Comparative Study of Antioxidant Potentials of Methanol Fruit Extracts of *Morinda citrifolia* and *Morinda lucida*

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ABSTRACT

Herbal preparations of different *Morinda* plant species had been used as supplements and for therapeutic purposes with less emphasis on the bioactive components. This study is aimed at investigating the *in vitro* antioxidant potentials of methanol fruit extracts of *Morinda citrifolia* (MCE) and *Morinda lucida*. The potent bioactive agents in MCE and MLE were extracted with 90% methanol. Both plants species did not have catalase activity but scavenged 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) in concentration dependent manner against percentage inhibition showing effective concentration at 50% inhibition (EC₅₀) of 716.09 µg/ml and 910.24 µg/ml for MCE and MLE compared to that of vitamin C standard with the EC₅₀ of 54.61 µg/ml. The MCE and MLE scavenged superoxide (SO) radical in concentration dependent manner with the EC₅₀ of 72.77 µg/ml and 82.43 µg/ml respectively compared to that of vitamin C standard with EC₅₀ of 176.08 µg/ml. Also, MCE and MLE scavenged nitric oxide (NO) radical in concentration dependent manner with the EC₅₀ of 2198.32 µg/ml and 3734.26 µg/ml compared to that of vitamin E standard with EC₅₀ of 1700.73 µg/ml. Both MCE and MLE scavenged hydroxyl (OH) radical in concentration dependent manner with the EC₅₀ of 134.32 µg/ml and 155.07 µg/ml respectively compared to that of vitamin E standard with EC₅₀ of 54.33 µg/ml. These might imply that MCE is a better antioxidant than MLE. The MLE contained more vitamin A (2,438±0.1 i.u) than MCE (1,323±0.3 i.u) while MCE vitamin C (10.80±0.05 i.u) and vitamin E (0.15±0.2 i.u) contents was higher than that of MLE (vitamin C (7.40±0.02 i.u) and vitamin E (0.14±0.01 i.u)). These could also imply that MCE had more antioxidant activity and could be preferred in Nigeria folk medicine.

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INTRODUCTION

Plants contain biochemical components that can be extracted and used in the preparation of drug, or used directly as medication. These medicinal plant preparations are administered to man with the hope of promoting health and treating various diseases. Immense benefits have been derived by man from using medicinal herbs in disease management because they are relatively safer, more affordable and sometime offer better therapeutic values than synthetic drugs (Oyewole and Akigbala, 2011). One of these medicinal plants is *Morinda*. It is in a genus of flowering plants in the madder family, Rubiaceae. Its generic name is derived from the Latin words *morus* "mulberry",

from the appearance of the fruits, and *indica*, meaning "of India". There are about 80 species of *Morinda* trees although the species of interest in this work are *Morinda citrifolia* and *Morinda lucida*. *Morinda citrifolia* Linn Rubiaceae is one of the most important traditional Polynesian medicinal plant commercially known as Noni, Indian mulberry, Cheese fruit and indigenously found in open coast region at sea level and in forest areas about 1,300 feet above sea level. It is a small tropical evergreen shrub or tree, three to twelve meters high. It has straight trunk, large green leaves and distinctive grenade like yellow fruit. The size of the fruit is about 12cm due to coalescence of the interior ovaries of many closely packed flowers and it has a foul taste and a soapy smell when ripe. The fruit of this plant has been used as food, drink,

medicine, colourful dye, cosmetics purpose and has a high demand in medicines for different kinds of illnesses like diabetes, high blood pressure, AIDS, arthritis, cancer, gastric ulcer, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problem etc., (Kochuthressia and Jaseentha, 2015). On the other hand, *M. lucida* is an evergreen shrub or small to medium-sized tree bearing a dense crown of slender, crooked branches. It can grow from 2.4 - 18 metres tall, although plants of 25 metres are recorded in coastal Cote D'Ivoire. The branches are often crooked or gnarled and it is a multipurpose species yield dyes, timber, fuel and traditional medicines. The roots are sold in local shops and markets, both as dyestuff and medicine, whilst the leaves and twigs are sold as a medicinal tonic for young children.

The ability of *Morinda* to perform these curative actions is because of the presence of phytochemicals and antioxidants (Webbi, 2013). Antioxidants are a class of molecules that are capable of inhibiting the oxidation of another molecule (Becker *et al.*, 2004). The body naturally circulates various nutrients due to their antioxidant properties. It also manufactures antioxidant enzymes in order to control free radical chain reactions. Some antioxidants are produced by the body, but some are not. The body's natural antioxidant production can decline with age. Antioxidants play a significant role in one's health, as they can control how fast a man age by fighting free radicals.

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules (Ananya, 2016). Once formed these highly reactive radicals can start a chain reaction, like dominoes. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cell damage caused by free radicals appears to be a major contributor to aging and diseases like cancer, heart disease, decline in brain function, decline in immune system etc.

The *M. citrifolia* and *M. lucida* contain antioxidants like vitamin C, vitamin E, and A (Adejo *et al.*, 2015). Vitamin C is the most important water-soluble antioxidant in the extracellular fluids. Vitamin C helps to neutralize reactive oxygen species (ROS) in the water or aqueous phase before it can attack the lipids. Vitamin E is the most important lipid soluble antioxidant. It is important as the chain-breaking antioxidant within the cell membrane. It can protect the membrane fatty acids from lipid peroxidation. Vitamin C in addition is capable of regenerating vitamin E. Beta carotene and other carotenoids also have antioxidant properties. Carotenoids work in synergy with vitamin E. Both *Morinda* species also scavenged superoxide, nitric oxide, hydroxyl and 1, 1-diphenyl-2-picrylhydrazyl radical.

Most plants with high antioxidant potentials are believed to be medicinal plants. Thus, this work will compare the antioxidant potentials of methanol fruit extracts of *Morinda citrifolia* and *Morinda lucida* to determine the specie that will be preferred in traditional medicine.

Plant Materials Sample (*Morinda citrifolia*)

The *M. citrifolia* and *M. lucida* fruits were obtained from Hezekiah Dike Street, Obinagu, Awka and Nnamdi Azikiwe University Awka plantation. These plants were identified in the Botany laboratory of Nnamdi Azikiwe University. A voucher specimen was deposited in the herbarium of the University and was authenticate by Dr. B. O. Aziagba with the herbarium numbers, page 14, cabinet 2 and shelf 35. The fruits were dried in an oven at 50°C, soaked for 48 hours and extracted with 90% Lobachem methanol.

Preparation of fruit Extract

Morinda fruits were rinsed with distilled water and dried in an oven at 50°C for twelve (12) hours. The dried fruits were grinded into powder with a manual blender. Four hundred and fifty grams (450 gms) of the dried fruit powder was macerated in seven hundred millilitres (700 ml) of 99% Lobachem methanol, agitated for 5 minutes with an electric blender and left for 48 hours at room temperature. The mixture was pressed with a muslin cloth and then filtered with Whatmann No.1 filter paper. Then, different quantities of the methanolic extract of *Morinda* were used for the vitamins and antioxidant analysis.

Chemicals: All chemicals used were of analytical grade.

METHODS

The catalase activity was assayed using colorimeter as described by Beers and Sizer, 1952.

DPPH radical scavenging activity was measured using spectrophotometric method as described by Ahmed *et al.*, (2015).

Hydroxyl radical scavenging activity was measured according to method described by Halliwell *et al.*, (1987).

Nitric oxide scavenging activity was determined according to Rozina *et al.*, 2012.

Superoxide (SO) radical scavenging assay determined by Yu *et al.*, 2003.

Determinations of antioxidant vitamins (A, C and E) in the extract were done using the following methods;

Vitamin A; Spectrophotometry method as described by Plummer, (1978).

Vitamin C; Titration method as described by John and Bernard, 1990.

Vitamin E; Titration method as described by Kayden *et al.*, (1973).

Statistical Analysis

Data were reported as means $\pm$ S.D, where appropriate. Student T-test and correlation analysis were used to analyse

the research data using Statistical Packages for Social Sciences (SPSS) version 16. Differences were considered significant when $p < 0.05$ and $p < 0.01$.

RESULTS

Percentage yield (*M. citrifolia*)

$$= \frac{\text{Extract weight}}{\text{Crude weight}} \times 100$$

$$= \frac{23.08}{452.32} \times 100 = 5.10\%$$

Percentage yield (*M. lucida*) = $\frac{\text{Extract weight}}{\text{Crude weight}} \times 100$

$$= \frac{27.54}{468.57} \times 100 = 5.88\%$$

Table 1: Scavenging effect of extracts on DPPH radical

Concentration (µg/ml)	Percentage Inhibition (%)		
	MCE	MLE	Vitamin C (Standard)
1000	64.51	54.48	96.01
500	44.33	28.51	95.04
250	23.69	14.25	92.32
200	11.40	11.40	92.14
100	9.47	5.70	91.56

EC₅₀ for MCE=716.09 µg/ml, EC₅₀ for MLE= 910.24µg/ml

EC₅₀ for vitamin C (Ascorbic acid) = 54.61µg/ml

Table 1 shows the scavenging effect of MCE and MLE on DPPH radical. The quantitative DPPH assay showed a decolouring effect of the purple colour of the DPPH radical to yellow after both extracts were incubated with it for 30 minutes. From this table, it was deduced that the two extracts scavenged DPPH radical in concentration dependent manner with the highest percentage inhibition of 64.51 and 54.48 for MCE and MLE respectively. The effective concentration at which the extract can scavenge 50% of the free radical present (EC₅₀) are 716.09µg/ml and 910.24 µg/ml for MCE and MLE respectively compared with that of vitamin C (Ascorbic acid) of 54.61µg/ml. Both MLE and MCE concentration against percentage inhibition showed a statistically positive significant correlation ($p < 0.01$) with correlation coefficient (R^2) of 0.986 and 0.971 indicating that MCE could be a better antioxidant than MLE.

Table 2: Scavenging effect of extracts of MCE and MLE on Superoxide radical.

Concentration (µg/ml)	Percentage Inhibition (%)		
	MCE	MLE	Vitamin C (Standard)
1000	92.85	88.23	70.35
500	88.56	76.37	65.80
250	85.56	68.57	52.40
200	84.56	65.01	50.74
100	68.75	60.66	43.80

EC₅₀ for MCE = 72.77µg/ml, EC₅₀ for MLE = 82.43µg/ml

EC₅₀ for vitamin C (Ascorbic acid) = 176.08µg/ml

Table 2 shows the results of the scavenging effect of MCE and MLE on superoxide radical. From this table, it was deduced that the two extracts scavenged superoxide radical in concentration dependent manner with the highest percentage inhibition of 92.85% and 88.23% for MCE and MLE respectively. The effective concentration at which the extract scavenged 50% of the free radical present (EC₅₀) for MCE and MLE were 72.77µg/ml and 82.43µg/ml respectively compared with that of vitamin C (Ascorbic acid) of 176.08µg/ml. The MLE extracts concentration against percentage inhibition showed a statistically positive significant correlation ($p < 0.01$) with correlation coefficient (R^2) of 0.988 while that of MCE showed non positive statistically correlation ($p > 0.01$) with correlation coefficient (R^2) of 0.756 indicating that MCE has more antioxidant potential.

Table 3: Scavenging effect of MCE and MLE on Hydroxyl radical.

Concentration (µg/ml)	Percentage Inhibition (%)		
	MCE	MLE	Vitamin E (Standard)
100	49.45	48.84	85.98
64	47.94	46.88	79.67
32	47.49	46.27	69.10
12.8	47.22	46.21	41.80
6.4	47.12	46.00	15.74

EC₅₀ for MCE = 134.32 µg/ml, EC₅₀ for MLE = 155.07µg/ml

EC₅₀ for vitamin E (Alpha-tocopherol) = 54.33µg/ml.

Table 3 represents the scavenging effect of MCE and MLE on hydroxyl radical. The analysis showed that the MCE and MLE scavenged hydroxyl radical in concentration dependent manner with the highest percentage inhibition of 49.45 and 48.84 respectively. The effective concentration at which the extracts can scavenge 50% of the free radical present (EC₅₀) for MCE and MLE were 134.32µg/ml and 155.07 µg/ml respectively compared with that of vitamin C (Ascorbic acid) of 176.08µg/ml. The MCE extracts concentration against percentage inhibition showed a statistically positive significant correlation ($p < 0.01$) with correlation coefficient (R^2) of 0.963 while that of MLE showed significant statistically positive correlation ($p < 0.05$) with correlation coefficient (R^2) of 0.946. Here, vitamin E was more effective scavenger than the extracts but MCE has more antioxidant power than MLE.

Table 4: Scavenging effect of MCE and MLE on Nitric oxide radical.

Concentration (µg/ml)	Percentage Inhibition (%)		
	MCE	MLE	Vitamin E (Standard)
1000	26.50	18.50	31.70
500	26.14	14.16	4.12
250	17.10	11.92	13.00
200	16.95	11.29	0.00
100	8.60	6.41	3.61

EC_{50} for MCE = 2198.32 μ g/ml, EC_{50} for MLE = 3734.26 μ g/ml,

EC_{50} for vitamin E = 1700.73 μ g/ml

Table 4 represents the scavenging effect of MCE and MLE on nitric oxide radical. The analysis showed that the MCE and MLE weakly scavenged nitric oxide radical in concentration dependent manner with the highest percentage inhibition of 18.50 μ g/ml and 26.50 μ g/ml respectively. The effective concentration at which the extracts can scavenge 50% of the free radical present (EC_{50}) for MCE and MLE were of 2198.3 μ g/ml and 23734.26 μ g/ml respectively compared with that of vitamin E of 157.73/ml). The MLE extracts concentration against percentage inhibition showed a statistically positive significant correlation ($p < 0.05$) with correlation coefficient (R^2) of 0.931 while that of MCE showed non-significant statistically positive correlation ($p > 0.05$) with correlation coefficient (R^2) of 0.836 indicating that MCE has more antioxidant power.

Comparison of the Anti-Radical Power (ARP) of the extracts Against 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Radical, Nitric oxide Radical, Superoxide Radical and Hydroxyl Radical Scavenging Activity

Fig. 1 represents the anti-radical power of MCE and MLE fruit against different free radicals. ARP were calculated as the inverse of EC_{50} of those radicals. The ARP of MCE against superoxide radical was the highest, showing that MCE extract had the ability to quench superoxide radical more than the DPPH, nitric and hydroxyl radicals when compared to that of MLE extract while MLE had the least ability to scavenge nitric oxide radical more than DPPH, superoxide and hydroxyl radicals when compared to that of MCE.

Fig. 1: Anti Radical Power of MCE and MLE against different free radical.

Table 5: Vitamin content of MCE and MLE.

Vitamins	Values (I.U)	
Vitamin A	1,323 $\pm$ 0.3	2,438 $\pm$ 0.1
Vitamin C	10.80 $\pm$ 0.051	7.40 $\pm$ 0.02
Vitamin E	0.15 $\pm$ 0.21	0.14 $\pm$ 0.01

Table 4 displays the vitamin concentrations of MCE and MLE fruits. The MLE had higher vitamin A concentration (2,438 $\pm$ 0.1 I. U) than MCE (1,323 $\pm$ 0.3 I. U) while MCE had higher vitamin E concentration (0.15 $\pm$ 0.2 I. U) than MLE (0.14 $\pm$ 0.01 I. U). Also, MCE had higher water soluble vitamin C concentration (10.80 $\pm$ 0.05 I. U) than MLE (7.40 $\pm$ 0.02 I. U).

DISCUSSION

Different plant parts are made up of bioactive compound which avail them the ability to mop-up free radicals. Antioxidants are mainly responsible for this effect. This work compares the *in vitro* antioxidant potentials of methanol fruit extract of *Morinda citrifolia* (MCE) and *Morinda lucida* (MLE).

The results of the *in vitro* antioxidant potentials showed that MCE and MLE had free radical scavenging activities for 1, 1-diphenyl-2-picryl-hydrazyl (DPPH), nitric oxide, hydroxyl and superoxide radicals. There was no catalase activity in MCE and MLE. This was not in line with Srinivasanan and Durairaj, (2014) that detected catalase activity in *M. citrifolia*. The absence of catalase is because methanol provokes a decrease in the activity and concentration of antioxidant enzymatic as well as non-enzymatic parameters thereby causing enhanced membrane peroxidation of phospholipids (Skrzydowska, 2003). Catalase converts hydrogen peroxide into water and oxygen, using iron and manganese as cofactors (Chelikani *et al.*, 2004) thereby, finished up the detoxification process started by superoxide dismutase (SOD).

The result showed that the effective concentration of extract that caused 50 % inhibition of free radicals (EC_{50}) of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical for MLE (910.24 μ g/ml) was significantly higher ($p < 0.05$) than that of MCE (716.09 μ g/ml). This could imply that MCE had more scavenging activity or antioxidant activity than MLE although their scavenging activity was lower when compared with the standard vitamin C of EC_{50} (54.61 μ g/ml). The EC_{50} of superoxide (SO) radical for MLE (82.43 μ g/ml) was significantly higher ($p < 0.05$) than that of MCE (72.77 μ g/ml). This could suggest that MCE had higher superoxide radical scavenging activity than MLE. But both extracts were better superoxide radical scavengers when compared with the standard vitamin C of EC_{50} (197.08 μ g/ml). The EC_{50} of hydroxyl (OH $\cdot$) radical for MCE (141.45 μ g/ml) was non-significantly lower ($p > 0.05$) than that of MLE (EC_{50} 172.98 μ g/ml). This could indicate that the antioxidant activity of MCE was slightly higher than that of MLE. The EC_{50} of nitric oxide (NO) radical for MLE (3676.60 μ g/ml) was significantly higher ($p < 0.05$) than that of MCE of EC_{50} (2198.32 μ g/ml). This could mean that the antioxidant activity of MCE was higher than that of MLE. Meanwhile, both extracts were weak antioxidants when compared with the standard vitamin E of EC_{50} (157.61 μ g/ml).

From the above results both extracts scavenged DPPH, SOD, OH and NO radicals so, it could be suggested that they had antioxidant activity (Rahman and Moon, 2007; (Hasan *et al.*, 2008). When there is an imbalance between the free radicals and the antioxidant, it results to oxidative injury which is the

fundamental mechanism underlying a number of human neurologic and other disorders such as inflammation, viral infections, autoimmune pathologies, and digestive system disorders including gastrointestinal inflammation and ulcer (Aruoma, 2003).

Vitamin A, C and E are non-enzymatic antioxidants (Shahidi and Zhong, 2010). They play crucial roles in preventing peroxidation damage in the biological system (Fogliano *et al.*, 1999; Mantena *et al.*, 2003). The result of vitamin concentrations of the extracts supported Dixon *et al.* (1999) report which stated that the vitamins in the MCE are mainly ascorbic acid and provitamin A. Extracts from both plant species had very high vitamin A concentrations of $1,323 \pm 0.3$ I.U and $2,438.48 \pm 0.1$ I.U for MCE and MLE respectively with vitamin A content of MLE being significantly higher ($p < 0.05$) than that of MCE indicating that MLE could be a better antioxidant than MCE. The vitamin C concentration of MCE (10.80 ± 0.05 I.U) was significantly higher ($p < 0.05$) than that of MLE (7.4 ± 0.02 I.U). This result supported the claims of anticancer effect of both extracts since vitamin C plays a laudable role in the prevention and treatment of cancer (Carr and Frei, 1999; Li and Schellhorn, 2007). The vitamin E concentrations of 0.15 ± 0.2 I. U for MCE was not significantly higher ($p > 0.05$) than that of MLE with 0.14 ± 0.01 I. U. Both extracts had low vitamin E content when compared with the recommended daily allowance (RDA) of 28 I.U (National Academy Press, 2000). This result also is suggestive of the fact that both extracts should possibly have low effect in preventing the formation of blood clots that could lead to heart attack or venous thromboembolism (Glynn *et al.*, 2007).

CONCLUSION

Morinda citrifolia and *Morinda lucida* plants are used in folk medicine because of their high antioxidant activity. These plants contained the fat soluble vitamin A (a good source of vitamin A) and the water soluble vitamins B and C. They have moderate to strong scavenging activity for DPPH, SO₂, OH and NO radicals with no catalase activity. These antioxidants might have contributed to the use of these plants both as supplement and for therapeutic purposes. Nevertheless, MCE might be a better antioxidant than MLE and could be preferred in folk medicine.

Contribution to knowledge

Morinda citrifolia is more potent than *Morinda lucida*.

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