 5 years, respectively. Therefore, in this study the authors conclude that, trees of 5 years have better nutritional, anti-nutritional and proximate composition compared to all age categories included in this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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