

Evaluation of Nutritional Properties of dried Moringa (Moringa stenopetala) Leaves and Dried Moringa Leaves Infusion

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ABSTRACT

Moringa demand is currently increased in Ethiopia due to its nutritional and medicinal values. However, there is no as such information about their dried leaves and infusion nutritional properties. The purpose of this study was to evaluate the dried leaves and infusion nutritional properties. The dried leaves of *Moringa* were characterized for proximate, minerals, phytochemicals, and antioxidants. The result shows dried *Moringa* leaf had 6 g/100 g moisture, 11.2 g/100 g ash, 25.3 g/100 g crude protein, 6.9 g/100 g crude fat, 9.3 g/100 g crude fiber, 41.30 g/100 g carbohydrate, 1300.7 mg/100 g Ca, 128.9 mg/100 g Na, 330.9 mg/100 g K, 1056.6 mg/100 g Mg, 47.7 mg/100 g Fe, 1.8 mg/100 g Zn, 0.8 mg/100 g Cu, 3.4 mg/100 g Mn, 2.6 mg GAE*/g total phenolic content, 11.9 mg CE/g total flavonoid content, 3.3 mg CE/g Condensed tannin content, 0.48 (IC-50, g/mL) DPPH scavenging, 2.27 mg AAE/g Ferric reducing power and 1.23 mg BHTE/g total antioxidant. This study provides evidence on the dried *Moringa* leaves nutritional properties and its leave infusion antioxidant and phytochemicals properties.

Keywords: Antioxidant activity; Moringa; Phenolic content; Infusion

INTRODUCTION

Moringa (M. stenopetala) is often named as African Moringa tree because it is native to southern Ethiopia, North Kenya and Eastern Somalia [1,2]. Moringa is widely cultivated and distributed in Southern Ethiopia mainly in Gamo Gofa, Wolavita, Konso, Sidama, Keffa, Borana, South Omo, Dherashe areas and the adjoining provinces [3,4]. Moringa plants are among the high value and multi-purpose trees with medicinal, nutritional, and socioeconomic values [4,5]. Moringa leaves have been found to have potential health benefits which include removing different kinds of intestinal worms, exhibit anti-tumor, anti-inflammatory, antiulcer, anti-atherosclerotic, increasing food appetite, protecting abdominal constipation, a cure for different kinds of respiratory diseases such as bronchitis and influenza, because the leaves being rich in biologically active compounds such as phytochemicals, antioxidants, carotenoids, tocopherols, and vitamin C [5,6]. The fresh and dried leaf of Moringa is widely sold for vegetable and herbal use and consumed in cities of Ethiopia, due to its perceived awareness of health and nutritional benefits [4,7]. The dried Moringa leaf is eaten fresh, cooked, or stored as dried powder for further uses [6]. The leaves of the Moringa tree are potential sources to improve nutrition, boost food security and foster rural development, due to its immense nutritional value such as minerals,

antioxidants, protein, and vitamins [8,9]. However, there is no as such compiled information in Ethiopia about dried *Moringa* leaves and its infusion nutritional (proximate, minerals, phytochemicals and antioxidants) properties. Therefore, evaluation of dried *Moringa* leaves and its infusion nutritional properties and compile information are important for the user in the form of dried and tea.

MATERIALS AND METHODS

Sample collection and preparation

Fresh *Moringa* leaves were obtained from Arba Minch Agriculture Research Center (Ethiopia) and the samples were packed in polyethylene (plastic) bags and transported to Wondo Genet Natural Product Research and Hawassa University Food Science and Technology Laboratory.

The fresh leaves of uniform shape, color and size were spread thinly on paper-lined wooden trays and subjected to shade drying at ambient temperature to prevent the loss of volatile aroma compounds and also photooxidation for five days according to method reported by killedar et al. [10]. The dried samples were separately milled using an electric Blender (Model BLG401, Zhejiang YiLi Tool Co., Ltd., China). The milled samples were sieved using 2 mm sieve size to separate the milled leaves and

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the large size sample. The sieved sample was kept in an air-tight container and stored at room temperature until further analysis (Figure 1).

Preparation of extracts

The extract was prepared from the sieved samples according to the method reported by Koh et al. [11] and Mingarro et al. [12]. The infusion was boiled in tape water for 5 min at 97°C as usual for normal tea preparation. The infusion was filtered through a double-layered muslin cloth to get rid of the large particles and filtered through a filter paper (Whatman no. 1). The filtered product was then allowed to concentrate at 45°C for three consecutive days by evaporating excess water and obtain the dried. The extract was weighed and its representative percentage yield was recorded. The crude extract 1 g was dissolved in 50 mL of the solvent (99.9 analytical grade methanol) to make a stock solution of 20 mg/ mL. The prepared stock solution was kept at 4°C in a refrigerator, to serve as the working solution for all the phytochemicals and antioxidant tests.

Proximate analyses

Moisture: The moisture content of milled dried leaves of *Moringa* was determined using the method used by Nielsen [13]. A dry sample of 2 g was weighed in triplicates and placed in a forced-air oven at 105°C for 3 hrs. The samples were removed and cooled in desiccators. The loss in weight was determined and recorded as the moisture content. The percentage of moisture was calculated using equation 1.

Where W_1 = weight of fresh samples, W_2 =weight of fresh samples and crucibles and W_3 = weight of dried samples and crucible.

Ash: The ash content was determined according to the method used by Nielsen (13). A dry sample of 2 g was measured into crucibles of a known weight and incinerated in a muffle furnace at 550°C for 3 hrs. The samples were then cooled in desiccators and weighed. The percentage of total ash was calculated using equation 2.

Where; W_1 = Weight of empty crucible, W_2 = Weight of fresh samples and W_3 =Weight of ashed samples and crucibles.

Protein: The crude protein was determined using the Kjeldahl

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method as described by Nielsen [13]. Dry Sample of 2 g was introduced into the digestion flask. Then, 10 mL of concentrated sulphuric acid (H₂SO₄), a mixture of 2.5 g of copper sulphate (CuSO₄), Potassium sulphate (K,SO₄), and Titanium dioxide (TiO₂) were added into each sample and digested in a Kjeldahl digestion flask (KDN-20C, China) at 380°C for 6 hrs., until the mixture clears. The digest was filtered into 500 mL volumetric flask and made up to mark with 100 mL deionized water and connected for distillation. Ammonia was steam distilled for an hour to which had been added 20 mL of 40% NaOH solution. The distillates 200 mL were collected in 250 mL conical flask containing 20 mL of $0.2 \text{ N} \text{ H}_2\text{SO}_4$ and methyl red indicator. The ammonia that distilled into the receiving conical flask was reacted with 0.2 N H₂SO₄ and the excess acid in the flask was estimated by back titration against 20 mL of 0.1 N NaOH with a color change from red to yellow. A blank distilled was collected in 250 mL conical flask containing 20 mL of 0.2 N H₂SO₄ and methyl red indicator. The distillate was titrated against 20 mL of 0.1 N NaOH. Total nitrogen and crude protein content of the sample was then determined and calculated using equation 3 and 4, respectively.

Where, W₁=sample weight, M=molar mass

Crude fat: The crude fat content was determined using the Soxhlet method according to the method used by Nielsen [13]. In this method, 2 g of the sample was added into a flat bottom flask of known weight with the extractor mounted on it. The thimble was held halfway into the extractor and the weighed sample was transferred into the thimble and the thimble was plugged with cotton wool and extracted with 200 mL of petroleum ether for 6 hours. The solvent-free fat in the flux was dried in an oven for an hour at 105°C, cooled in desiccators, and fat content was then calculated using equation 5.

Crude fat (%) = $(W_{e}+W_{e})-W_{e}/W_{s} \times 100....5$

Where $W_e = Weight$ of extract, $W_f = Weight$ of flux and $W_s = Weight$ of sample.

Crude fiber: The crude fiber was determined according to the method used by Nielsen [13]. The dried samples of 2 g were introduced into the extraction unit, $150 \text{ mL of hot } 0.2 \text{ N H}_2\text{SO}_4$ was



Figure 1: Fresh, dried and powder Moringa leaves.

added and digested for 30 min. Then, the acid was drained and the sample was washed with hot deionized water. Finally, the fiber was extracted and dried by moistening with a small portion of acetone which was then allowed to drain. The sample was incinerated at 550°C for 3 hours. Until all carbonaceous matter was burnt. The crucible containing the ash was cooled in the desiccators and weighed. The percentage of crude fiber was calculated using equation 6.

Where: W_1 = weight of sample used W_2 = Weight of sample and crucible before ashing W_3 = Weight of crucible and ash.

Carbohydrate (CHO): Carbohydrate content of the milled leaves of *Moringa* was determined by difference as described by Ihekoronye & Ngoddy [14] and calculated using equation 7.

%CHO = 100 - % (ash + protein + fat + crude fiber + moisture) 7

Mineral content: The mineral contents such as: Sodium (Na), Potassium (K), Magnesium (Mg), Calcium (Ca), Zinc (Zn), Iron (Fe), Copper (Cu) and Manganese (Mn) of *Moringa* were analyzed as described by Marcinek & Krejpcio [15] using ash method. The minerals were analyzed using an Atomic Absorption Spectrophotometer (Spectra AA 220, USA Varian). Sample of *Moringa* 2 g was put in crucibles and then in a muffle furnace for 3 hr. to obtain ash. The residue was dissolved in 10 mL of HNO₃: HCl (2:3 v/v) then heated until fumes disappeared. The solution was transferred separately in 250 mL volumetric flasks by filtration using Whatman filter paper No 42, then volume made up to 250 mL with distilled water. The concentration of mineral was calculated and expressed as mg/100 g as described in equation 8.

Element (mg/100 g)=
$$C \times V \times df/W$$

.....

8

Where C = The concentration of the element in the sample solution in mg/L; V = The volume of the undiluted sample solution in mL; W = The sample weight in grams, and df = is the dilution factor.

Phytochemicals

Total Phenolic Content (TPC): The total phenolic content of the extract was estimated according to the method used by Shan et al. [16] using gallic acid as a standard. Folin-Ciocalteu reagent (diluted ten times) (1 mL) was added to 0.1 mL of the extract (1 mg/mL). The mixture was left for 5 min and 1 mL (7.5% w/w) of sodium carbonate was added. The absorbance of the resulting blue color was measured at 765 nm with a UV-visible double beam spectrophotometer (Spectronic 20, UK) after incubation for 90 min at room temperature. The TPC was estimated from gallic acid calibration curve (y = 0.0073x -0.0462, R² = 0.973) and results were expressed as milligram gallic acid equivalent/gram of dried extract (mg GAE/g).

Total Flavonoid Content (TFC): Total flavonoid content (TFC) of extract was determined according to the method used by Ayoola et al. [17]. The extracts (1 mg/mL) were diluted with 1.25 mL distilled water and 0.75 μ L 5% NaNO₂ was added to the mixture. After 6 min, 150 μ L 10% AlCl₃ was added and after another 5 min, 1mL Na OH was added to the mixture. Immediately the absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 510 nm. All the calculations were done using standard equation Catechin obtained from standard calibration curves (y=0.0014x+0.0192, R²=0.97 (p<0.001)). Results were expressed as milligram of catechin equivalents per gram of dry extract (mg CE/g).

Condensed Tannins Content (CTC): The condensed tannins content of the exract was assayed as described by Chew et al. [18]. The undiluted crude extract 0.5 mL was first mixed with 3 mL of vanillin reagent (4%, w/v, in absolute methanol), and followed by the addition of 1.5 mL of concentrated HCl (37%). The mixture was stored in a dark at room temperature for 15 min. Blank was prepared by replacing 0.5 mL of the undiluted crude extract with 0.5 mL of deionized water. The absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 500 nm. Catechin was used for calibration of the standard curve (y=0.0042x+0.0331, R²=0.995 (p<0.001) and the results were expressed as mg catechin equivalent per 100 g dry weight sample (mg CE/100 g).

Antioxidant activities

DPPH radical scavenging activity: The 2, 2-diphenyl-1picrylhydrazyl (DPPH) radical scavenging activity of the extract was determined as described by Brand-Williams et al. [19]. Different concentrations (50 to 1000 μ g/mL) of the extracts were taken in different test tubes. Freshly prepared DPPH solution (2 mL, 0.06%, and w/v) in methanol was added in each of the test tubes containing 1 mL of the extract. The reaction mixture and the reference standards (ascorbic acid and BHT) were vortexed and left to stand at room temperature in the dark for 30 min. The absorbance of the solutions was measured using a UV-visible spectrophotometer (Janeway, 96500, UK) at 520 nm. Methanol (100%) was used as a blank. The ability to scavenge the DPPH radical was calculated using equation 9:

Radical scavenging effect (%)= $A_c - A_c \times 100$ 9

Where $A_c = Absorbance$ of the control; $A_s = Absorbance$ of the sample

The antioxidant activity of the extract was expressed as IC-50 (Inhibitory Concentration 50%) and value is the concentration in (μ g/mL) of extracts that scavenges the DPPH radical by 50%.

Ferric reducing antioxidant power: This assay was carried out according to the method used by Safdar et al. [20]. One milliliter of the extract with a concentration of 1 mg/mL was mixed with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. Then the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 mL, 10%) was added to the mixture. Finally, 2.5 mL of the supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL FeCl₃ (0.1%) and the absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 700 nm. The reducing power was expressed as mg of ascorbic acid equivalents/g of dried extract (mg AAE/g) using the calibration curve (y=0.0063x+0.148, R²=0.99 (p<0.01)).

Total antioxidant activity using phosphomolybdenum assay: The total antioxidant activity of extract was determined by phosphomolybdenum assay according to the method used by Prieto et al. [21]. Sample of 0.3 mL extract (1 mg/mL) in the solution was mixed with 3 mL phosphomolybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulphuric acid) in capped test tubes. The samples were incubated for 90 min in a water bath at 95°C. After cooling to room temperature,

Table 1: The proximate composition of dried Moringa leaves in (g/100 g db).												
Sample	Moisture		Ash	Crude protein	Crude fa	Crude fiber		Carbohydrate				
Moringa	6.0 ±	0.5	11.2 ± 0.3	25.3 ± 0.2	6.9 ± 0.2	9.3	3 ± 0.7	41.3 ± 0.7				
Table 2: Mineral contents of dried Moringa leaves.												
Sample	Ca	Na	K	Mg	Fe	Zn	Cu	Mn				
Moringa	1300.7 ± 0.1	128.9 ± 0.1	330.9 ± 0.1	1056.6 ± 0.1	47.7 ± 0.3	1.8 ± 0.1	0.8 ± 0.1	3.4 ± 0. 3				

 Table 3: Total Phenolic Contents (TPC), Total Flavonoid Contents (TFC), Condensed Tannin Contents, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) scavenging

 IC 50 values, ferric reducing power (FRAP), and total antioxidant activities (TAA) of dried Moringa leaves.

Sample	DPPH scavenging (IC 50, g/mL)	Ferric reducing power (mg AAE/g)	Total antioxidant (mg BHTE/g)	TPC (mg GAE*/g)	TFC (mg CE/g)	CTC (mg CE/g)
Infusion	0.48 ± 0.12	2.27 ± 0.02	1.23 ± 0.02	2.6 ± 0.1	11.9 ± 0.2	3.3 ± 0.1
AA	0.03 ± 0.02		~	~	~	~

Values are mean \pm SD (n=2), Total Phenolic Content (TPC) expressed as gallic acid equivalent per g of the dried extract; 2: Total Flavonoid Content (TFC) expressed as catechin equivalent per g of the dried extract, AAE/g: Ascorbic acid equivalents per gram of dried extract; BHTE/g: Butylated hydroxytoluene equivalents per gram of dried extract and whereas AA (Ascorbic acid).

the absorbance of the solutions was measured using a UV-visible spectrophotometer (JANEWAY, 96500, UK) at 695 nm against a blank (3 mL methanol without plant extract). The total antioxidant activity was expressed as milligram butylated hydroxytoluene equivalent/gram of dried extract (mg BHTE/g) using a calibration curve (y=0.0094x+0.112, R²=0.99 (p<0.001)).

Statistical analysis

All data were analyzed using one-way ANOVA with traits as an independent variable using SAS 9.3 software and the Values are expressed in mean \pm SD

RESULTS AND DISCUSSION

Proximate composition

The level of moisture, ash, crude protein, crude fat, crude fiber and carbohydrate in food is of utmost importance for many scientific, technical and economic reasons. The chemical composition of dried leaves of *Moringa* is shown in Table 1. Dried *Moringa* leaf had moisture level (6 g/100 g), ash (11.2 g/100 g), crude protein (25.3 g/100 g), crude fat (6.9 g/100 g), crude fiber (9.3 g/100 g) and carbohydrate (41.30 g/100 g). This finding is similar to the reports of nutritional assessment of dried *Moringa* leaves (moisture, crude fat, and carbohydrate), higher (proteins) and lower (fiber content) by Abuye et al. [22]. The proximate composition of the *Moringa* leaves in the current study was lower (moisture, protein, and fiber) and higher (ash fat and carbohydrate) than that the report by Ilyas et al. [23]. The variability of this finding and others scholars is might be due to climatic, seasonal and processing methods [24].

Mineral content

The minerals of dried *Moringa* leaves results are showed (Table 2). The dried *Moringa* leaves had level of Ca (1300.7 mg/100 g), Na (128.9 mg/100 g), K (330.9 mg/100 g), Mg (1056.6 mg/100 g), Fe (47.7 mg/100 g), Zn (1.8 mg/100 g), Cu (0.8 mg/100 g) and Mn (3.4 mg/100 g). The mineral levels of *Moringa* were lower than that of findings of Abuye et al. [22] and higher for all minerals than from the finding of Seifu [25]. The variability of this finding and others scholars is might be due to climatic, seasonal and processing methods [24].

Phytochemical properties of infusion

The phytochemical properties of infusion of dried *Moringa* leaves results are showed (Table 3). The dried *Moringa* leaves infusion had level of 2.6 \pm 0.1 mg GAE*/g Total Phenolic Content (TPC), 11.9 \pm 0.2 mg CE/g) Total Flavonoid Content (TFC) and 3.3 \pm 0.1 mg CE/g Condensed Tannin Content (CTC). The phytochemical contents of the dried *Moringa* leaves infusion of this study is higher than the *Moringa* herbal tea infusion done by Ilyas et al. [23] and Okiki et al. [26]. The difference could be due to the difference in tea plants, the method used and climatic condition.

Antioxidant Activities of dried Moringa infusion

The antioxidant properties of dried *Moringa* leaves infusion results are showed (Table 3). The dried *Moringa* leaves had level of 0.48 \pm 0.12 (IC-50, g/mL) DPPH scavenging, 2.27 \pm 0.02 mg AAE/g Ferric Reducing Power (FRAP) and 1.23 \pm 0.02 mg BHTE/g and Total Antioxidant (TAA). The concentration of an antioxidant needed to decrease the initial DPPH concentration by 50% (IC-50) is a parameter widely used to measure antioxidant activity [27]. The lower the IC-50 the higher is the antioxidant activity [19]. This finding is lower than the finding of Ilyas et al. [23] and higher than the finding of Tebeka and Libsu [28] on assessment of antioxidant potential of *Moringa* leaf extract. The difference could be due to the difference in tea plants, the method used and climatic condition.

CONCLUSION

From this study, it can be concluded that scientific evidence of dried *Moringa* (*Moringa stenopetala*) leave and its nutritional properties such as proximate (Moisture, Ash, crude protein, crude fat, crude fiber and carbohydrate), minerals (Ca, Na, K, Mg, Fe, Zn, Cu and Mn), phytochemicals (total phenolic, total flavonoid, and condensed tannin) content, and antioxidant (DPPH scavenging activity, ferric reducing assay power and total antioxidant) capacity of dried *moring* leaves and its infusion were done and the results were compiled as shown under results and discussion parts. However, there is a need to carry out further composition profile of the dried *Moringa* leave infusion using GS-MS, HPLC and UPLC to explore the potential chemicals present in the infusion.

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