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Comparative *in vitro* Assessment of Drumstick (*Moringa oleifera*) and Neem (*Azadiracta indica*) Leaf Extracts for Antioxidant and Free Radical Scavenging Activities

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ABSTRACT

The current research was aimed at comparing the leaf extracts of two medicinal plants (*Moringa oleifera* and *Azadiracta indica*) for antioxidant and free radical scavenging potentials in different extracting solvents (absolute ethanol, 70 and 50% ethanol). Different *in vitro* assays such as total phenolic and flavonoid content, 2-2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, metal chelating activity, reducing power and total antioxidant capacity were employed in the study. The results revealed that *A. indica* contained more phenols and flavonoids than *M. oleifera* with the different extracting solvents. The amount of phenols and flavonoids in *A. indica* played a pivotal role in scavenging more of the DPPH radical at a lower inhibitory concentration, IC_{50} of 77.94 μg mLG^{1} than in *M. oleifera* at 118.96 μg mLG^{1} in absolute ethanol. *Moringa oleifera* was a better scavenger of the DPPH radical in 70 and 50% ethanol. In absolute ethanol, *A. indica* also chelated 50% of the metal ion at IC_{50} of 0.22 μg mLG^{1} which was even better than ascorbic acid (5.95 μg mLG^{1}) and gallic acid (0.503 μg mLG^{1}) standards. The values for *A. indica* were also comparably better than those of *M. oleifera* for reducing power and total antioxidant capacity at the respective concentrations. The results are indicative of the antioxidant and free radical scavenging potentials of *M. oleifera* and *A. indica*. Comparatively, *A. indica* was better than *M. oleifera* in doing the job and absolute ethanol extracts were better than 70 and 50% ethanol extracts in the scavenging potential.

Key words: Antioxidants, scavenging, free radical, *M. oleifera*, *A. indica*, phenols, flavonoids

INTRODUCTION

Over the years, plants have been the major source of products for prevention and treatment of ailments in traditional medicine. This is because plants are naturally endowed with inherent bioactive compounds with medicinal properties capable of preventing or mitigating disease conditions. In recent time, with the advancement in molecular biology which has provided an indebt understanding of the molecular structures and actions of these bioactive compounds, much interest has been paved to medicinal plants occasioned by their invaluable medicinal properties. Worthy of note is the fact that some of these medicinal plants have been reported to exhibit oxidative stress mitigating properties on oxidative stress related diseases such as cancer, asthma, cardiovascular diseases, diabetes, arthritis, inflammation etc. (Kottaimuthu, 2008; Gomez-Flores *et al*., 2008; Koffi *et al*., 2009; Tripathy *et al*., 2010; Oluwole *et al*., 2011). This

oxidative stress occur when there is an imbalance between the production of reactive oxygen species (free radicals) and the mechanism of detoxifying them (antioxidant production) in the body. When this occurs, the generated free radicals which are unstable atoms with unpaired valence electrons (Kadam *et al*., 2010; Aluko *et al*., 2013) will attack bio-molecules in the body transforming them into free radicals such as hydrogen peroxides (H_2O_2). This subsequently proliferates into the aforementioned disease conditions. Infertility has also been highly linked to the production of free radicals (Agarwal *et al*., 2008).

Generally, synthetic antioxidants are the commonest ways used in mopping the deteriorating effects of these free radicals in the body. The growing panics on the use of these synthetic antioxidants are the reported side effects orchestrated by their consumption (Kukic *et al*., 2006; Vijayakumar *et al*., 2012; Ikpeme *et al*., 2013). Due to this, there have been shifts from the use of synthetic antioxidants to natural antioxidants sourced from medicinal plants following reports on their safety, accessibility and affordability (Calixto, 2000; Ikpeme *et al*., 2011, 2012). Undoubtedly, the reported safety, cost effectiveness and accessibility of these medicinal plants on health has opened up a new field of research allowing research scholars to study different plants for their antioxidant potency as alternative measure to the synthetic antioxidants.

Moringa oleifera is one of such medicinal plant reported with antioxidant properties (Siddhuraju and Becker, 2003; Iqbal and Bhanger, 2006). *Moringa oleifera* is reported to be effective in the treatment of rheumatism, infections, hiccough, influenza and internal abscess (Anwar *et al*., 2007; Mishra *et al*., 2011). The leaf extract is capable of reducing hyperglycemia (Mbikay, 2012). Nutritionally, the leaves contain essential amino acids, vitamins, minerals and $-carotene which have rendered it an invaluable commodity in the food industries (Sabale *et al*., 2008; Sharma *et al*., 2012). *Azadirachta indica* commonly known as neem is another medicinal plant of great importance in traditional medicine and fertility studies (Ekaluo *et al*., 2010). Neem oil, bark and leaf extracts are therapeutic in folk medicine for the control of leprosy, intestinal helminthiasis, respiratory disorders, constipation and skin infections (Biswas *et al*., 2002). Although antioxidant properties of *M. oleifera* and *A. indica* have been reported in recent researches, however, comparative reports of the antioxidant properties of these two commonly used medicinal plants are rare. Thus, the aim of the present research was to assess and compare the *in vitro* antioxidant activities and free radical scavenging potentials of *M. oleifera* and *A. indica* in different extracting solvents (absolute ethanol, 70 and 50% ethanol) to ascertain a more reliable antioxidant source between the two plants.

MATERIALS AND METHODS

Collection of plant materials and extraction: Fresh leaves of *M. oleifera* and *A. indica* were obtained from Staff Quarters, University of Calabar, Calabar and authenticated in the Herbarium Unit of the Department of Botany, University of Calabar. The fresh leaves were freed from dirts, air dried at room temperature for one week and then finely milled separately using a blender (Model: 5KSB655CCSO). Ten grams of the milled sample was soaked in 100 mL of the three different solvents (absolute ethanol, 70 and 50% ethanol) for 72 h at room temperature. The soaked samples were shaken intermittently during the extraction period and subsequently filtered using Whatman No. 1 filter paper. The resulting extracts were concentrated under vacuum in a rotary evaporator at 45°C for complete solvent removal. A stock solution of each crude extract was prepared and desired working concentrations were made by appropriate dilutions.

Determination of extract yield (%): The percentage yield of each extract was obtained by dividing the weight of the concentrated crude extract by the initial weight (10 g) of dry milled starting material and multiplying the ratio by 100.

Determination of Total Phenolic Content (TPC): The total phenolic contents of the extracts were determined by the Folin-Ciocalteau method according to Duarte-Almeida *et al*. (2006). One hundred microliter of Folin-Ciocalteau reagent was added to 500 µL of the different extract solutions containing 1000 µg mL^{-1} +6 mL of distilled water and shaken for one minute. Thereafter, 2 mL of 15% sodium carbonate was added to the mixture and shaken again for 30 sec. Finally, distilled water was added to the solution to make it up to 10 mL, then left to incubate for 1.5 h at room temperature. Thereafter, the absorbance at 750 nm was evaluated using a spectrophotometer (LABTECH UV/VIS Spectrophotometer, India-Single beam 295). Gallic acid monohydrate, a standard phenol, in the range of 5-150 µg mL^{-1} was used to prepare standard reference curve. The Total Phenol Contents (TPC) of the extracts were expressed as Gallic Acid Equivalents (GAE) from the linear regression curve of gallic acid.

Determination of Total Flavonoid Content (TFC): The total flavonoid contents of each extract concentration were determined using the aluminum chloride colorimetric method, according to Dewanto *et al*. (2002). The different extract solutions (1 mL containing 1000 µg mL^{-1}) were diluted with 4 mL of distilled water in a 10 mL volumetric flask. Thereafter, 0.3 mL of 5% sodium nitrite ($NaNO_2$) solution was added to each extract solution. Five minutes later, 0.3 mL of 10% aluminium chloride ($AlCl_3$) was added; 1 min later, 2 mL of 1.0 M sodium hydroxide (NaOH) was added and finally, 2.4 mL of distilled water was added and mixed properly. Absorbance of the reaction mixture was read at 510 nm. Rutin, a standard flavonoid in the range of 10-150 µg mL^{-1} was used to prepare the standard reference curve. Total Flavonoid Content (TFC) of the extracts were expressed as Rutin Equivalents (RE) from the linear regression curve of Rutin.

DPPH radical scavenging activity: The ability of *M. oleiferia* and *A. indica* leaf extracts to scavenge stable DPPH radical was measured using the method of Mensor *et al*. (2001). Five different concentrations of each test extracts were prepared in methanol (20, 40, 60, 80, 100 µg mL^{-1}). One milliliter of 0.3 mM of freshly prepared DPPH solution in methanol was added to 2.5 mL solution of each extract concentration and allowed to react in the dark at room temperature for 30 min. Absorbance of the resulting solution was measured at 518 nm. Methanol (1 mL) was added to 2.5 mL of each extract concentration was used as blank, while 1 mL of 0.3 mM DPPH solution added to 2.5 mL of methanol served as a negative control. Ascorbic acid and gallic acid were used as standard reference compounds (positive controls) for comparison. Percentage DPPH scavenging activities of the extracts and standards were determined using the following equation:

$$\text{Scavenging activity (\%)} = 100 - \left| \frac{A_s - A_b}{A_c} \times 100 \right|$$

Where:

A_s = Absorbance of sample (extracts or reference standard)
A_b = Absorbance of blank
A_c = Absorbance of negative control

Results were expressed as inhibitory concentration, IC_{50} (concentration of extract or standard required to scavenge 50% of DPPH radicals) which were determined from a linear regression curve of concentration versus scavenging activity (%).

Metal (ferrous ion) chelating activity: The ferrous ion chelating activity of *Moringa oleifera* and *A. indica* leaf in different extracting solvents (Absolute ethanol, 70 and 50% ethanol) concentrations (Absolute, 70 and 50% ethanol) were determined by the method of Ebrahimzadeh *et al*. (2009). Here, the ability of the extracts to chelate ferrous ion (Fe^{2+}) was estimated. Different concentrations (20-100 μg mL^{-1}) of each extract were prepared and 1 mL of each concentration were mixed with 1 mL of $FeSO_4$ (0.125 M) and 1 mL of ferrozine (0.3125 mM) and shaken vigorously. After incubating for 10 min at room temperature, the mixture solution was measured using a spectrophotometer at 562 nm against a blank containing the same components as stated above but the extracts were replaced with distilled water (1 mL of distilled water). The blank was incubated under the same conditions as the test samples. Sodium EDTA (Na_2EDTA) was used as control. The percentage inhibitions of ferrozine (Fe^{2+}) by the extracts were determined using the following equation:

$$\text{Inhibition of ferrozine (\%)} = \frac{A_c - A_s}{A_c} \times 100$$

Where:
A_c = Absorbance of control
A_s = Absorbance of sample

Results were expressed as IC_{50} (concentration of extract or standard required to chelate 50% of ferrous ions) which were determined from a linear regression curve of concentration versus chelating activity (%).

Total Antioxidant Capacity (TAC) assay: The Total Antioxidant Capacity (TAC) of *M. oleifera* and *A. indica* leaf extract in different extracting solvents (absolute ethanol, 70 and 50% ethanol) were determined by the phosphomolybdate method according to Jayaprakasha *et al*. (2002). An aliquot (30 μL) of different concentrations (20, 40, 60, 80 and 100 μg mL^{-1}) of the test extracts were mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) taken in test tubes. The tubes were capped with aluminium foil and incubated in a boiling water bath at 95°C for 90 min. The reaction mixture was allowed to cool to room temperature and the absorbance of the solution was measured at 695 nm against a blank containing 3 mL of reagent solution and the appropriate volume of the dissolving solvents. The blank was incubated under the same conditions as the test samples. Ascorbic acid and gallic acid were used as standard reference compounds to compare the activities of the extracts.

Reducing power assay: Antioxidant activity of the leaf extract of *Moringa oleifera* and *A. indica* in different extracting solvents (absolute ethanol, 70 and 50% ethanol) were determined to assess their ferric ion (Fe^{3+}) reducing ability according to the method of Anandjiwala *et al*. (2007). Different concentrations (20, 40, 60, 800, 100 μg mL^{-1}) of each extract were prepared and 1 mL of each concentration was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.8) and 2.5 mL of

potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 20 min. To this mixture, 2.5 mL of 10% trichloroacetic acid was added and then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride was added. Absorbance of the Pert Prussian blue solution formed was measured at 700 nm. Ascorbic acid and gallic acid were used as standard reference compounds for comparison and prepared in same concentrations as the extracts.

Statistical analysis: Analysis of variance (ANOVA) was used to analyze absorbance values for total phenolic content, total flavonoid content, reducing power and total antioxidant capacity of the two medicinal plants against the reference standards. Mean separation was done using the Least Significant Difference (LSD) test.

RESULTS

Extract yield, total phenolic content, total flavonoid content and inhibitory concentration (IC_{50}) of *M. oleifera* and *A. indica*: Following the differences in concentration of the extracting solvents (absolute ethanol, 70 and 50% ethanol), there were concomitant differences in the percentage yield of the extracts. *Moringa oleifera* had the highest yield in 50% ethanol (23.12%) followed by 70% ethanol (21.89%) and absolute ethanol (11.79%). *Azadiracta indica* yield also increased as the concentration of the extracting solvent reduced; absolute ethanol (11.34%), 70% ethanol (17.84%), 50% ethanol (20.14%) as shown in Table 1. Results for total phenolic and flavonoid content revealed a concentration dependent relationship. *Azadiracta indica* had significant amount ($p<0.05$) of phenols than *M. oleifera* in the different extracting solvents. Flavonoid content of *A. indica* was significantly higher ($p<0.05$) in absolute ethanol (128.39 μg RE mgG^{1}), 70% ethanol (42.83 μg RE mgG^{1}), 50% ethanol (30.89 μg RE mgG^{1}) than absolute ethanol (85.06 μg RE mgG^{1}), 70% ethanol (25.05 μg RE mgG^{1}) and 50% ethanol (8.95 μg RE mgG^{1}) of *M. oleifera*. In DPPH radical scavenging, it required 77.94 μg mgG^{1} absolute concentration of *A. indica* to scavenge 50% of the radical compared to 118.96 μg mgG^{1} of *M. oleifera* at the same concentration. *Azadiracta indica* was also a better metal chelator ($p<0.05$) than *M. oleifera* since it required a lesser amount of its extract to chelate 50% of ferrous ion (Fe^{2+}) in the respective extraction solvent concentration compare to *M. oleifera* (Table 1).

Concentration effect on free radical scavenging properties of *M. oleifera*, *A. indica* and standards: The result revealed significant differences in the free radical scavenging potential of *M. oleifera* and *A. indica* at the different concentration as shown in Table 2. Although

Table 1: Extract yield, phenolic content, flavonoid content, DPPH radical scavenging and metal chelating activities of *M. oleifera* and *A. indica*

	M. oleifera			*A. indica*		
Parameters	Absolute ethanol	Ethanol (70%)	Ethanol (50%)	Absolute ethanol	Ethanol (70%)	Ethanol (50%)
Extract yield (%)	11.97	21.89	23.12	11.34	17.84	20.14
Phenol content (μg GAE mLG^{1})	30.94 ± 0.64^{d}	29.90 ± 0.51^{e}	29.09 ± 0.05^{e}	58.31 ± 0.31^{a}	51.40 ± 1.32^{b}	43.24 ± 1.71^{c}
Flavonoid content (μg RE mLG^{1})	85.06 ± 0.76^{b}	25.05 ± 0.29^{e}	8.95 ± 1.25^{f}	128.39 ± 0.73^{a}	42.83 ± 0.48^{c}	30.89 ± 2.04^{d}
DPPH radical scavenging (μg mLG^{1})*	118.96 ± 2.57^{e}	120.77 ± 1.03^{d}	152.79 ± 1.76^{b}	77.94 ± 1.94^{f}	139.51 ± 1.88^{c}	193.89 ± 4.82^{a}
Metal chelating activity (μg mLG^{1})**	568.20 ± 0.01^{c}	3369.00 ± 0.07^{b}	3812.50 ± 0.09^{a}	0.22 ± 0.01^{f}	9.07 ± 0.06^{e}	9.97 ± 0.041^{d}

Means with different superscript along the same horizontal array differ significantly ($p<0.05$) from each other. *IC_{50} values for ascorbic acid and gallic acids are 5.95±0.13 and 0.50±0.02 μg mLG^{1}, respectively. **IC_{50} value for Na_2 EDTA is 0.02±0.001 mg mLG^{1}

Table 2: Concentration effect on reducing power and antioxidant capacity of *M. oleifera*, *A. indica* and standards

Conc. of extracts (µg mLG^{1})	*M. oleifera*			*A. indica*			Standards	
	Absolute ethanol	Ethanol (70%)	Ethanol (50%)	Absolute ethanol	Ethanol (70%)	Ethanol (50%)	Ascorbic acid	Gallic acid
Reducing power								
20	0.685±0.002^{e}	0.726±0.006^{b}	0.706±0.005^{c}	0.696±0.006^{d}	0.635±0.015^{f}	0.702±0.005^{c}	1.909±0.074^{a}	-
40	0.711±0.003^{d}	0.728±0.007^{c}	0.717±0.003^{d}	0.729±0.007^{c}	0.748±0.018^{b}	0.728±0.007^{c}	2.149±0.078^{a}	-
60	0.727±0.007^{d}	0.734±0.007^{d}	0.758±0.001^{b}	0.733±0.007^{d}	0.757±0.001^{b}	0.747±0.021^{c}	2.239±0.098^{a}	-
80	0.735±0.007^{e}	0.762±0.160^{c}	0.773±0.172^{b}	0.744±0.151^{d}	0.761±0.160^{c}	0.750±0.151^{d}	2.459±1.020^{a}	-
100	0.757±0.080^{e}	0.765±0.140^{de}	0.807±1.004^{b}	0.767±0.140^{cd}	0.775±0.170^{c}	0.758±0.060^{e}	2.464±1.004^{a}	-
Antioxidant capacity								
20	0.027±0.011^{d}	0.032±0.011^{d}	0.026±0.011^{d}	0.054±0.004^{c}	0.056±0.003^{c}	0.039±0.011^{d}	0.651±0.898^{a}	0.400±0.040^{b}
40	0.032±0.001^{d}	0.032±0.001^{d}	0.028±0.001^{d}	0.059±0.005^{c}	0.060±0.005^{c}	0.050±0.005^{c}	0.778±1.024^{a}	0.431±0.051^{b}
60	0.041±0.002^{e}	0.041±0.002^{e}	0.030±0.002^{e}	0.071±0.006^{cd}	0.077±0.008^{d}	0.057±0.004^{c}	0.849±1.004^{a}	0.434±0.051^{b}
80	0.053±0.003^{e}	0.042±0.003^{ef}	0.031±0.002^{f}	0.085±0.030^{c}	0.085±0.030^{c}	0.065±0.009^{d}	1.011±1.051^{a}	0.459±0.058^{b}
100	0.053±0.003^{e}	0.048±0.004^{ef}	0.037±0.004^{f}	0.088±0.060^{c}	0.089±0.060^{c}	0.071±0.031^{d}	1.050±1.310^{a}	0.460±0.061^{b}

Means with different superscript along the same horizontal array differ significantly ($p<0.05$) from each other. -: Particular standard was not used

ascorbic acid, a standard reference was a better reducing agent than the test medicinal plants, however, the absorbance values of the medicinal plants indicated their scavenging potentials. At 20 µg mLG^{1}, 70% ethanol extract of *M. oleifera* reduced significant amount (0.726) of the ferric ion ($p<0.05$) than *A. indica* but at 40 µg mLG^{1}, 70% ethanol extract of *A. indica* was better than *M. oleifera*. There was no significant difference ($p>0.05$) in the reducing power of *M. oleifera* at 60 µg mLG^{1}, absolute ethanol and 70% ethanol extracts (0.727 and 0.734) and 60 µg mLG^{1}, absolute ethanol extract (0.733) of *A. indica*. At 100 µg mLG^{1}, 50% ethanol extract of *M. oleifera* was better in reducing free radical while *A. indica* was better at 70% ethanol extract of the same concentration. Ascorbic and gallic acid standards showed significant antioxidant capacity than the test medicinal plant extracts. At 20 µg mLG^{1}, 70 and 50% ethanol extracts of *A. indica* were the same ($p>0.05$). Absolute ethanol, 70 and 50% ethanol extracts of *M. oleifera* (0.027, 0.032 and 0.026) and 50% ethanol extract of *A. indica* (0.039) showed no significant difference at 20 µg mLG^{1}. At 40, 60, 80 and 100 µg mLG^{1}, *A. indica* showed high total antioxidant capacity than *M. oleifera* in the different extracting solvents.

DISCUSSION

Medicinal plants are regularly screened for free radical scavenging properties basically from the reports on their safety, efficacy and cost effectiveness (Calixto, 2000; Ikpeme *et al*., 2011, 2012) over synthetic antioxidants reported with side effects upon their consumption (Vijayakumar *et al*., 2012). As a result of this, various medicinal plants reported with antioxidant properties have been recommended for pharmaceutical industries and traditional medicine for the control and treatment of different kinds of ailments.

The results of the current research revealed the presence of reasonable amount of phenols and flavonoids in both medicinal plant extracts. Polyphenolic and flavonoid compounds are very important secondary metabolites in plants and are reported to be responsible for the variation in antioxidant activities in plants (Demiray *et al*., 2009; Basma *et al*., 2011; Uyoh *et al*., 2013) and are capable of fighting against free radicals by inactivating lipid free radicals or preventing decomposition of hydrogen peroxide into free radicals due to their redox properties, chelate metal

ions, quenching singlet and triplet oxygen (Pokorny *et al*., 2001; Maisuthisakul *et al*., 2007; Balasundram *et al*., 2006; Javanmardi *et al*., 2003). This may undoubtedly suggest that plants with high quantity of polyphenols and flavonoids are good antioxidant sources although quantifying phenols and flavonoids are not the only yardstick for measuring antioxidant capacity of a substrate as many *in vitro* antioxidant assays are always required for a more categorical conclusion. Approximately, *Azadiracta indica* contained more phenols and flavonoids in absolute ethanol extract than *Moringa oleifera* which suggest why it required lesser amount of the extract (97.94 μg mL^{-1}) to scavenge 50% of DPPH radical compare to *M. oleifera* at the same concentration (Table 1). The DPPH, a synthetic free radical have been used to measure *in vitro* ability of a test substance to scavenge free radicals. The effects of phenolic compounds on DPPH radical scavenging are thought to be due to their hydrogen donating ability (Ghimeray *et al*., 2009) to the unstable DPPH free radical that accepts an electron or hydrogen to become a stable diamagnetic molecule (Siddaraju and Dharmesh, 2007). It is most likely that the decrease in absorbance of DPPH radical caused by phenolic compound in our result is due to reaction between antioxidant molecules in the extracts and the radicals. Although the amount of DPPH scavenged by the two plants in the respective extracting solvents are not equivalent to ascorbic and gallic acid standards, however, this amount is adequate to suggest the extract potential in scavenging free radicals.

According to Ghimeray *et al.* (2009) transition metals have played a pivotal role in the generation of oxygen free radicals in living organisms. These transition elements such as iron and copper are able to kick start free radical generation because they are oxidation reaction catalyst due to unpaired electrons on their valence shells which makes them structurally unstable. The unstable nature of these metals often orchestrate the conversion of H_2O_2 to OH in the Fenton reaction and in decomposition of alkyl peroxides to heavy reactive alkyl and hydrogen radicals (Hsu *et al*., 2006). Interestingly, chelating agents such as antioxidants may inactivate metal ions and potentially inhibit the metal dependent processes (Finefrock *et al*., 2003) by donating electrons to these unstable-electron-deficient metals. From Table 1, with only 0.22 μg mL^{-1}, *A. indica* chelated 50% of ferrous ions in absolute concentration of ethanol showing even more chelating capacity than ascorbic and gallic acid standards which chelated 50% of the metal ion at higher concentrations (5.95 and 0.503 μg mL^{-1}, respectively). In 70 and 50% ethanol, *A. indica* extracts also showed good metal chelating capacity comparable to the two standards. This result suggest that rather than depending completely on ascorbic acid, gallic acid and other synthetic antioxidant sources to mitigate the deteriorating effect of free radicals in the body, a more readily and cost effective source such as *A. indica* leaf extracts could be adopted. Although *M. oleifera* extracts did not show good metal chelating potential, its antioxidant properties is worth recommending as reflected from its DPPH scavenging capacity in absolute ethanol.

Reducing power is the measure of the extract ability to donate electrons in order to facilitate the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}). The absorbance values indicate the concentration of Fe^{2+}, thus, the higher the absorbance values the higher the concentration of Fe^{2+} which indicate the ability of the extract to donate electrons as an antioxidant reservoir (Laandrault *et al*., 2001; Yen *et al*., 2000). This may probably suggest that both *M. oleifera* and *A. indica* extracts are good electron donors (antioxidant sources) following their absorbance values at the respective concentration of the extracting solvents.

It has been reported that damages mediated by free radicals such as superoxide anion (O_2^-), hydroxyl radical (OH) and peroxyl radical (ROO$^-$) result in the disruption of membrane fluidity, protein denaturation, lipid peroxidation which brings about generation of oxidative stress evidenced

in many chronic diseases (Biglari *et al*., 2008; Ikpeme *et al*., 2014). Polyphenols from plant origin have the capacity to quench these radicals due to their ability to stabilize unpaired electrons (Anokwuru *et al*., 2011) and may have implication in prevention and/or curing oxidative stress diseases (Shukla *et al*., 2009). Although antioxidant activities of *M. oleifera* and *A. indica* obtained from the various *in vitro* assays in this study may not be completely obtainable *in vivo*, the results probably indicate that the extract of these two medicinal plants can scavenge and/or prevent free radical generation thereby mitigating oxidative stress mediated diseases in the body.

CONCLUSION

Comparatively, the leaf extract of *A. indica* had more phenols and flavonoids than *M. oleifera* in all the extracting solvents. This culminated why *A. indica* was able to scavenge 50% of the DPPH radical in absolute ethanol and also chelated 50% of the metal ion at a lower concentration than *M. oleifera*. The absorbance values for reducing power and total antioxidant capacity of *A. indica* were also comparably better than *M. oleifera*. Thus, the results indicate that *A. indica* leaf extract is a better antioxidant and free radical scavenger over *M. oleifera* mostly in absolute ethanol although more comparative antioxidant studies of the two plant extracts are required to further authenticate this claim.

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