

Effects of Boiling Time on the Antioxidant Activity of Moringa Leaf Extract

Obiajulu, Emmanuel Ifeanyi^{1*}, Asogwa, Ifeyinwa Sabina²

¹Department of Food Technology, Federal Polytechnic, P.M.B. 1012, Kaura Namoda, Zamfara State.

²Department of Food Science and Technology, University of Nigeria, Nsukka, Enugu State.

***Corresponding Author:** Obiajulu, Emmanuel Ifeanyi, Department of Food Technology, Federal Polytechnic, P.M.B. 1012, Kaura Namoda, Zamfara State

Abstract: The effect of boiling time on the antioxidant activity of Moringa leaf extracts was determined by producing an aqueous extracts from Moringa oleifera leaves which was harvested, sorted weighed, washed and properly homogenized with distilled water using blender, the extracts were divided into segments; which are unboiled, boiled for five, ten and fifteen minute. The antioxidant activity of the extracts were determined using 2,2 Diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) methods at four different concentrations (4, 6, 8 and 10mg/ml); the DPPH and the FRAP value increased significantly with increase in both the boiling time and reagent concentration. The nutritional composition, moisture was 84.915% for MLE15 to 97.580% for MLE0, protein was 0.630% for MLE0 to 1.395% for MLE15, fat 0.835% for MLE0 to 2.955% for MLE15, for fibre nothing was detected for all the samples, carbohydrate 0.470% for MLE0 to 9.280% for MLE15, the β -carotene was 1.447mg/100g for MLE0 to 10.346% for MLE15 and vitamin C 20.850% for MLE15 to 32.970% for MLE0. All the data are expressed in mean of duplicate values and were subjected to ANOVA using SPSS version 16 at $p < 0.05$.

Keywords: moringa, antioxidant, phytochemical, extracts, leaf, beta-carotene, vitamins.

1. INTRODUCTION

Africa is blessed with a sufficient array of phytochemical rich plant resources; unfortunately many are underutilized thereby limiting their health promoting benefits. Moringa is one of such underutilized crops, in order to promote its utilization and consumptions, studies aimed at investigating its antioxidant activity are pertinent.

Moringa oleifera is the most widely cultivated species in the genus *Moringa*, the only genus in the plant family Moringaceae. Common names include moringa, Drumstick tree, Ben oil tree or Benzoil tree [1] and it is also called “Zogale” in Northern Nigeria.

M. oleifera is a fast-growing, drought-resistant tree, native to the southern foothills of the Himalayas in Northwestern India, and widely cultivated in tropical and subtropical areas where its young seed pods and leaves are used as vegetables, and many parts of the tree are used in traditional herbal medicine. Moringa can also be used for water purification and hand washing [2]. The leaves are the most nutritious part of the plant, being a significant source of B vitamins, vitamin C, pro vitamin A as beta-carotene, vitamin K, manganese, and protein, among other essential nutrients [3]. The leaves are cooked and used like spinach and are commonly dried and crushed into powder used in soups and sauces. [1].

Moringa oleifera are grown and used in many countries around the world is a multi-purpose tree with medicinal, nutritional and socio-economic values. In Senegal and Benin, *M. oleifera* leaves are dispensed as powder at health facilities to treat moderate malnutrition in children, which establishes the medicinal uses of *M. oleifera* leaves by local communities in Uganda. More than twenty-four medicinal uses of *M. oleifera* leaves were established and the medicinal abilities were as a result of the phytochemicals found in them. The local communities in Uganda use *M. oleifera* leaves to treat common ailments. Presence of phytochemicals in the extracts, indicate possible preventive and curative property of *M. oleifera* leaves [4].

Antioxidants are compounds that inhibit oxidation, oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions to balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase), produced internally, or the dietary antioxidants vitamin C, and vitamin E [5].

The term "antioxidant" is mostly used for two entirely different groups of substances: industrial chemicals that are added to products to prevent oxidation, and naturally occurring compounds that are present in foods and tissue. The former, industrial antioxidants have diverse uses: acting as preservatives in food and cosmetics, and being oxidation-inhibitors in fuels [5].

Antioxidants are the molecules that prevent cellular damage caused by oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from one molecule to an oxidizing agent which is known to produce free radicals. These free radicals are highly reactive species which contains one or more unpaired electrons in their outermost shell. Once they are formed, the chain reaction starts. Antioxidant reacts with these free radicals and terminates this chain reaction by removing free radical intermediates and inhibits other oxidation reactions by oxidizing themselves.

Although oxidation reactions are crucial for life, they can also be damaging, plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases [6]. Oxidative stress plays a key role in causing various human diseases, such as cellular necrosis, cardiovascular disease, cancer, neurological disorder, Parkinson's dementia, Alzheimer's disease, inflammatory disease, muscular dystrophy, liver disorder, and even aging. Besides, there are some antioxidants in the form of micronutrients which cannot be manufactured by the body itself such as vitamin E, β -carotene, and vitamin C, and hence these must be supplemented in the normal diet [7], and some of which can be affected by processing.

Food processing is the transformation of agricultural products into food, or of one form of food into other forms. Food processing includes many forms of treating foods, from grinding grain to make raw flour to home cooking to complex industrial methods used to make convenience foods [8]. It is known that processing affects the nutritional and phytochemical composition of plants.

2. MATERIALS AND METHODS

Fresh Moringa leaves were harvested from a farm in Eha-Alumona, Nsukka Local Government, Enugu State. Other chemicals and equipment used were from the Department of Nutrition and Dietetics Laboratory of the University of Nigeria Nsukka

2.1. Preparation of the Aqueous *moringa* Leave Extracts

Moringa leaf (1.2 kg) was used for the experiment, the leaves was divided into four (4) different treatment groups of 300 g each; unboiled, boiled for 5mins, 10mins and 15mins respectively. An aqueous extraction was done on the individual groups by homogenizing the leaves with 1.2 ltrs of water in a blender (Binatone, Germany 8219sp) followed by filtration with muslin cloth then boiling at varying length of time as stated before for use. The samples were stored in separate dark coloured bottles, allowed to cool and later used for analysis.

2.2. Proximate Analysis

The following proximate analysis was determined, the moisture, ash, protein, fibre and carbohydrate contents of the leaf extracts.

2.3. Determination of Moisture Content

The moisture content of the samples was determined using the hot oven method of AOAC [9]. Exactly 5ml of each sample was put into a washed, dried and pre-weighed crucible dish and placed in a Phoenix oven at a temperature of 70-80°C for 2hrs and at 100-105°C until the weight was constant. The samples were cooled in desiccators and weighed. The weight loss was obtained as the moisture content and was calculated as:

$$\% \text{ Moisture content} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where; W_1 = initial weight of empty crucible, W_2 = weight of crucible + sample before drying

W_3 = final weight of crucible + sample after drying

2.4. Determination of Crude Protein

The crude protein of the samples was determined by the semi-micro Kjeldahl technique described by AOAC [9]. Exactly 2 ml of the sample was put into a Kjeldahl flask. Three grams (3 g) anhydrous sodium sulphate and one (1 g) of hydrated copper sulphate (catalyst) were added into the flask. Then 20ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) was added to digest the sample.

The digestion continued under heat until a clear solution was observed. The clear solution was then cooled and made up to 100 ml with distilled water and a digest of about 5 ml was collected for distillation. Then 5ml of 60 % sodium hydroxide (NaOH) was put into the distillation flask and distillation was allowed to take place for some minutes. The ammonia distilled off was absorbed by boric acid indicator and this was titrated with 0.01M hydrochloric acid (HCl). The titre value of the end point at which the colour changed from green to pink was taken. The crude protein was calculated as:

$$\% \text{ Crude protein} = \frac{0.0001401 \times T \times 100 \times 6.25}{W \times 5}$$

Where; T= titre value, W= weight of sample dried

2.5. Determination of Fat Content

The Soxhlet extraction method as described by AOAC [9] was used. The extraction flasks were washed with petroleum ether, dried and cooled and weighed. Two (2) ml of the samples were collected into the extraction thimble. It was then placed back in the Soxhlet apparatus. The washed flask was filled to about three quarter of its volume with petroleum ether (that has the boiling temperature range of 40-60 oC). The apparatus was then set-up and extraction carried out for a period of 4-6 hours after which complete extraction was made. The petroleum ether was recovered leaving only oil in the flask at the end of the extraction. The oil in the extraction flask was dried in the oven, cooled and finally weighed. The fat content was expressed as a percentage of raw materials. The difference in weight of empty flasks and the flask with oil content which was calculated as:

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

Where B = Weight of empty flask, A = Weight of sample, C = Weight of flask + Oil.

2.6. Determination of Crude Fibre

The crude fibre content of the samples was determined using the method described by AOAC. [9]. Ten (10) ml of sample was digested with 200 ml of 0.22M H_2SO_4 , filtered and washed severally with quantitatively into another conical flask and dissolved in a 200 ml of 1.25 % NaOH solution, boiled for 30 mins, cooled filtered and washed with boiling water.

The residue will be dried at 105 oC for 2 hrs, cooled in a desiccators and weighed (W_1). W_1 will be incinerated at 550 oC for 2 hrs in a muffle furnace, cooled in a desiccators and reweighed (W_2). Percentage crude fibre will be calculated thus:

$$(W_1 - W_2 / S) \times 100$$

Where: W_1 =weight of sample before incineration, W_2 = weight of sample after incineration,

S= weight of sample use.

2.7. Determination of Ash Content

The ash content of the sample was determined by the method recommended by AOAC [9]. A silica dish was heated to about 60 oC, cooled in desiccators and weighed. 5 ml of the sample was put into the silica dish and transferred to the furnace. The temperature of the furnace was then allowed to reach about 525 oC after placing the dish in it. The temperature was maintained until whitish-grey colour was obtained indicating that all the organic matter content of the sample has been destroyed. The dish

was then brought out from the furnace and cooled in the desiccators and re-weighed. The percentage ash content was calculated as:

$$\% \text{ ash content} = (C-A/B) \times 100$$

Where; A = weight of empty dish, B = weight of empty dish + sample before ashing

C = weight dish + ash

2.8. Determination of Carbohydrate

Carbohydrate was determined as the nitrogen free extraction calculated by difference as described by [9]. The formula below was used:

$$\% \text{ Carbohydrate} = 100 \% - (\text{protein} + \text{fat} + \text{fibre} + \text{ash} + \text{moisture})$$

2.9. Determination of Some Selected Vitamins

Bearing in mind the food sample used for this experiment is a vegetable it was necessary to determine some predominant vitamins that are present in it and to also study the effect of boiling time to their availability to the consumers. Some of the determined vitamins were β -carotene (Vitamin A precursor) and Vitamin C.

2.10. Determination of β -Carotene

Beta-carotene will be determined using spectrophotometric method described by Pearson, [10]. One (1 ml) of the sample was dissolved with 10ml of acetone in a 50ml conical flask, allowed to stand for 20mins and shake gently at 4mins interval to extract the colour substance in the sample. About 10ml of water was added after agitation and allowed to settle. The upper layer was separated. The solution was cleared into a test tube; 5ml of hexane was added, allowed to settle in 2 layers. The layers were separated using a separating funnel. The upper layer was used for β -carotene analysis. Then 2 ml of the supernatant was pipetted into a cuvette and read in the spectrophotometer (Janway-6300, England) at 453nm.

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of standard}}{\text{Weight of sample}}$$

2.11. Vitamin C Content Determination

Vitamin C content was determined by titration following the AOAC [9] protocol. Standard ascorbic acid solution (Sigma, USA) was made by dissolving 0.05 g of pure acid in 45 ml of extraction solution in a volumetric flask and the solution was made up to the 50 ml mark. The vitamin C extraction solution (5 % trichloroacetic acid) was made by dissolving 50 g of the pure acid in one litre of distilled water. Ten (10) ml of the sample was transferred into a volumetric flask. More extraction solution was added until the 100 ml mark, the contents mixed thoroughly and filtered immediately. Aliquots (10 ml) of the extract were titrated against standard 2,6 dichlorophenol indophenols (DCPIP) obtained by dissolving 0.05 g of DCPIP in 100 ml distilled water and standardized by titration against 2 ml of standard ascorbic acid. An equivalent amount of the extraction solution taken as the blank was titrated against standard DCPIP and correction for it made in the final titre. The ascorbic acid content of the sample was calculated using the formula;

$$\text{Vitamin C content (mg/100g DM)} = \frac{\text{Titre} \times V_e \times V_1 \times 100 \times 100}{V_2 \times S \times 1000 \times Y}$$

Where V_e = vitamin C equivalent of 1ml of DCPIP (mg/ml)

V_1 = total extract volume (ml), V_2 = titrated extract volume (ml), S = sample weight

Y = sample dry matter (%)

2.12. Determination of Antioxidant Activity

The antioxidant activity of the moringa leaf extracts were determined using the free radical scavenging activity and the ferric reducing antioxidant power.

2.13. Determination of Free Radical Scavenging Activity

The DPPH free radical scavenging activity of aqueous extracts of *M. oleifera* was determined according to the method reported by Brand-Williams et al., [11] with slight modification. The stock solution of the radical, prepared by dissolving 24 mg DPPH in 100 ml methanol, was kept in a refrigerator until further use. The working solution of the radical was prepared by diluting the DPPH stock solution with methanol to obtain an absorbance of about 0.98 (± 0.02) at 517 nm.

In a test tube, 3 ml DPPH working solution was mixed with 100 μ l plant extract (1 mg/ml). The absorbance was measured with a UV-visible spectrophotometer (Janway-6300, England) at 517 nm for a period of 30mins. The percent antioxidant or radical scavenging activity was calculated using the following formula:

$$\% \text{Antioxidant activity} = [(Ac - As)/Ac] \times 100$$

Where, Ac and As are the absorbance of control and sample, respectively. The control contained 100 μ l methanols in place of the plant sample. The procedure was repeated at different DPPH concentrations (4, 6, 8 and 10mg/ml).

2.14. Measurement of the Reducing Power of the Extracts

The reducing power of the aqueous sample extracts was determined according to the method of Yen and Chen [12]. The extracts (2ml) were mixed with an equal volume of 0.2M phosphate buffer, pH6.6 and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20mins after which an equal volume of 1% trichloroacetic acid (TCA) was added to the mixture, which was then centrifuged (Gallenkamp, England) at 5,000rpm for 10mins. The upper layer of the solution was mixed with distilled water and 0.1% FeCl₃ in a ratio of 1:1:2 and the absorbance of the upper layer were measured with UV-visible spectrophotometer (Janway-6300, England) at 700nm, at varying concentrations and using ascorbic acid as control. Increased absorbance of the reaction mixture indicated increased reducing power. Total reducing power was expressed as absorbance units per total phenolics per gram of sample. The procedure was repeated at different FRAP concentrations (4, 6, 8 and 10mg/ml).

3. RESULTS AND DISCUSSIONS

The effect of boiling time on the proximate composition of the moringa leaf extracts is shown in table 1. Boiling time brought about a significant ($p < 0.05$) variation in the proximate properties of the moringa leaf extracts. The ash content of the extracts appreciated significantly ($p < 0.05$) with the boiling time, the content ranged from 0.485 % for MLE₀ to 1.455 % for MLE₁₅, the increase in the ash content could attribute to evaporation of water with subsequent concentration of constituents. Udousoro and Etuk, [13] had similar results in their research work on fresh *Telfairia occidentalis*. The ash content of a food or substance tells us the mineral content of that substance and minerals are nutrients that are needed in the body for body metabolism and tissue formations.

The moisture content of the extracts reduced significantly ($p < 0.05$) as the boiling time increases; the moisture content ranged from 84.915 % for MLE₁₅ to 97.580 % for MLE₀, the decrease in the moisture content of the extracts is as a result of the heating process which brings about evaporation in the extracts and also the high moisture content is attributed to the extraction method (aqueous extraction) of the moringa leaf extracts. Navarro-Gozalez *et al.*, [14] observed same trends of values for *Tropaelum majus* and *Spilanthes oleracea* which has a high moisture value be declines as the heating time progresses and Asogwa *et al.*, [15] observed same on boiling Moringa leaf.

The protein content of the moringa leaf extract increased significantly ($p < 0.05$) with the boiling time, the proteins content ranged from 0.630 % for MLE₀ and 1.395 % for MLE₁₅, the increase in the protein level would be as a result of the concentrating of the extracts due to heating, similar increase was also seen in the work done by Bakari *et al.*, [16] on *Cactus cladode* extracts, the protein content will beneficial to the body even though it's in small amount.

Table1. Proximate Compositions of the Moringa Leaf Extracts (%)

Samples	MLE ₀	MLE ₅	MLE ₁₀	MLE ₁₅
ASH	0.485 \pm 0.01 ^a	0.485 \pm 0.01 ^a	0.960 \pm 0.04 ^b	1.455 \pm 0.02 ^c
MOISTURE	97.580 \pm 0.01 ^c	96.925 \pm 0.23 ^c	90.185 \pm 0.13 ^b	84.915 \pm 0.53 ^a
PROTEIN	0.630 \pm 0.01 ^a	0.640 \pm 0.01 ^a	0.900 \pm 0.08 ^b	1.395 \pm 0.08 ^c

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FAT	0.835±0.01 ^a	0.865±0.01 ^a	2.825±0.06 ^b	2.955±0.02 ^c
CRUDE FIBRE	ND	ND	ND	ND
CARBOHDRATE	0.470±0.03 ^a	1.085±0.23 ^a	5.130±0.07 ^b	9.280±0.49 ^c

Values in the same row bearing different superscript are significantly difference at $p < 0.05$; data are expressed as means of duplicates \pm standard deviation (SD)

ND = Nothing Detected. MLE₀ = Unboiled Moringa Leaf Extracts, MLE₅ = Five Minutes Boiled Moringa Leaf Extracts, MLE₁₀ = Ten Minutes Boiled Moringa Leaf Extracts, MLE₁₅ = Fifteen Minutes Boiled Moringa Leaf Extracts.

Table 2. Vitamin Composition of Moringa Leaf Extracts

SAMPLES	β -CAROTENE (mg/100g) (Pro-Vitamin A)	VITAMIN C (mg/100g)
MLE ₀	1.447±0.31 ^a	32.970±0.83 ^c
MLE ₅	6.600±0.02 ^b	29.385±1.01 ^b
MLE ₁₀	7.668±0.02 ^c	21.990±1.32 ^a
MLE ₁₅	10.346±0.06 ^d	20.850±0.1 ^a

Values in the same column bearing different superscript are significantly difference at $p < 0.05$; data are expressed as means of duplicates \pm standard deviation (SD)

MLE₀ = Unboiled Moringa Leaf Extracts, MLE₅ = Five Minutes Boiled Moringa Leaf Extracts.

MLE₁₀ = Ten Minutes Boiled Moringa Leaf Extracts, MLE₁₅ = Fifteen Minutes Boiled Moringa Leaf Extracts.

The fat content of the extracts increases with the boiling time significantly ($p < 0.05$), the contents are 0.835 % for MLE₀ to 2.955 % for MLE₁₅, the increase in the fat content of the leaf extract is as a result concentrating of the extracts and also that moringa is slightly an oil containing plant, related trends was noticed in the work done by Sunday *et al.*, [17] on fresh *Curcuma longa* extract its lipid contents also increased with heating, the fat content of the extract is highly beneficial because the oil found in *Moringa oleifera* is an essential oil.

The fibre content of the moringa leaf extracts was significantly ($p < 0.05$), at increasing boiling time, but the result of the analysis shows that nothing was detected for all the extract samples, this result could be attributed to high moisture content and the filtration process.

The carbohydrate content of the extracts increased significantly ($p < 0.05$) with boiling time, the carbohydrate contents of the extracts ranges from 0.470 % for MLE₀ to 9.280 % for MLE₁₅, the increase in the content is as a result that the extract is carbohydrate based and the concentrating of the extracts which was brought by the boiling of the extracts. Adeniyani *et al.*, [18] observed the similar increase in the carbohydrate content of *Sesamum indicum*.

The effect of boiling time on the vitamin content of the moringa leaf extracts was seen in Table 2. The boiling time of the extracts brought about significant ($p < 0.05$) deviations in the vitamin contents of the moringa leaf extract. The β -carotene content of the extract samples increased significantly ($p < 0.05$) with boiling time, the β -carotene content of the samples increased from 1.447 mg/100g for MLE₀ to 10.346 mg/100g for MLE₁₅, the increase in the β -carotene content of the extracts is attributed to the concentration of the extracts during boiling and also mild heating process disrupts the cell walls of the plant extracts thereby releasing the β -carotene content of the samples and Anjum, [19] had a similar trends of results on his work on spinach and lettuce

The vitamin C content of the leaf extracts decreases significantly ($p < 0.05$) as the boiling time increases, the vitamin C content of the extracts ranged from 20.850 mg/100g for MLE₁₅ to 32.970 mg/100g for MLE₀, the reduction in the vitamin C content of the extracts was because Vitamin C is a water soluble vitamins and that it is heat labile. Some of the vitamin C would have leached out during the washing process. The work done by Igwemmar *et al.*, [20] on some vegetables portrays similar trends of vitamin C content decrease when heat processed.

The effect of boiling time on the DPPH (2,2, Diphenyl -1- picrylhydrazyl) activity of the moringa leaf extracts at varying DPPH concentrations (4, 6, 8 and 10 mg/ml) is presented in Figure 1. Both the DPPH concentration and the boiling time led to significant ($p < 0.05$) increases in the DPPH activity of the moringa leaf extracts. For instance at 4 mg/ml, the DPPH activity increased from 15.140 % for MLE₀ to 35.490 % for MLE₁₅, at 6 mg/ml it increase from 17.690 % for MLE₀ to 45.465 % for

MLE₁₅, at 8 mg/ml it was from 18.265 % for MLE₀ to 47.905% for MLE₁₅ and at 10 mg/ml it was 28.150 % for MLE₀ to 54.035 % for MLE₁₅. The increase in the DPPH activity of the moringa leaf extracts came as expected because of the results got from the flavonoid and phenol of the moringa leaf extracts Obiajulu *et al.*, [21], so their increase is as a result of the high content of the phenolics compounds in the leaf. Phenolic compounds are known to exhibit good antioxidants. Shukla *et al.*, [22] also observed an increase in the DPPH activity of *Dracaena reflexa* leaves, when subjected to heating over time and also similar trends was noticed in the investigation of the DPPH activity of *Reinwardtia indica* leaves extract [23]. The increase in the activity for both DPPH and FRAP are highly beneficial to human health because they are great indicators that moringa leaf extracts is a rich source of natural antioxidants and can be used for the reduction of the high rate of oxidative stress related illnesses like cancer, obesity, diabetes and host of others.

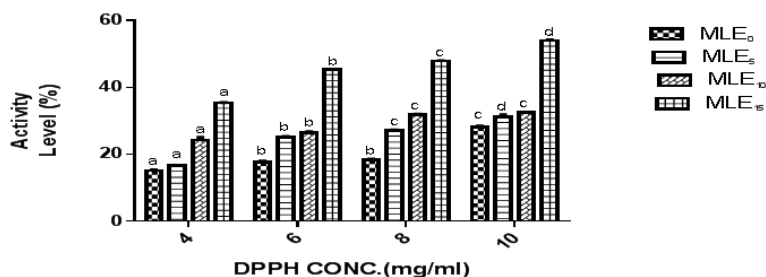


Figure1. Effect of Boiling Time on the DPPH Activity (%) of Moringa Leaf Extracts at varying DPPH Concentration

Bars of the same pattern bearing different superscript are significantly difference at $p < 0.05$; data are expressed in mean of duplicates \pm standard deviation (SD)

MLE₀ = Unboiled Moringa Leaf Extracts, MLE₅ = Five Minutes Boiled Moringa Leaf Extracts,

MLE₁₀ = Ten Minutes Boiled Moringa Leaf Extracts, MLE₁₅ = Fifteen Minutes Boiled Moringa Leaf Extracts.

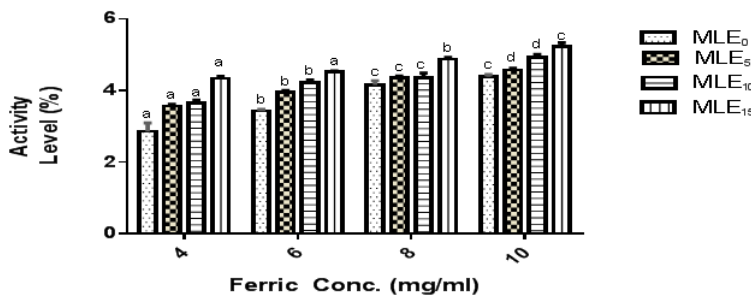


Figure2. The Effect of Boiling Time on the Ferric Reducing Antioxidant Power of the Moringa Leaf Extracts at Different FRAP Concentration

Bars of the same pattern bearing different superscript are significantly difference at $p < 0.05$; data are expressed in mean of duplicates \pm standard deviation (SD)

MLE₀ = Unboiled Moringa Leaf Extracts, MLE₅ = Five Minutes Boiled Moringa Leaf Extracts.

MLE₁₀ = Ten Minutes Boiled Moringa Leaf Extracts, MLE₁₅ = Fifteen Minutes Boiled Moringa Leaf Extracts.

The effect of boiling time on the FRAP (Ferric Reducing Antioxidant Power) of moringa leaf extracts at varying FRAP reagent concentrations (4, 6, 8 and 10 mg/ml) is shown in figure 2. Both the FRAP concentration and the boiling time increment had led to significant ($p < 0.05$) increase in FRAP potentials of the moringa leaf extracts. For example at 4 mg/ml the FRAP activity appreciated from 2.868 % for MLE₀ to 4.335 % for MLE₁₅, at 6 mg/ml it move from 3.430 % for MLE₀ to 4.520 % for MLE₁₅, at 8 mg/ml it was from 4.165 % for MLE₀ to 4.875% for MLE₁₅ and at 10 mg/ml it was 4.390 % for MLE₀ to 5.225 % for MLE₁₅. The increase in the FRAP activity of the moringa leaf extracts did not come as surprise bearing in mind the results of the flavonoid and phenol of the moringa leaf extracts [21], so their increase is as a result of the rich sources of the phenolics compounds in the moringa leaf. A similar trend was observed in the investigation done by Shukla *et al.*, [22] on *Dracaena reflexa* leaves and also that of Abdul-rasheed *et al.*, [24] on the extracts some commonly used spices in India when heated over time.

4. CONCLUSION

The research showed that the moringa leaf extracts contain phenol and flavonoids in appreciable quantities [21]. Boiling time also caused significant increase in all the proximate composition of the leaf extracts except for moisture that decreased and crude fibre which was not detected. Results of vitamin analysis showed that while vitamin C content of the extracts decreased with boiling time, Pro-vitamin A content increased as boiling progressed.

The antioxidants properties determined using DPPH and FRAP at various concentrations; showed high antioxidant activities. The antioxidant activities increased with both the boiling time and concentration of the DPPH and FRAP reagents.

This research has shown that boiled moringa leaf extracts possessed higher antioxidant activity than the raw sample. This implies that boiled leaf extracts would possess higher health promoting potentials than the raw extracts. It is recommended therefore that for moringa leaf extracts should be boiled for at least 15mins for optimum antioxidant activity.

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