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Nutritional composition, bioactive components and antioxidant activity of *Moringa stenopetala* and *Moringa oleifera* leaves grown in Gaborone, Botswana

Katso Twinkle Ntshambiwa, Eyassu Seifu* and Gaone Mokhawa

Abstract

Moringa is a multipurpose tree and an important vegetable crop elsewhere. However, it is recently introduced to Botswana and grown in the backyards of households as a shade. Its uses are generally unknown to the community, and it is underutilized despite its huge nutritional and non-food uses. In this study, the nutritional composition, bioactive components and antioxidant activity of *Moringa stenopetala* (MS) and *Moringa oleifera* (MO) leaves grown in Gaborone Botswana were determined. Except for moisture content, no significant difference ($p > 0.05$) was observed in proximate composition between MS and MO leaves. The moisture content of MS leaves was significantly ($p < 0.05$) higher than that of MO leaves. MS leaves had significantly ($p < 0.05$) higher Na and K contents than MO leaves. However, MO leaves had significantly ($p < 0.05$) higher Zn content than MS leaves. The two Moringa leaves had comparable Fe, Ca and Mg contents. The Vitamin C content of MO leaves was significantly ($p < 0.05$) higher than that of MS leaves. However, MS leaves had significantly ($p < 0.05$) higher total phenolic, total flavonoid contents (mg/100 g) and antioxidant activity ($\mu\text{g/mL}$) as compared to MO leaves. The Moringa leaves can be used for development of functional foods with improved nutrition and health benefits.

Keywords: Flavonoids, Mineral content, Proximate composition, Total phenolics, Vitamin C

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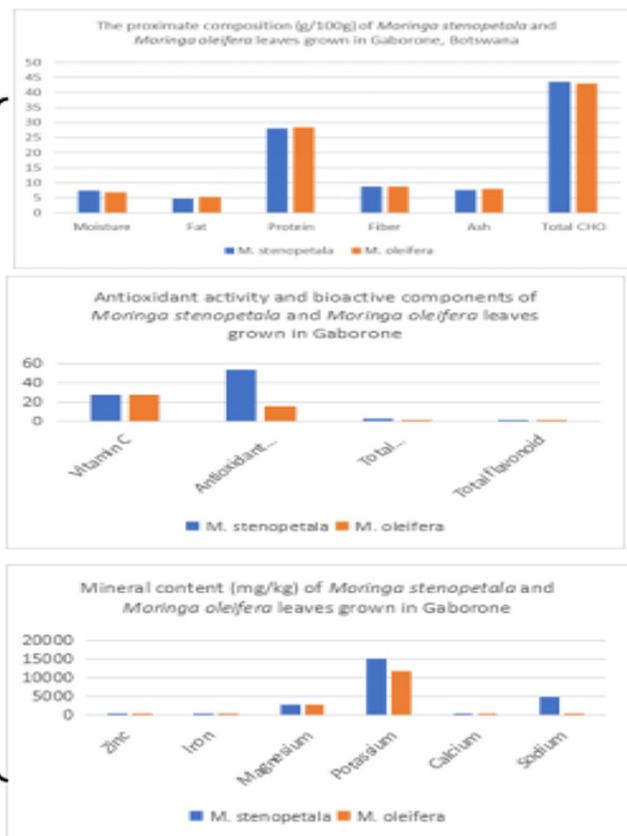
Graphical Abstract



Moringa stenopetala leaf powder



Moringa oleifera leaf powder



Introduction

Moringa is a tropical plant that belongs to the family *Moringaceae* and grows throughout the tropics. The genus *Moringa* consists of 13 species (NRC 2006) of which only *Moringa oleifera* (*M. oleifera*) has been accorded research and development attention. *M. oleifera* originates from the sub-Himalayan tracts of northern India and is commonly referred to as ‘horseradish tree’ or ‘drumstick tree’ (Jahn 1991). Moringa is a multipurpose tree of significant economic importance as it has vital nutritional, industrial and medicinal applications (Jahn, 1991; NRC 2006).

The rest of the species of Moringa, on the other hand, have not been studied in detail and their potential uses have not been fully understood. *Moringa stenopetala* (*M. stenopetala*) is indigenous to southern Ethiopia and it is called Haleko in the region (Seifu 2014). *M. stenopetala* is mainly cultivated for its edible leaves which are consumed as a vegetable in southern Ethiopia (Seifu 2014). It is very drought resistant and the leaves have excellent nutritional composition. *M. stenopetala* leaves contain

high protein, calcium and iron contents when compared to other fruits and vegetables (Luoh et al. 2017). Moreover, in addition to its superior nutritional value, *M. stenopetala* leaves possess antioxidant activity (Luoh et al. 2017) and therapeutic properties against a number of human ailments owing to their possession of the bio-molecule rutin which has antioxidant and antidiabetic properties (Habtemariam 2016).

M. stenopetala leaves are an important vegetable source for millions of people in southern Ethiopia, especially during dry seasons (Abuye et al. 2003; Habtemariam 2016). It is a fast-growing multipurpose tree that can easily establish on marginal lands and less-fertile soils in drier habitats and thus, it could serve as a reliable source of food and income to many communities (Habtemariam 2016). The leaves of *M. stenopetala* are eaten like cabbage; most of the plant parts are used for curing various ailments; the dried leaves are traded as nutritional supplement and as medicine particularly for diabetes and associated disorders; the seeds are good sources of edible oil as well as sources of biofuel; the seeds are excellent

flocculent agents and employed for water purification (Habtemariam 2016).

M. oleifera is the most popular species of the genus Moringa and it is widely distributed in many tropical and subtropical countries. It is produced on a commercial scale in several parts of the world including India, Africa, South and Central America and Southeast Asia. Nearly every part of the tree is consumed or used to make traditional herbal treatments. The leaves are edible and consumed as a staple diet in many regions and reported to contain very high antioxidant activity (Mbikay 2012).

M. oleifera requires minimal care and management to grow, and it can survive under harsh climatic conditions. It is well-known for its medicinal properties in addition to its nutritional value. The tree has a wide range of therapeutic values, with almost every part of the tree being used for treatment of various ailments.

Moringa is a multipurpose tree and an important vegetable crop in many parts of the tropics. However, it is recently introduced to Botswana and grown in the backyards of households as a shade. Its uses are generally unknown to the community, and it is underutilized despite its huge nutritional and non-food uses. It was first introduced and has become popularly known for its health benefits in Botswana since 2003 (Seifu & Teketay 2020).

M. stenopetala and *M. oleifera* have not yet been widely used by people in Botswana. Most people in Botswana have minimal knowledge on Moringa's nutritional value but rather know only the medicinal benefits of Moringa (Seifu & Teketay 2020). People in Botswana use Moringa leaves in tea and for medicinal purposes, but they do not consume the leaves as green vegetable (Seifu & Teketay 2020). Seifu & Teketay (2020) indicated that there is a huge potential to commercialize Moringa in Botswana. They also indicated the need to promote its cultivation and utilization in order to fully exploit its food and non-food applications.

The nutritional value of Moringa varies with cultivar and geographical locations where the plant has grown (Olson et al. 2016; Oyeyinka & Oyeyinka 2018). To date, no information exists on the nutritional composition and bioactive components of *M. stenopetala* and *M. oleifera* species grown under Botswana's climatic conditions. This study was designed to generate information on the nutritional value, bioactive components, and antioxidant activity of *M. stenopetala* and *M. oleifera* grown in Botswana. Such information will help create awareness about the nutritional benefits of Moringa leaves in the country and thereby promote its consumption as vegetable by Botswana. It will also be used for possible future improvement of the plants and/or their utilization in Botswana.

The objectives of this study were to determine the proximate composition of the leaves of *M. stenopetala* and *M. oleifera* grown in Gaborone, Botswana; evaluate selected bioactive components (vitamin C, total phenolic content, total flavonoid content, and antioxidant activity) in the leaves of *M. stenopetala* and *M. oleifera*; and determine selected mineral contents (sodium, potassium, magnesium, calcium, zinc, iron) of *M. stenopetala* and *M. oleifera* leaves.

Materials and methods

Study site and source of Moringa leaves

Two kg each of *M. stenopetala* and *M. oleifera* leaves were collected from backyards of households in Sebele and Block 10 areas, respectively in Gaborone city, which are about 3 km apart. Gaborone is situated at 24° 39' 29' S and 25° 54' 44' E – 24.66° S and 25.9° E in the southeastern corner of Botswana (Morton et al. 2008). It is located 15 km away from the South African border. The city lies at an elevation of 1010 m above sea level and has a hot semi-arid climate. Gaborone has a mean annual temperature of 20.7 °C and a mean annual precipitation of 538 mm (Jonsson 2004). Of this amount, 84% falls from October to the beginning of April, suggesting that clear skies are predominant during the rest of the year. The mean wind speed reaches its maximum in November and its minimum in April. The former is a result of the frequent cold-front passages on the subcontinent in this transitional month (Jonsson 2004). Daytime wind speed is high in the subtropics due to the intense insolation (Jonsson 2004) causing strong convection and turbulence. Soils in Gaborone are mainly residual soils, which are well-drained loamy-sand soils with depth of 0 to > 1.5 m (Zhai et al. 2003).

Preparation of sample

The *M. stenopetala* and *M. oleifera* leaf samples preparation prior to the analysis included removing the leaves from the twigs, washing the Moringa leaves with distilled water to remove dirt and other foreign matter. Sorting of the leaves was then done to get rid of discolored and damaged leaves. The leaves were then placed in a tray and put in the oven at 35 °C for 24 h. The dried leaves were ground (to less than 0.1 mm size) into powder using a laboratory blender. The powder obtained was then stored in airtight glass containers (Fig. 1) awaiting analysis for proximate composition, mineral content and bioactive components while vitamin C content was analyzed using fresh leaves. The experiment was repeated three times.

Proximate composition

The moisture and ash contents of the Moringa leaf samples were determined according to AOAC (2000) method



A. Neatly spacing leaves to ensure uniform drying



B. Sample drying in oven dryer at 35°C for 24 h



C. Sample storage in airtight glass container

Fig. 1 Preparation and drying of Moringa leaf samples

925.10 and method 923.03, respectively. The crude protein (CP) content in percentage was determined by micro-Kjeldahl method as described in AOAC (1996) (method 960.5). The crude fat content was determined by Soxhlet method as described by AOAC (1996) (method 920.39 C). The crude fiber content was determined by non-enzymatic gravimetric method and total carbohydrate was estimated by difference according to AOAC (1996).

Mineral analysis

The mineral content of the Moringa leaf samples was determined by atomic absorption spectrophotometer method after digestion of samples with concentrated sulfuric acid and selenium catalyst. Selenium-sulphuric acid digestion solution was prepared by mixing 5 g of selenium powder (catalyst) with 500 mL of sulphuric acid in a beaker. After mixing the two were stirred over a hot

plate to dissolve the selenium. After dissolving, the mixture was then left to cool (Sahrawat et al. 2002). Digestion of the samples was done by weighing about 1 g of the Moringa leaf sample and wrapping it with a lens tissue after which it was placed in digestion tubes. After that, the selenium-sulphuric acid (25 mL) mixture was poured on to the samples on the digestion tubes, from there the samples were taken to the block digester which was preheated at 370°C and digested for 4 h (Sahrawat et al. 2002). Complete digestion was marked by the clear mixture of digests after 4 h. After complete digestion, the mixture was allowed to cool and after cooling, approximately 4 mL of hydrogen peroxide was added to each digest in the digestion tube and they were left for 4 h in the digestion tubes. After adding hydrogen peroxide, the Moringa leaf digests were transferred into 200 mL volumetric flasks which were clearly labelled according to the type of sample. The volume of the digests was filled to

the meniscus using distilled water and the mixture was swirled for complete mixing (Sahrawat et al. 2002). After digestion, individual mineral (Mg, Ca, Fe, Zn, Na and K) contents were analyzed using the Atomic Absorption Spectrophotometer at the Department of Agricultural Research Laboratory located in Sebele, Gaborone.

Determination of Ca. and Mg contents

First, lanthanum chloride solution was prepared to suppress interference by phosphorus. About 29 g of lanthanum chloride was slowly dissolved into 250 mL of concentrated hydrochloric acid in a volumetric flask, the mixture was then diluted to 500 mL with deionized distilled water (Environmental Protection Agency 1979).

$$\text{The number of moles of vitamin} = [\text{molarity (M) of KIO}_3, \text{ x volume in L consumed on titration}]/3]$$

$$\text{Vitamin C mass (g)} = \text{number of moles} \times \text{formula mass (formula mass is 176.12 g/mol)}.$$

After preparation of the lanthanum chloride solution, 2 mL digested Moringa leaf sample was mixed with 18 mL of lanthanum chloride solution and then absorbance of samples and blank was measured at 422.7 nm for Ca and at 285.2 nm for Mg by atomic absorption spectrophotometer. Standard Ca solution (25, 50 and 125 ppm) and Mg solution (10, 20 and 50 ppm) were used for the construction of the calibration lines for each from which the contents were determined and expressed as mg/kg.

Determination of Fe, Zn, K and Na contents

The Fe, Zn, Na and K contents were determined from the digested Moringa leaf samples and absorbance for Fe, Zn, Na and K were measured at 248.3, 213.8, 279.5 and 324.75 nm, respectively using atomic absorption spectrophotometer. The standard solutions used for calibration line construction were for Fe (2, 4 and 12 ppm), Zn (2, 4 and 12 ppm), Na (2, 4 and 12 ppm) and K (2, 4 and 12 ppm) from which the contents were determined and expressed as mg/kg.

Evaluation of bioactive components

Vitamin C content

To determine the amount of vitamin C in Moringa leaves, 75 g of each of the fresh Moringa leaves was crushed with a mortar and pestle and then added to 50 mL of distilled water, mixed and then the mixture was strained using a cheesecloth to extract the filtrate. The extracted solution was poured into a volumetric flask and then distilled water was added to it up to the 100 mL mark. The redox titration with potassium

iodate (KIO₃) was used to determine the vitamin C content in the Moringa leaves (AOAC 1996). The extract of both Moringa species was pipetted (5 mL) into a 250 mL conical flask each, after which 150 mL of distilled water, 5 mL of 0.6 KIO₃, 5 mL of 1 M HCL and 1 mL starch indicator solution (0.5%) were added to each sample. These samples were titrated with 0.002 M potassium iodate standard solution from burette until a permanent trace of dark blue color (due to starch –iodine complex) appears. From there, the volume of KIO₃ used was noted and used to calculate the content of vitamin C. The titration was carried out three times and average values were calculated and then the vitamin C content was expressed in mg/100 g. In the titration reaction that was used, one mole of iodate generates 3 moles of iodine and one mole of iodine reacts with one mole of L-ascorbic acid.

Total phenolic content

The total phenolic content of the Moringa leaf samples was determined by the Folin–Ciocalteu method (Singleton & Rossi 1965). The total phenols were extracted from the Moringa leaves samples (0.1 g) using distilled water at a dilution factor of 1;10 and added to 1% HCL in methanol with shaking at low speed. The extracts were centrifuged at 4200 rpm for 30 min. The supernatant (0.5 mL) of the sample (pipetted into a clean dry test tube) was collected and used for the total phenolic content analysis. An aliquot of the extract (0.1 mL) was diluted with 7 mL of distilled water and reacted with 0.5 mL of Folin–Ciocalteu reagent, swirled and incubated for 5 min at room temperature. Then 1.5 mL of 20% (w/v) sodium carbonate solution was added including water to make up the volume to 10 mL. The mixture was shaken and then incubated at room temperature for 2 h. Absorbance was read at 735 nm using a UV Spectrophotometer (Jenway 6300 Spectrophotometer, Cole-Palmer Ltd, UK). Blank absorbance value was subtracted from the sample absorbance value. Gallic acid was used to construct a standard calibration line in the range of 0.0 to 0.6 mg GAE mL⁻¹ ($y = 1.0591x - 0.012$, $R^2 = 0.9954$) from which the total phenolic content was expressed as milligrams of gallic acid equivalents per 100 g of the Moringa leaves solution.

Total flavonoids content

The Moringa samples were extracted as described for the determination of total phenolic content. Total flavonoids content was determined by aluminum chloride

method using quercetin as the standard (Marthur & Vijayvergia 2007). One mL of the extracted sample of the Moringa leaves and 4 mL of water were added into a test tube of 10 mL volume. 0.3 mL of 5% sodium nitrate and 0.3 mL of 10% aluminum chloride were added into the mixture after 5 min. The mixture was then incubated at room temperature and then 1 mL of 1 M sodium hydroxide was added to the reaction mixture. Distilled water was used to adjust the final volume to 10 mL. Absorbance of the samples was measured against blank at 510 nm using spectrophotometer (Jenway 6300 Spectrophotometer, Cole Palmer Ltd, UK). Blank absorbance was subtracted from the sample absorbance and the total flavonoid content was evaluated from quercetin calibration line in the range of 0 to 0.6 mg QE/mL ($y = 0.4521x + 0.0051$ $R^2 = 0.9796$) and expressed in quercetin equivalent per 100 g of the sample mixture.

DPPH radical scavenging activity

DPPH radical scavenging activity was used to determine the antioxidant activity of the Moringa leaf samples. For determination of the DPPH scavenging activity, sample of the Moringa leaf powder of about 0.1 g was extracted in a mixture of ethanol-water (50:50, v/v) at room temperature shaking the content in a water bath for 30 min, centrifuged (4200xg for 30 min) and the supernatant solution was used for reaction with DPPH solution (Sigma Aldrich) (Kim et al. 2002). Sample extract, L-AA or blank (10 mL of ethanol-water solution only) solutions each 1.5 mL was mixed with 1.5 mL of DPPH solution in a clean test tube, incubated in a dark at room temperature for 30 min and absorbance was then measured at 517 nm with UV-Vis Spectrophotometer (Jenway 6300 Spectrophotometer, Cole-Palmer Ltd, UK) and the blank absorbance value was subtracted. The DPPH antioxidant activity was then expressed as LAA equivalent from the L-AA standard calibration line. Blank absorbance was subtracted from the sample absorbance and the antioxidant activity was determined from the calibration line in the range of 0.2, 0.4, 0.6, 0.8 and 1 to ($y = 0.209x + 0.11$ $R^2 = 9642$).

Statistical analysis

All analyses were done in triplicates and the data generated for the variables considered for each Moringa species were given as mean \pm standard deviation. The variations between the two Moringa species for the parameters examined were determined by one-way Analysis of Variance and significant differences were declared at $p < 0.05$.

Results and discussion

Proximate composition of Moringa leaves

The proximate composition for the *M. stenopetala* and *M. oleifera* leaves are given in Table 1. Except for moisture content, no significant difference ($p > 0.05$) was observed between the two Moringa species for the other proximate composition parameters.

The moisture content (on dry matter basis) of *M. stenopetala* (7.40%) was significantly higher ($p < 0.05$) than that of *M. oleifera* (6.73%) (Table 1). The moisture content of *M. oleifera* leaves observed in the present study agrees with the findings of Mikore & Mulugeta (2017) who reported a moisture content of 6.60% and 6.88% for *M. oleifera* leaf samples collected from Karat and Secha areas in southern Ethiopia, respectively. The moisture content of *M. oleifera* leaves observed in the present study is also comparable to the findings of Peñalver et al. (2022) and Moyo et al. (2011) who reported 7.23% and 9.53%, respectively for moisture content of *M. oleifera* leaves. However, it is higher than the findings of Ogbe & Affiku (2011) who recorded 3.21% for moisture content of *M. oleifera* leaves.

The moisture content of *M. stenopetala* observed in the current study is comparable to the results of Mikore & Mulugeta (2017) who reported a moisture content of 7.73% and 7.92% for *M. stenopetala* leaves collected from Gato and Sikela areas in southern Ethiopia, respectively. Also, the moisture content of *M. stenopetala* leaves observed in the present study is comparable to the findings of Raghavendra et al. (2016) who recorded 8.10% moisture content for *M. stenopetala* leaf samples collected from Ambo city in Ethiopia. The moisture contents of the two Moringa leaves are less than the 10% recommended moisture content for dried vegetables (Emelike & Ebere 2016) and hence they will have a stable shelf life under normal storage conditions.

Table 1 The proximate composition (g/100 g) of *Moringa stenopetala* and *Moringa oleifera* leaves grown in Gaborone, Botswana

Parameter	<i>M. stenopetala</i>	<i>M. oleifera</i>
Moisture	7.40 ^a \pm 0.32	6.73 ^b \pm 0.21
Fat content	4.66 \pm 0.01	5.21 \pm 0.42
Crude fiber	8.83 \pm 1.09	8.72 \pm 0.82
Crude protein	28.02 \pm 1.12	28.41 \pm 1.16
Ash content	7.64 \pm 0.22	7.92 \pm 0.17
Total carbohydrate	43.44 \pm 0.93	43.01 \pm 0.89

Means that are followed by different superscript letters in the same row are significantly different at 0.05 significance level; The values in the Table are means \pm standard deviations of triplicate observations

The variations in moisture contents of the Moringa leaves examined in the present study could be attributed to difference in soil moisture content where the plants have grown. Soil is the main reservoir of water and nutrients, and thus controls the availability of most essential plant nutrients for crop growth and establishment (Ewetola et al. 2019). A water deficit in the soil can cause water stress in plants, triggering morphological and physiological changes (Vasconcelos et al. 2019). The moisture content of the soil significantly influenced the relative water content of *M. stenopetala* leaves (Galgaye et al. 2020). In our study, the *M. stenopetala* and *M. oleifera* leaf samples were obtained from backyards of households located at Sebele and Block 10, respectively in Gaborone city, which are about 3 km apart from each other. Thus, the observed difference in moisture content between *M. stenopetala* and *M. oleifera* leaf samples could be attributed to differences in moisture content of the soil in which the plants have grown since the moisture content of the soil could vary depending on litter cover and/or whether the owners water their Moringa plants or not. Lamidi et al. (2017) reported that the difference in the moisture content of *M. oleifera* leaves grown in two different areas could be attributed to the composition of the soil of the areas. Plant species vary in their ability to absorb and translocate nutrients to edible parts (Kumssa et al. 2017). Thus, the observed difference in moisture content between the two moringa species could partly be due to genetic factors.

The results of the current study showed that *M. stenopetala* and *M. oleifera* leaves have comparable macronutrient (crude protein fat, ash, crude fiber and total carbohydrate) contents. The absence of significant difference in proximate composition between the two Moringa species observed in the present study is in agreement with earlier published work. Udofia et al. (2020) reported no significant difference in crude protein and fat contents in the leaves of *M. stenopetala* and *M. oleifera*. Moreover,

the respective concentrations of the macronutrients observed in the leaves of the two Moringa species analyzed in the present study are within the range of published work reported in the literature for similar species. Both Moringa species had high protein and ash contents suggesting that consumption of the leaves either as fresh vegetables or in powdered form could significantly improve nutrition of communities where protein-energy malnutrition is prevalent.

Mineral content of Moringa leaves

Among the minerals analyzed in the present study, K, Na and Mg were the predominant minerals present in the leaves of both Moringa species. *M. stenopetala* leaves had significantly ($p < 0.05$) higher K and Na contents than *M. oleifera* leaves (Table 2). Whereas *M. oleifera* leaves had higher ($p < 0.05$) Zn content as compared to *M. stenopetala* leaves (Table 2). No significant differences ($p > 0.05$) were observed in Ca, Mg and Fe contents between the two Moringa species (Table 2).

The difference in mineral contents observed between *M. stenopetala* and *M. oleifera* leaves could mainly be attributed to genetic differences between the two Moringa types. Genetic differences have been reported to be the major sources of variation in nutrient composition including mineral contents between *M. stenopetala* and *M. oleifera* leaves (Jiru et al. 2006; Kumssa et al. 2017). Olson et al. (2016) reported that genetic factors account for significant variations in mineral composition and protein content among different Moringa species. Plant species vary in their ability to absorb and translocate mineral elements to edible parts (Kumssa et al. 2017). In our experiment, the *M. stenopetala* and *M. oleifera* leaf samples were obtained from backyards of different households in Gaborone city. Thus, the observed difference in mineral contents between *M. stenopetala* and *M. oleifera* leaf samples could also be partly attributed to differences in management practices such as watering of the plants

Table 2 Mineral content (mg/kg) of *Moringa stenopetala* and *Moringa oleifera* leaves grown in Gaborone

Mineral	<i>M. stenopetala</i>	<i>M. oleifera</i>	RDDI (1–3 yrs) (mg/day)	RDDI (19–30 yrs) (mg/ day)*
Zinc	3.72 ^a ± 1.31	17.14 ^b ± 4.17	4.1	7.0–4.9
Iron	155.71 ± 9.70	141.46 ± 27.92	5.8	13.7–29.4
Magnesium	2790.15 ± 376.66	2760.84 ± 304.79	80	100–310
Potassium	15275.55 ^a ± 1175.60	11815.95 ^b ± 1159.70	3000	4700
Calcium	79.45 ± 13.35	113.10 ± 23.25	500	1000
Sodium	4865.30 ^a ± 373.67	627.68 ^b ± 69.56	1000	1500

Means that are followed by different superscript letters in the same row are significantly different at 0.05 significance level; The values in the Table are means ± standard deviations of triplicate observations; RDDI = Recommended daily dietary intake according to Pan American Health Organization (2020); *Figures in a range indicate values for male and female adults, respectively

and application of fertilizers by the households who grew the respective plants. Management regimes, such as, lone trees, hedgerow, woodlot, pollarding, lopping, watering, fertilizing and intercropping can cause variations in the elemental concentration of the different edible parts of *M. stenopetala* and *M. oleifera* trees (Kumssa et al. 2017).

The K content of *M. stenopetala* leaves observed in the present study is comparable to the results reported by Mikore & Mulugeta (2017) for Gato area ($14,047 \pm 97.14$ mg/kg) but is more than those reported by the same authors for Sikela area ($12,247 \pm 434.90$ mg/kg). The K content of the present study is also comparable to the findings of Melesse (2011) who reported a K content of 16,600 mg/kg for *M. stenopetala* leaves. However, the K content of the present study is higher than the findings of Abuye et al. (2003) who reported a K content of 4530 mg/kg. On the other hand, the K content obtained in the current study is less than those reported by Raghavendra et al. (2016) (18426.75 mg/kg) for the same species.

The K content of *M. oleifera* leaves obtained in the current study is comparable to the findings of Mikore & Mulugeta (2017) who reported a K content of 12,160 mg/kg for *M. oleifera* leaf samples collected from Secha area; however, it is higher than the K content (9570 mg/kg) reported by the same authors for samples collected from Karat area. The K content observed in the current study is also higher than the K content (17.5 mg/kg) of *M. oleifera* leaves reported by Peñalver et al. (2022). However, the K content observed in the present study for *M. oleifera* leaves is less than the findings of Yaméogo et al. (2011) who reported a K content of (19,220 mg/kg) for the same species.

The Ca contents of leaves of both Moringa species analyzed in the present study are much less than the Ca content reported by Mikore & Mulugeta (2017) for the same species. Mikore & Mulugeta (2017) reported Ca content of $18,342 \pm 1010.0$ mg/kg for *M. stenopetala* leaves collected from Sikela area and $18,230 \pm 990.2$ mg/kg for *M. stenopetala* leaves collected from Gato area. The results of this study are also less than the findings of Raghavendra et al. (2016), Abuye et al. (2003) and Melesse (2011) who reported Ca contents of 9716.03 mg/kg, 7928 mg/kg and 18,500 mg/kg, respectively for *M. stenopetala* leaves.

The Ca content of *M. oleifera* leaves observed in the present study is less than the findings of Mikore & Mulugeta (2017) who reported a Ca content of 19,026 mg/kg and 18,803 mg/kg for *M. oleifera* leaves collected from Secha and Karat areas, respectively. The Ca content of *M. oleifera* observed in the current study is also less than the findings of Yaméogo et al. (2011) who reported a Ca content 20,981 mg/kg for the same species.

However, it is higher than the Ca content (14.8 mg/kg) reported by Peñalver et al. (2022) for *M. oleifera* leaves.

The Na content of *M. stenopetala* leaves analyzed in the present study is much higher than the findings of Mikore & Mulugeta (2017) who reported Na contents of 1293 ± 99.02 mg/kg and 1298 ± 99.50 mg/kg for *M. stenopetala* leaves collected from Sikela and Gato areas, respectively. The Na content of *M. stenopetala* leaves observed in the present study is comparable to the findings of Abuye et al. (2003) and Melesse (2011) who reported a Na content of 4035 mg/kg and 4100 mg/kg, respectively for *M. stenopetala* leaves. However, the Na content of the current study is higher than the value (1519.68 mg/kg) reported by Raghavendra et al. (2016) for *M. stenopetala* leaves.

On the other hand, the Na content of *M. oleifera* leaves analyzed in the present study is much lower than the findings of Mikore & Mulugeta (2017) who reported Na contents of 1296 ± 101.5 mg/kg and 1287 ± 108.1 mg/kg for *M. oleifera* leaf samples collected from Secha and Karat areas, respectively. It is also less than the findings of Peñalver et al. (2022) who reported Na content of 1331.1 mg/kg for *M. oleifera* leaves. However, the Na content of *M. oleifera* leaf obtained in the present study is higher than the findings of Yaméogo et al. (2011) who reported a Na content of 288 mg/kg for *M. oleifera* leaves grown in Ouagadougou.

The Mg content of *M. stenopetala* leaves observed in the current study is much lower than values reported by Mikore & Mulugeta (2017). Mikore & Mulugeta (2017) reported Mg content of 4550 ± 310.5 mg/kg and 4500 ± 242.7 mg/kg for *M. stenopetala* leaves collected from Sikela and Gato areas, respectively. The Mg content of *M. stenopetala* leaves observed in the present study is also less than the value (86.1 g/kg) reported by Melesse (2011) for the same species. However, it is higher than the value (101.93 mg/kg) reported by Raghavendra et al. (2016) for Mg content of *M. stenopetala* leaves.

On the other hand, the Mg content of *M. oleifera* leaves observed in the present study is less than the findings of Mikore & Mulugeta (2017) who reported a Mg content of 4370 mg/kg and 4273 mg/kg for *M. oleifera* leaf samples collected from Secha and Karat areas, respectively. The Mg content of *M. oleifera* leaves observed in the present study is comparable to the findings of Yaméogo et al. (2011) who reported a Mg content of 4060 mg/kg for *M. oleifera* leaves grown in Ouagadougou. However, it is higher than the value (3010 mg/kg) reported by Peñalver et al. (2022) for the same species.

The Zn content of *M. stenopetala* leaves considered in the current study is comparable to the findings of Abuye et al. (2003) who reported a Zn content of 5.3 mg/kg for the same species. The Zn content of the leaves of

M. stenopetala species analyzed in the current study is much lower than that reported by Mikore & Mulugeta (2017) and Raghavendra et al. (2016). Mikore & Mulugeta (2017) found Zn content of 57.63 ± 4.45 mg/kg and 27.90 ± 1.59 mg/kg for *M. stenopetala* leaves obtained respectively from Sikela and Gato areas in southern Ethiopia while Raghavendra et al. (2016) found a Zn content of 27.32 mg/kg leaves of *M. stenopetala*.

The Zn content of the *M. oleifera* leaves examined in the present study is generally lower than values reported in the literature. Peñalver et al. (2022) reported a slightly higher (20.4 mg/kg) Zn content for leaves of *M. oleifera*. Mikore & Mulugeta (2017) found a Zn content of 31.87 ± 2.11 mg/kg and 21.30 ± 2.09 mg/kg for *M. oleifera* leaves collected, respectively from Secha and Gato areas. The Zn content of *M. oleifera* leaves collected from Secha area is higher than the Zn content observed in the present study while that of Gato area was comparable to the Zn content reported in the current study. Yaméogo et al. (2011) reported Zn content of 109 mg/kg for *M. oleifera* leaves grown in Ouagadougou.

The iron content *M. stenopetala* leaves observed in the current study is less than those reported (962.50 mg/kg) by Raghavendra et al. (2016) for *M. stenopetala* species. However, the Fe content of the leaves of *M. stenopetala* considered in the present study is much higher than that reported by Mikore & Mulugeta (2017) and Abuye et al. (2003). Mikore & Mulugeta (2017) reported Fe contents of 80.03 ± 2.50 mg/kg and 82.30 ± 2.65 mg/kg for *M. stenopetala* leaves collected, respectively from Sikela and Gato areas in southern Ethiopia while Abuye et al. (2003) reported an iron content of 30.8 mg/kg for leaves of *M. stenopetala*.

On the other hand, the Fe content of *M. oleifera* leaves analyzed in the present study is less than the reported Fe content (283 mg/kg) by Yaméogo et al. (2011) for *M. oleifera* leaves. The Fe content observed in the current study is also less than the Fe content (251.4 mg/kg) reported by Peñalver et al. (2022) for the same species. Mikore & Mulugeta (2017) reported an Fe content of 81.60 ± 1.14 mg/kg and 81.37 ± 2.11 mg/kg, respectively for *M. oleifera* leaves collected from Secha and Karat areas in southern Ethiopia which is less than the Fe content reported in the present study.

The discrepancies in mineral concentration observed between *M. stenopetala* and *M. oleifera* leaves analyzed in the present study and the respective values reported in the literature for the two Moringa species could be due to environmental factors such as soil type, soil fertility and climatic conditions (Kumssa et al. 2017; Kim & Kim 2019). The potential soil nutrient capacity determines to a large extent the mineral concentrations in Moringa leaves (Olson et al. 2016; Kumssa et al. 2017). It was also

reported that the mineral content in Moringa leaves is significantly positively correlated with elemental mineral concentration of the soil (Kumssa et al. 2017). Difference in the stage of growth of the Moringa leaves could also contribute to the variations of the elemental mineral concentrations in Moringa leaves (Kumssa et al. 2017).

The results of the mineral analysis showed that the leaves of both Moringa species could meet the recommended daily dietary intake of most of the minerals analyzed in the present study. The recommended daily dietary intake of K for children (1–3 years) is 3.0 g (Pan American Health Organization 2020). The leaves of both Moringa species considered in the present study contain much higher amounts of K and hence can meet the daily K intake of a child aged between 1 and 3 years. Furthermore, the leaves of the Moringa species considered in this study can also meet the daily K intake (4700 mg per day) of adult male and female individuals aged between 19 and 30 years old (Pan American Health Organization 2020). A high amount of K is helpful for people taking diuretics to control hypertension and it also increases the utilization of iron (Arinathan et al. 2003; Adeyeye & Fagbohun 2005).

The Moringa species considered in the present study can provide up to 22.62% of the recommended daily intake of Ca for children aged between 1 and 3 years. The recommended daily intake of calcium for children (1–3 years) is 500 mg per day (Pan American Health Organization 2020). Calcium is important in the building of skeletal structures and muscle function.

According to Pan American Health Organization (2020), the daily Na requirement for children aged between 1 and 3 years is 1 g per day. The results show that the *M. stenopetala* leaves considered in the current study could serve as an important source of sodium in children's diets. Furthermore, the *M. stenopetala* leaves analyzed in current study can also meet the daily Na intake (1500 mg per day) of adult male and female individuals aged between 19 and 30 years (Pan American Health Organization 2020). Sodium is an important macro-mineral in the human body, which is used to conduct nerve impulses, contract and relax muscles, and maintain the proper balance of water and minerals in the body.

The results showed that the Moringa species considered in the present study could meet the daily Mg intake of children aged between 1 and 3 years old. According to Pan American Health Organization (2020), the daily Mg requirement for children (1–3 years) is 80 mg per day. Furthermore, the leaves of the Moringa species considered in the present study can also meet the daily Mg intake of adult males (100 mg per day) and adult females (310 mg per day) aged between 19–30 years old (Pan American Health Organization 2020). Magnesium

is an important co-factor for many enzyme activities in the human physiological function. It is also important for plant growth as it facilitates trapping of sunlight necessary for photosynthesis (Cakmak & Yazici 2010).

The recommended daily intake of Zn for children (1–3 years) is 4.1 mg per day (Pan American Health Organization 2020). The results showed that leaves of *M. oleifera* species can provide Zn requirements in the diets of children aged between 1 and 3 years whereas *M. stenopetala* leaves can provide 90.7% of the Zn requirement of children. *M. oleifera* can also provide Zn requirements in the diets of adult males (7.0 mg per day) and adult females (4.9 mg per day) aged between 19 and 30 years old (Pan American Health Organization 2020). Zinc is an essential trace element that is needed only in small amounts by the human body for vital biochemical functions and it plays a very important role in human growth. Zinc is one of the limiting micronutrients in human nutrition. Zinc is also important in plant growth as it helps in the production of chlorophyll (Hafeez et al. 2013).

Leaves of the Moringa species considered in the present study could serve as an excellent source of iron in children's diet. The recommended daily intake of Fe for children (1–3 years) is 5.8 mg per day (Pan American Health Organization 2020). Furthermore, these Moringa species can also meet the daily Fe intake of adult males (13.7 mg per day) and adult females (29.4 mg per day) aged between 19 and 30 years old (Pan American Health Organization 2020). Iron is vital in human nutrition as part of hemoglobin and myoglobin for transport of oxygen to the cells.

Bioactive components of Moringa leaves

Vitamin C content

The vitamin C content (27.94 mg/100 g) of *M. oleifera* leaves was significantly higher ($p < 0.05$) than that of *M. stenopetala* leaves (27.16 mg/100 g) (Table 3). The vitamin C content of *M. stenopetala* leaves observed in the present study is in line with the findings of Abuye et al. (2003) who reported a vitamin C content of 28 mg/100 g for the same species. On the other hand, the vitamin C

content of *M. oleifera* leaves observed in the present study is higher than the value (15.2 mg/100 g) reported by González-Burgos et al. (2021); however, it is much lower than the values 257 mg/100 g reported by Palada et al. (2017), 220 mg/100 g reported by Mahmood et al. (2010) and 162 mg/100 g reported by Witt (2020) for vitamin C contents of leaves of the same species. The variations in vitamin C content observed between the two Moringa species could be attributed to genetic differences; however, the difference in vitamin C content of Moringa leaves analysed in the present study and values reported in the literature for the two Moringa types may be due to difference in environmental factors such as soil nutrients and climatic conditions as suggested by Mengel & Kirkby (1987).

The recommended daily intake of vitamin C for children (1–3 years) is 30 mg per day (Pan American Health Organization 2020). Thus, the leaves of the Moringa species considered in the present study can provide over 90% of the daily vitamin C requirements of children. Moreover, the leaves of the Moringa species considered in the present study can provide 60% of the daily vitamin C requirements (45 mg per day) of adult males and females aged between 19 and 30 years old (Pan American Health Organization 2020). From the results of the present study, it can be extrapolated that consumption of about 200 g of Moringa leaves can provide the daily vitamin C requirements of adult individuals.

The body requires vitamin C for the synthesis and metabolism of tyrosine, folic acid and tryptophan, hydroxylation of glycine, proline, lysine, carnitine and catecholamine (Chambial et al. 2013). Vitamin C also helps facilitate the conversion of cholesterol into bile acids and hence lowers blood cholesterol levels (Chambial et al. 2013). Chambial et al. (2013) reported that consumption of vitamin C increases the absorption of iron in the gut by reducing ferric to ferrous state. In addition to serving as a common enzymatic cofactor in the synthesis of collagen and being involved in various physiological functions, ascorbic acid is a well-known small molecular weight dietary antioxidant (Habtemariam 2017).

Table 3 Antioxidant activity and bioactive components of *Moringa stenopetala* and *Moringa oleifera* leaves grown in Gaborone

Parameter	<i>M. stenopetala</i>	<i>M. oleifera</i>
Vitamin C (mg/100 g)	27.16 ^a ± 0.36	27.94 ^b ± 0.24
Total phenolic content (mg/100 g)	0.67 ^a ± 0.002	0.35 ^b ± 0.004
Total flavonoid content (mg/100 g)	0.50 ^a ± 0.008	0.33 ^b ± 0.004
Antioxidant activity (µg/mL)	53.21 ^a ± 0.005	16.24 ^b ± 0.009

Means that are followed by different superscript letters in the same row are significantly different at 0.05 significance level; The values in the Table are means ± standard deviations of triplicate observations

Total flavonoid content

The total flavonoid content of *M. stenopetala* leaves (0.50 mg/100 g) was significantly higher ($p < 0.05$) than that of *M. oleifera* leaves (0.33 mg/100 g) (Table 3). The total flavonoid content of *M. stenopetala* leaves observed in the present study is lower than the findings of Toma et al. (2014) who reported a total flavonoid content of 71.73 mg/g for *M. stenopetala* leaves. Similarly, the total flavonoid content of *M. oleifera* leaves observed in the present study is much lower than the value (27 mg/g) reported by Sreelatha & Padma (2009) for *M. oleifera* leaves. The observed difference between the present study and literature values could be attributed to the different geographical locations where the plants have grown. According to Adhikari et al. (2020), geographical location plays a critical role in the presence of bioactive constituents and the antioxidant activity of plants.

Total phenolic content

The total phenolic content of *M. stenopetala* leaves (0.67 mg/100 g) was significantly higher ($p < 0.05$) than that of *M. oleifera* leaves (0.35 mg/100 g) (Table 3). The total phenolic content of *M. stenopetala* leaves observed in the present study is lower than the findings of Toma et al. (2014) and Tebeka & Libsu (2014) who reported total phenolic contents of 79.81 mg/g and 92.8 mg/100 g, respectively for total phenolic content of the same species.

The total phenolic content of *M. oleifera* leaves observed in the present study is lower than the findings of Peñalver et al. (2022) who reported a total phenolic content of 32.90 mg/g for the same species. The total phenolic content of *M. oleifera* leaves observed in the present study is also lower than the value (45.81 mg/g) reported by Sreelatha & Padma (2009) for *M. oleifera* leaves. The difference in total phenolic contents observed between the two Moringa leaves in the present study could mainly be attributed to genetic differences between the two species. The difference in the observed total phenolic contents between the present study and literature values might be attributed to environmental factors.

Antioxidant activity

The antioxidant activity of *M. stenopetala* leaves (53.21 µg/mL) was significantly higher ($p < 0.05$) than that of *M. oleifera* leaves (16.24 µg/mL) (Table 3). This observation is in line with the report of Habtemariam (2017) who indicated that the antioxidant potential of *M. stenopetala* is significantly higher than *M. oleifera*. The antioxidant activity of *M. stenopetala* leaves observed in the present study is lower than the findings of Habtemariam (2015) who reported antioxidant activity of 59.5 µg/mL for *M. stenopetala* leaves. Similarly, the antioxidant

activity of *M. oleifera* leaves observed in the present study is lower than the value (18.15 µg/mL) reported by Sreelatha & Padma (2009) for *M. oleifera* leaves. The difference in antioxidant activity observed between the two Moringa leaves in the present study could mainly be attributed to genetic differences between the two species. The difference in the observed antioxidant activity between the present study and literature values might be attributed to environmental factors. According to Zahra et al. (2019), environmental conditions can affect the synthesis of secondary active substances and plant chemicals in plants that have antioxidant properties. Zahra et al. (2019) further stated that in addition to changing climatic conditions, the soil conditions can also affect the plant's antioxidant activity. Antioxidant activity is an important property as it prevents or slows damage to cells caused by free radicals which have the potential of causing heart diseases, cancers and other diseases (Lobo et al. 2010). Antioxidant compounds found in Moringa leaves can be useful for the photoprotection against oxidative stress caused by UV exposure, which after prolonged exposure could lead to skin cancer (Peñalver et al. 2022).

Conclusion

The results showed that the leaves of both Moringa species contained high concentrations of proteins, vitamin C and minerals especially of the essential microminerals Zn and Fe and the macro minerals Mg, K and Na. The Moringa leaves considered also showed high antioxidant activities and contained appreciable quantities of the bioactive components phenolics and flavonoids. This suggests that the leaves of both Moringa species can be used for fortification of foods with low micronutrient content to improve their protein and minerals contents. The observed antioxidant activity and bioactive components of the Moringa leaves means that they can be used in the development of nutraceuticals and functional foods with potential health promoting properties. Further studies on the amino acid profiles and antinutritional factors of Moringa leaves are recommended.

Abbreviations

AOAC: Association of Official Analytical Chemists; CF: Crude fiber; CP: Crude protein; DPPH: 2,2-diphenyl-1-picrylhydrazyl; MS: *Moringa stenopetala*; MO: *Moringa oleifera*; Zn: Zinc; Fe: Iron; Mg: Magnesium; K: Potassium; Ca: Calcium; Na: Sodium.

Supplementary Information

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Additional file 1: Supplementary Material 1.

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Authors' contributions

KTN: collected data, analyzed data and wrote thesis, ES: conceived the research idea, designed the study, supervised thesis research work, drafted the manuscript and reviewed the manuscript, GM: co-supervised thesis and edited the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

All data supporting this study are included in this manuscript. Further details are available upon request from the corresponding author.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there is no competing interest associated to this work.

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