# TOTAL POLYPHENOLIC CONTENT AND ANTIOXIDANT PROPERTIES OF *MORINGA OLEIFERA* LEAF EXTRACTS

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**ABSTRACT**

***The study was carried out to evaluate the relative antioxidant properties and polyphenol contents of partially purified fractions of Moringa oleifera leaves extracts. The total phenolic, total flavonoid, anthocyanin, proanthocyanidine and tannin contents of the crude methanolic extract, aqueous fraction and ethyl acetate fraction were determined using established methods, while the antioxidant properties of the test fractions were evaluated using five in vitro radical scavenging assays: 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, nitric oxide inhibitory assay, lipid peroxidation assay, reductive potential assay, and the ferric reducing ability of plasma (FRAP) assay. The highest radical scavenging effect and polyphenol contents were observed in the ethyl acetate fractions than the other fractions: the order of activity for all the assays was ethyl acetate reaction > crude extract > aqueous extract. The results obtained in the present study indicates that M. oleifera could be a potential source of natural antioxidant and could be applied as a functional food as regard its relatively low tannin content.***

**Keywords**: 2, 2-Diphenyl-1-picryl hydrazyl, Antioxidant, Polyphenols, Moringa

## INTRODUCTION

*Moringa oleifera* is the most widely cultivated species of the family, Moringaceae that is native to the sub-Himalayan tracts of India, Pakistan and Bangladesh. This rapidly-growing tree (also known as the horseradish tree), was utilized by the ancient Romans, Greeks and Egyptians. It is now widely cultivated and has become naturalized in many locations in the tropics (Luqman *et al.,* 2012).

*M. oleifera* has in recent times been advocated as an outstanding indigenous source of highly digestible protein, calcium, iron, vitamin C and carotenoids suitable for utilization in many of the developing regions of the world where undernourishment is a major concern (Aktar and Ahmad, 1995). *Moringa* leaves contain more vitamin A than carrots, more calcium than milk, more iron than spinach, more vitamin C than oranges, and more potassium than bananas. The protein quality of *Moringa* leaves competes with that of milk and eggs (Ezeamuzie *et al*., 1996; Anwar and Bhanger, 2003). Moreover, this plant is rich in compounds containing the simple sugar and rhamnose, as well as glucosinolates and isothiocyanates. These constituents strengthen its hypotensive, anticancer, and antibacterial activity. Ethnomedically, *M. oleifera* is an antioxidant material used to combat different diseases associated with oxidative stress.

Oxidative stress is caused by an imbalance between the production of reactive oxygen and the biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. All forms of life maintain a reducing environment within their cells. This reducing environment is preserved by enzymes that maintain the reduced state through a constant input of metabolic energy. Disturbances in this normal redox state can cause toxic effects through the production of peroxides and free radicals that damage all components of the cell, as well as proteins, lipids and DNA (Aruoma, 1998). Moderate oxidative stress can trigger apoptosis, whereas severe stress can cause necrosis (Ames, 1983).

In humans, oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease, myocardial infarction, Alzheimer's disease and chronic fatigue syndrome, amongst others. However, it is beneficial, as regards the prevention of aging through a process named mitohormesis. A particularly destructive aspect of oxidative stress is the production of reactive oxygen species (ROS), which include free radicals and peroxides. Some of the less reactive of these species (such as superoxide) can be converted by oxidoreduction reactions into radical species that can cause extensive cellular damage (Duvivier *et al.,* 2010). In addition, ROS are involved in cell signaling and immune system by attacking and killing pathogens (Thannickal and Fanburg, 2000).

In order to combat oxidative stress, an antioxidant is required. Antioxidants delays or reduces oxidation of the substrate by reacting with free radicals and other reactive oxygen species. During this reaction the antioxidant sacrifices itself by becoming oxidized. However, antioxidant supply is not unlimited as one antioxidant molecule can only react with a single free radical. Therefore, there is a constant need to replenish antioxidant resources, whether endogenously or through supplementation (Sarr and Tsai, 2008).

Recently there has been upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical tissue injury. The purpose of the present study was to evaluate the *in vitro* antioxidant activities and polyphenol contents of leaves extracts of *M. oleifera.*

## MATERIALS AND METHODS

***Moringa oleifera:*** Fresh leaves of *M. oleifera* were collected from the lawn in front of the Department of Chemical Engineering/Food Science and Technology, Obafemi Awolowo University, Ile-Ife, Osun, State, Nigeria. The plant material was identified (TPL, 2010) and authenticated by taxonomist in the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria, where the voucher specimen (MO-123) was deposited.

Fresh leaves of *M. oleifera* were air dried under room temperature until a constant weight was obtained, powdered and extracted by soxhlet apparatus. 50 gram batches, of the powdered leaves were extracted with 10 volumes of 80 % (v/v) methanol to obtain the methanolic extract. This extract was concentrated to dryness in vacuum at 40° C using a rotary evaporator. The crude methanolic extract was then suspended in 100 ml of distilled water and subsequently fractionated with ethyl acetate (3 x 100 ml). The ethyl acetate fraction was concentrated to dryness in vacuum at 40° C using a rotary evaporator and freeze dried. The two fractions were then used for the study.

**Radical Scavenging Activity:** The hydrogen or radical scavenging properties of the extracts, was determined using the stable radical DPPH (2, 2-diphenyl-1-piccrlhydrazyl hydrate) according to the method of Blois (1958) as describe by Brace *et al.* (2005). When DPPH reacts with an antioxidant compound which can donate hydrogen, it is reduced (DPPH + RH → $DPPH_2$ + R). The change in color from deep violet to light yellow was measured at 517 nm.

To 1 ml of different concentrations (0.5, 0.25, 0.125, 0.0625, 0.003125 mg/ml) of the extract or standard was added 1 ml of 0.3 mM DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes after which the absorbance was read at 517 nm against a DPPH control containing only 1 ml of methanol in place of the extract. The antioxidant activity (AA) was then calculated using the formula: AA = [(Ao – Ac)/Ao] x 100, where: Ao = absorbance without extract and Ac = absorbance with extract.

**Lipid Peroxidation:** The anti-lipid peroxidative properties of the extract was determined using a modified thiobarbituric acid reactive species (TBARS) assay of Ohkowa *et al.* (1979) as described by Dasgupta and De (2002). In this assay, the end product of lipid peroxidation using liver homogenate as lipid-rich media was quantified by determining the formed malonyldialdehyde (MDA) which reacts with the thiobarbituric acid (TBA) under acidic condition

to form an MDA-TBA adduct. This pink colored product was read at 532 nm (Ruberto *et al.*, 2000).

0.5 ml of 0.1 mg/ml liver homogenate was added 0.1 ml of varying concentration of the extract (0.5, 0.25, 0.125, 0.0625, 0.003125 mg/ml) in a test tube followed by the addition of 1 ml distilled water. Then 50 µl of $FeSO_4$ (0.07 M) was added to the reaction mixture. The reaction mixture was vortexed and allowed to stand for 30 minutes at room temperature after which 1.5 ml of 20 % (v/v) acetic acid and 1.5 ml of 0.8 % (w/v) thiobarbituric acid in 1.1% (w/v) sodium dodecyl sulphate were added. The resulting mixture was then incubated in a water bath at $95^0$C for 1 hr. After cooling, 4.0 ml of butan-1-ol was added to each tube, shaken vigorously and centrifuged at 3000 rpm for 10 minutes. The absorbance of the organic upper layer was measured at 532 nm. The TBARS values were calculated using the extinction coefficient 1.56 x $10^{-5}$ M/cm. Inhibition of lipid peroxidation (%) by the extract was calculated using the formula: (1-E/C) x 100), where: C = absorbance value of the fully oxidized control

**Nitric Oxide:** Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitric ions which were measured by Griess reaction (Marcocci *et al.*, 1994).

The reaction mixture (3 ml) containing sodium nitroprusside (10 mM) in phosphate buffered saline (PBS) and the varying concentrations (0.5, 0.25, 0.125, 0.0625, 0.003125 mg/ml) of the extract were incubated in a water bath at room temperature for 150 minutes. After incubation, 1.5 ml of the reaction mixture was removed and 1.5 ml of Griess reagent was then added. The absorbance of the chromophore formed was read using spectrophotometer at 546 nm. Inhibition of nitric oxide radical (%) by the extract was calculated using the formula: NO = [(1-E/C)] x 100, where: C= absorbance value of the fully oxidized control and E = absorbance in the presence of extract.

**Phenolic Content:** The total phenolic content of the extract was determined using the folin ciocalten assay method of Singleton and Rossi (1965) as described by Gulcin *et al.* (2003).

To 0.1 ml of 1 mg/ml of extract / standard was added 0.9 ml of distilled water. 0.2 ml of folin reagent was then added. The resulting mixture was then vortexed. After 5 minutes, 1 ml of 7 % $Na_2CO_3$ solution was then added to the mixture. The solution was then diluted to 2.5 ml and then incubated for 90 minutes at room temperature. The absorbance at 750 nm was then read against the reagent blank. Standard preparation was done by preparing a stock solution of gallic acid 1 mg/ml, aliquots of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken and made up to a total volume of 2 ml. The total phenolic content of the plants was then calculated as shown below in equations and expressed as mg gallic acid equivalent (GAE)/g fresh weight. Analysis was conducted in duplicates and mean value considered. The phenolic content was calculated thus: C = c*v/m, where: C = total content of phenolic compound in gallic acid equivalent (GAE), c = concentration of gallic acid established from the calibration curve (mg/ml), V = volume of extract (ml) and m = weight of the crude methanolic plant obtained.

**Flavonoids:** The determination of the total flavonoidal content of the plant extract was based on the aluminum chloride colorimetric method of Zhilen *et al*. (1999) as described by Miliauskas *et al*. (2004).

To 0.1 ml of extract/standard was added 0.4 ml of distilled water. This was followed by 0.1 ml of 5 % sodium nitrite. After 5 minutes, 0.1 ml of 10 % aluminum chloride and 0.2 ml of sodium hydroxide were added and the volume was made up to 2.5 ml with distilled water. The absorbance at 510 nm was measured against the blank.

A stock solution of quercetin (1 mg/ml) was prepared. Aliquots of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken and the volume made up to 2 ml with distilled water. The total flavonoid content of the plant extract was then calculated as shown in the equation: $X = q \times V/w$, where X = total content of flavonoid compound in

quercetin equivalent, q = concentration of quercetin established from the standard curve, V = volume of extract (ml) and w = weight of the crude methanolic extract obtained. The total flavonoid content was expressed as mg quercetin equivalents per gram of the plant extract. The analysis was conducted duplicates and mean value considered.

**Proanthocyanidin:** The proanthocyanidin content of the extract was determined spectrophotometrically as described by Aksamit-Stachurska *et al*. (2008). Extracts were diluted to provide a spectrophometric reading between 0.1 and 0.8 absorbance units.

A 0.25 ml sample aliquot of adequately diluted extract was added to 2.25 ml of concentrated hydrochloric acid in n-butanol (10/90, v/v) in a screw top vial. The resulting solution was mixed for 10 to 15 seconds. Extracts were then heated for 90 minutes in an 85 $^{0}$ C water bath then cooled to 15 – 25$^{o}$ C in an ice bath. The absorbance at 550 nm was measured on a UV visible spectrophotometer. A control solution of each extract was prepared to account for background absorbance due to pigments in the extracts. The control solution consisted of the diluted extract prepared in the hydrochloric acid/n-butanol solvent without heating.

The proanthocyanidin content was expressed as mg cyaniding per Kg of sample using the formula: (ΔA ×MW) ×DF×1000 ÷ Є ×L, where: ΔA = $A_{550sample}$ – $A_{550control,}$ A550 sample = Sample absorbance at 550nm, A550control = control sample absorbance at 550nm, Є = Molar absorbance co efficient of cyanidin (17,360L-1M-1cm-1), L = pathlenght (1 cm), MW = molecular weight of cyaniding (287 g/mol) and DF= dilution factor to express as g/l.

**Anthocyanin:** Total anthocyanin content of the extract was determined by the pH differential method (Fuleki and Francis, 1968; Wrolstad, 1993). A pH 1.0 buffer solution was prepared by mixing 125 ml of 0.2 N KCl with 385 ml of 0.2 N HCl and 490 ml of distilled water. The pH of the buffer was adjusted to pH 1.0 with 0.2 N HCl. Another pH 4.5 buffer solution was prepared by mixing 440 ml of 1.0 M sodium acetate with 200 ml, 1.0 M HCl and 360 ml of distilled water. The pH of the solution was measured and adjusted to pH 4.5 with 1.0 MHCl. 0.5 ml of the extract was diluted to12.5 ml in the pH 1.0 and 4.5 buffers and allowed to equilibrate in the dark for 2 hours.

The absorbance of the samples at 512 nm (A512 nm) and 700 nm (A700 nm) was measured on a UV-visible spectrophotometer. The difference in absorbance (ΔA) between the anthocyanin extract diluted in pH 1.0 and pH 4.5 buffers was calculated using the equation: ΔA = (A512 pH 1.0 - A700 nm pH1.0) - (A512 nm pH 4.5 - A700 nm pH 4.5). The A700 nm was employed in the calculation of ΔA to correct for any background absorbance due to turbidity on the extracts. The anthocyanin content was expressed as mg cyaninidin 3-glucoside per 100 g berries using a molar absorbance coefficient (Є) of 26900 L-1M-1cm-1 (Guisti and Wrolstad, 2001).

TACY = (ΔA × MW) × DF × 1000 ÷ Є × 0.1 × 1, where: TACY = total anthocyanin expressed as mg cyaniding 3-glucoside/100g of plant material, MW = molecular weight of cyaniding 3-glucoside (449.2 g/l), DF = dilution factor to expressed the extracts on per gram of plant basis and Є = molar absorption co efficient of cyaniding 3-glucoside (26900 M-1cm-1).

**Statistical Analysis:** The results are expressed as mean ± SEM using Graph Pad Prism Graphical-Statistical Package version 5. The difference between groups was analyzed by one-way analysis of variance (ANOVA) followed by Dennett's test with 5 % level of significance ($p<0.05$).

## RESULTS

**Extracts Yield:** The percentage yield of the crude methanolic extract, aqueous and ethylacetate fraction obtained from the fresh plant material is given as 9.85 ± 0.33,7.42 ± 0.15 and 4.82 ± 0.09 %, respectively.

**Antioxidants:** The different fractions were assayed for total content of four major type of constiuents with antioxidant properties. The

antioxidant constituents were: total phenol, total flavonoid, proanthocyanidins and anthocyanins. The results showed that the total phenolic content in crude extract, aqueous fraction and ethylacetate fraction of *M. oleifera* were 1.622 ± 0.068, 1.058 ± 0.091 and 2.099 ± 0.087 mgGAE/g dry weight respectively with the ethylacetate fraction having the highest phenolics content.

The total flavonoids content of the different fractions expressed as quercetin equivalent per gram of the plant extract showed that the test materials had 0.921 ± 0.05, 0.66 ± 0.13, 1.311 ± 0.152 mg QUE/g dry weight for the crude extract, aqueous and ethylacetate fraction, respectively. As was observed in the total phenolic content, the ethylacetate fraction also had highest concentration of flavonoid contents (Table 1).

The concentration of anthocyanin in the test fractions were 0.126 ± 0.040, 0.06 ± 0.016 and 0.348 ± 0.006 cyanidin 3-glucoside/100 g for the crude extract, aqueous fraction and ethylacetate fraction, respectively, while the concentrations of proanthocyanin were 0.026 ± 0.012, 0.06 ± 0.011 and 0.291 ± 0.017 cyanidin 3-glucoside/100 g for the crude extract, aqueous fraction and ethylacetate fraction, respectively (Table 1).

**Antiradicals:** The results of the antiradical assays carried out on the different test fractions are shown in Table 2. In the DPPH assay the activity was concentration dependent i.e. activity increases with increase in concentration. Ethylacetate fraction had the highest activity with the least $IC_{50}$ value. The plant extract gave $IC_{50}$ values of 2.32 ± 18.30, 3.50 ± 28.13 and 1.78 ± 31.13 mg/ml for the crude extract, aqueous fraction and ethylacetate fraction, respectively (Table 2).

In the lipid peroxidation assay, the ethylacetate fraction had the highest inhibition as evidenced by the $IC_{50}$ values which were 4.33 ± 0.05, 3.76 ± 0.04 and 2.45 ± 0.07 mg/ml for the crude extract, aqueous fraction and ethylacetate fraction, respectively (Table 3).

The nitric oxide inhibition assay showed the ethylacetate fraction to be the most potent scavenger of nitric oxide. The $IC_{50}$ values were 3.59 ± 2.099, 4.43 ± 1.56 and 2.53 ± 5.65 mg/ml for the crude extract, aqueous fraction and ethylacetate fraction, respectively (Table 4).

The reductive potential of the test fractions at 0.5 mg/ml gave 0.818 ± 0.003, 0.688 ± 0.05 and 1.133 ± 0.03 for the crude extract, aqueous fraction and ethylacetate fraction, respectively (Table 5), with the ethylacetate fraction having the highest reducing capacity.

The FRAP assay result, showed a concentration dependent change when the FRAP values of the test fractions were determined. Results was expressed in mmol$Fe^{2+}$/L. The concentration of $Fe^{2+}$ in the reaction mixture at 0.5 mg/ml, was given as 1.088 ± 0.06, 0.888 ± 0.08 and 1.608 ± 0.09 mmol$Fe^{2+}$/l for the crude extract, aqueous fraction and ethylacetate fraction, respectively (Table 6).

## DISCUSSION

In recent times, many epidemiological studies have confirmed that intake of exogenous antioxidants is effective in the prevention of a number of human diseases which have been implicated to be due to oxidative stress. Synthetic antioxidants such as 2-3-ter-butyl-4-methoxyphenol (BHA), 2,6-di-ter-butyl-4 metylphenol (BHT) and tert-butylhydroquinone (TBHQ) are known to prevent oxidative stress related diseases but unfortunately, they have been proven to be toxic in chronic administration as evidenced by severe hemorrhage in rabbit after chronic administration of BHT (Valentao *et al*., 2002).

Consequently, the necessity for investigation and evaluation of safer antioxidants from natural sources becomes imperative. Antioxidants isolated from the plant source have proved to be safer even in chronic administration (Oyaizu, 1986; Adaramoye *et al*., 2008; Luqman *et al.,* 2012). Fruits, vegetables and oil seeds have increasingly been recognized as sources for antioxidant phenolics (Shahidi, 1997; Nack and Shahidi, 1989). Anedoctal evidence suggests that *M. oleifera* is useful in this regard, and could be boiled for its different health beneficts.

**Table 1: Concentration of some antioxidant constituents contained in *Moringa oleifera* leaves**

| Constituents | Crude extract | Aqueous fraction | Ethyl acetate fraction |
|---|---|---|---|
| **Total phenol** | 1.622 ± 0.068 | 1.058 ± 0.091 | 2.099 ± 0.087 |
| **Total flavonoids** | 0.921 ± 0.050 | 0.66 ± 0.130 | 1.311 ± 0.152 |
| **Anthocyanin** | 0.126 ± 0.040 | 0.060 ± 0.016 | 0.348 ± 0.006 |
| **Proanthocyanidine** | 0.026 ± 0.012 | 0.06 ± 0.011 | 0.291 ± 0.017 |
| **Tannins** | 1.332 ± 0.311 | 1.246 ±0.860 | 1.678 ± 0.987 |

*All values are expressed as mean ± SEM (n=3). The level of activity between the crude extract and the fractions are not significantly different (p>0.05)*

**Table 2: DPPH radical scavenging activity *of Moringa oleifera* leaf extracts**

| Concentration (mg/ml) | Percentage inhibition | | |
|---|---|---|---|
| | Crude extract | Aqueous fraction | Ethyl acetate fraction |
| **0.5** | 85.92 ± 2.22 | 60.72 ± 6.80 | 92.04± 10.01 |
| **0.25** | 54.43 ± 3.99 | 48.38 ± 5.22 | 84.59 ± 8.11 |
| **0.125** | 47.01 ± 2.00 | 28.55 ± 3.19 | 47.38 ± 6.24 |
| **0.0625** | 22.11 ± 2.61 | 20.02 ± 3.44 | 20.10 ± 3.88 |
| **0.003125** | 7.74 ± 2.12 | 6.81 ± 3.14 | 13.69 ± 3.86 |
| **$IC_{50}$ (mg/ml)** | 2.32 ± 18.30 | 3.50± 28.14 | 1.78± 31.13 |

*All values are expressed as mean ± SEM (n=3). The level of activity between the crude extract and the fractions are not significantly different (p>0.05)*

**Table 3: Antioxidant activity of *Moringa oleifera* leaf extracts using the lipid peroxidation**

| Concentration (mg/ml) | Crude extract (% inhibition) | Aqueous fraction (% inhibition) | Ethyl acetate fraction (% inhibition) |
|---|---|---|---|
| **5.0** | 52.01 ± 4.01 | 54.88 ± 2.01 | 64.61 ± 0.18 |
| **2.5** | 40.17 ± 1.11 | 46.05 ± 1.04 | 59.42 ± 0.80 |
| **1.25** | 34.61 ± 1.18 | 39.61 ± 1.29 | 44.64 ± 1.88 |
| **0.625** | 24.55 ± 1.09 | 30.81 ± 5.01 | 38.04 ± 0.25 |
| **0.3125** | 14.38 ± 0.004 | 20.99 ± 0.016 | 23.18 ± 0.90 |
| **$IC_{50}$(mg/ml)** | 4.33 ± 0.05 | 3.75 ± 0.04 | 2.45 ± 0.07 |

*All values are expressed as mean ± SEM. The level of activity between the crude extract and the fractions are not significantly different (p>0.05)*

**Table 4: Nitric oxide radical inhibition potentials of *Moringa oleifera* leaf extracts**

| Concentration (mg/ml) | Crude extract (% inhibition) | Aqueous fraction (% inhibition) | Ethyl acetate fraction (% inhibition) |
|---|---|---|---|
| **0.5** | 56.58 ± 0.04 | 50.24 ± 0.14 | 69.12±0.9 |
| **0.25** | 49.28 ± 0.09 | 44.01 ± 0.19 | 52.72±4.00 |
| **0.125** | 34.11 ± 0.07 | 30.28 ± 1.44 | 40.49±0.77 |
| **0.0625** | 28.84 ± 1.01 | 26.14 ± 0.62 | 32.71±1.82 |
| **0.03125** | 19.99 ± 0.61 | 17.14 ± 2.11 | 30.11±1.04 |
| **$IC_{50}$(mg/ml)** | 3.59 ± 0.98 | 4.43 ± 1.56 | 2.53±5.65 |

*All values are expressed as mean ± SEM. The level of activity between the crude extract and the fractions are not significantly different (p>0.05)*

**Table 5: Reducing power of *Moringa oleifera* leaf extracts**

| Concentration (mg/ml) | Crude extract | Aqueous fraction | Ethyl acetate fraction |
|---|---|---|---|
| **0.5** | 0.818 ± 0.008 | 0.688 ± 0.049 | 1.133 ± 0.0002 |
| **0.25** | 0.755 ± 0.134 | 0.670 ± 0.004 | 1.019 ± 0.004 |
| **0.125** | 0.704 ± 0.08 | 0.574 ± 0.008 | 0.791 ± 0.0041 |
| **0.0625** | 0.666 ± 0.04 | 0.541 ± 0.018 | 0.692 ± 0.008 |
| **0.03125** | 0.544 ± 0.03 | 0.508 ± 0.126 | O.680 ± 0.009 |

*All values are expressed as mean ± SEM. The level of activity between the crude extract and the fractions are not significantly different (p>0.05)*

**Table 6: Antioxidant activity of *Moringa oleifera* leaf extracts using the Frap assay**

| Concentration (mg/ml) | Crude extract | Aqueous fraction | Ethyl acetate fraction |
|---|---|---|---|
| **0.5** | 1.088±0.06 | 0.888±0.08 | 1.608±0.09 |
| **0.25** | 0.921±0.08 | 0.772±0.16 | 0.899±0.08 |
| **0.125** | 0.719±0.16 | 0.590±0.13 | 0.710±0.18 |
| **0.0625** | 0.692±0.04 | 0.512±0.09 | 0.521±0.28 |
| **0.03125** | 0.491±0.16 | 0.418±0.33 | 0.322±0.12 |

*All values are expressed as mean ± SEM. The level of activity between the crude extract and the fractions are not significantly different (p>0.05)*

That informed the use of polar-organic solvents for extraction; the solvents used, are close to water in polarity. The order of antioxidant activity based on values, was ethylacetate fraction > crude extract > aqeouse extract fraction. These results were similar to those of methanol extracts of some medicinal plants (Miliauskas *et al*., 2004), as well as ethanolic leaves and fruits extracts of Moringa (Luqman *et al.,* 2012).

Anthocyanin, proanthocyanidine, phenol and flavonoids possess hydroxyl groups which are resposible for free radical scavenging effect. It has been known that flavonoids posses antioxidant activity, and thus considered beneficial to human health and nutrition (Shahidi and Wanasundara, 1992). The relatively low tannnin content in the fractions qualifies *M. oleifera* as a functional food (Pari *et al*., 2007).

Phenolic compounds acts as free radical chain reaction terminator; thereby acting as antioxidant. Phenol also have a potential of combating oxidative stress, a syndrome causative of some neurodegenerative diseases and cardiovascular diseases. Phenolic compounds comprise a large group of biologically active substances.

Quercetin, catechin, ferrulic acid, caffeic acid, gallic acid, coumaric acid, and rutin are among the most common naturally occuring antioxidant phenolic compounds in foods. Other natural antioxidants include glutathione peroxidase, superoxide dismutase, tocopherols, and vitamin C and carotenoids (Duvivier *et al*., 2010). The level of antioxidant as evidenced in this study could speculate the presence of phenolics. In fact, the scavenging activity of aqueous extract by DPPH is lower than the crude extract and ethyl acetate fractions. This could mean polyphenolic compounds provided more potent DPPH scavenging activity; therefore, it is a useful guideline to design and develop natural antioxidants.

The mechanism of action of flavonoids are through scavenging or chelating process (Pourmorad *et al*., 2006). This could attest to the high level of scavenging activity expressed by the crude extract and the two other fractions (p>0.05). That, there was no significant change in the level of percentage inhibiton in the antiradical assay, could be as a result of the polarity in the solvent used in extraction and the partitioning with water.

**Conclusion:** On the basis of the results obtained in this present study, It may be concluded that *M.oleifera* leaf extracts have significant amount of

antioxidant activity and this is attributable to the polyphenol contents.

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