



Phytochemical constituents and *In vitro* Antioxidant Activities of Methanol leaf extracts of *Musanga cercropioides* and *Maesobotrya barteri*

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ABSTRACT

The quest for antioxidants of plant origin is growing at a massive rate due to their efficacy and affordability. This study examined the phytochemical constituents and *in vitro* antioxidant activities of methanol leaf extracts of *Musanga cercropioides* and *Maesobotrya barteri*. Standard methods were used to determine the phytochemical constituents of the leaves extracts. *In vitro* antioxidant activity of the methanol plant extracts was determined by a broad spectrum of antioxidant assays. Qualitative phytochemical screening revealed the presence of flavonoids, tannins, cardiac glycosides, saponins, steroids, alkaloids, coumarins, phenols and terpenoids in both plants. The quantitative analyses of the phytochemicals showed that *M. cercropioides* had higher levels ($p < 0.05$) of total phenol, total tannins, proanthocyanidin and lower total flavonoids as against *M. barteri*. DPPH scavenging activities of *M. cercropioides* (IC_{50} of $1.22.75 \pm 0.046$ mg/ml) were significantly higher ($p < 0.05$) than that of *M. barteri* (IC_{50} of $1.95.75 \pm 0.023$ mg/ml) but lower than that of ascorbic acid standard (IC_{50} 0.15 ± 0.003). *M. cercropioides* extracts had significantly higher ($p < 0.05$) capacity to reduce ferric ions to the ferrous form and also inhibit lipid peroxidation when compared with *M. barteri*. However, the reducing potential of *M. cercropioides* was comparable to the Ascorbic acid standard but significantly higher than that of *M. barteri* extracts. The study suggests that *M. cercropioides* and *M. barteri* contain bioactive compounds that may be effective in the management of oxidative stress related diseases.

Keywords: Phytochemical, Antioxidant, Oxidative stress, *Musanga cercropioides*, *Maesobotrya barteri*

INTRODUCTION

The use of natural products particularly medicinal plants by man for the treatment of diseases has been in existence since time immemorial. In recent times, research have shifted to the use of these medicinal plants because of their enormous therapeutic effects as well as their low or absence of side effects as seen in synthetic drugs (Omoriegie and Oikeh, 2015). The therapeutic effects of several medicinal plants have been attributed to their phytochemicals (Oriakhi *et al.*, 2014). These phytochemicals possess arrays of properties, the most common being their antioxidant activity. This property is particularly important for phenolic and flavonoid compounds because of their capacity to scavenge free radicals emanating from different reactive oxygen and nitrogen species (ROS/RNS), such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (HO^{\bullet}) peroxy radical (ROO^{\bullet}), nitric oxide (NO^{\bullet}), hypochlorite ion ($ClO^{\bullet-}$), hydrogen peroxide (H_2O_2), singlet oxygen (1O_2) and peroxyxynitrite ($ONOO^{\bullet-}$) (Halliwell, 1991; Ak and Gulcin, 2008; Royer *et al.*, 2011; Saurai, 2015; Kasanagana *et al.*, 2015). In most living cells, ROS/RNS are not only produced naturally as a result of stress or respiration but have known to be produced by radiation, bacterial and viral toxin, smoking and alcohol (Ojemekele *et al.*, 2017). If these reactive species are not trapped or eliminated by such cellular constituents, they may lead to serious diseases. Research have shown that ROS/RNS are associated with over 100 diseases, including Alzheimer's, cancer, atherosclerosis, diabetes mellitus, hypertension, aging and inflammation (Lopez-Alarcon and Denicola, 2013; Saurai, 2015).

Antioxidants are thus valuable in protecting cells from the damages associated with ROS and RNS. Synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have regularly been as food additives. However, they have been linked with liver damage and carcinogenesis (Halliwell, 1991; Stevanovic *et al.*, 2009; Cekic *et al.*, 2013). Natural antioxidants are an interesting alternative in view of their variety of structures and chemical interactions, as well as the numerous biological activities they can perform (Govindappa *et al.*, 2011). Indeed, several studies have reported that polyphenols, such as flavonoids, hydroxycinnamic acids and proanthocyanidins, act as powerful antioxidants (Martin-Sanchez *et al.*, 2014; Saurai, 2015).

Musanga cercropioides R. Br. a member of the *Moraceae* family is found mostly in the tropical forests of Africa stretching from Guinea to Congo (Ayinde *et al.*, 2003). The leaves have been shown to have oxytocic (Kamanyi *et al.*, 1992), antihypertensive (Kamanyi *et al.*, 1991; Kamanyi *et al.*, 1996; Dongmo *et al.*, 1996), antiglycaemic (Kamanyi *et al.*, 2000) and anti-inflammatory properties (Sowemimo *et al.*, 2015). Aqueous extract of the stem bark and the adventitious root sap have shown to have hypotensive and antimicrobial activity (Ayinde *et al.*, 2003; Uwah *et al.*, 2013).

Maesobotrya barteri, Baill. Hutch. (Euphorbiaceae), is the most widely distributed *Maesobotrya* species in Nigeria. It's a rainforest plant occurring in Sierra Leone, Southern Nigeria and Western Cameroon. It is an under exploited tropical fruit tree (Ogbuagu and Agu, 2008). *M. barteri*

bears fruit from April to June, which is up to 1 cm long, ovoid and often distinctly pointed. The fruits are succulent black-purple berries. They are edible and stain the tongue. The stems are used as chewing stick to treat toothache, the roots are cut and infused in gin for arthritis and the stems are also used for fencing and supporting yam tendrils on the farm (Ogbuagu and Agu, 2008). However, there is paucity of information on the quantitative phytochemical constituents and *in vitro* antioxidant properties of leaves these two plants. Thus, this study was aimed at evaluating the phytochemical constituents (qualitative and quantitative) and *in vitro* antioxidant activities of the methanol leaf extracts of *M. barteri* and *M. cecropioides*.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Fresh leaves of *Musanga cecropioides* and *Maesobotrya barteri* were harvested from the rain forest of Aladja, Delta State, Nigeria. The plant samples were identified at the Department of Plant Biology and Biotechnology, University of Benin, Benin city, Nigeria. Herbarium specimens with voucher numbers of MC/PBB/209/15 and MC/PBB/210/15 for *Musanga cecropioides* and *Maesobotrya barteri* respectively were deposited. Both plant samples were air-dried at ambient temperature, pulverised and kept in air tight containers prior to use.

Preparation of Methanol Extract

Methanol extracts of the leaves were prepared by soaking a weighed quantity (300g) of the crushed sample in a specific volume (2.5 litres) of methanol for 72 hours and then filtered using Muslin cloth. The extracts were then concentrated by using a rotary evaporator at 40°C and further freeze-dried.

Qualitative Phytochemical Screening

Chemical tests for the screening and identification of phytochemicals in the study plants were carried out in the extracts by using standard procedures as previously described by Sofowora (1993), Trease and Evans (1989) and Harborne (1998).

Quantitative Estimation of Phytochemicals

The total phenolic content was determined using the Folin - Ciocalteu method as described by Cicco *et al.* (2009) with slight modifications. The assay is based on the reduction of Folin-ciocalteu reagent (phosphomolybdate and phosphotungstate) by the phenolic compounds. The reduced Folin-ciocalteu reagent is blue and thus detectable with a spectrophotometer at 760 nm.

The total flavonoid content was determined using the method of Miliauskas *et al.* (2004). The assay is based on the reaction between flavonoids and aluminum chloride to generate a characteristic mixture that has maximum absorption at 510 nm. The determination of proanthocyanidin was carried out according to the method of Sun *et al.* (1998). It is based on oxidative depolymerization of condensed tannins in butanol-HCl reagent. Tannins content was determined by Folin-Denis method (Polshettiwar *et al.*, 2007). The method is based on the measurement of a blue colour formed by the reduction of phosphotungsto-molybdic acid by tannin-like compounds in alkaline medium.

Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the leaf extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). The assay is based on the ability of the antioxidant compounds to reduce DPPH by donation of hydrogen resulting in colour change from deep violet to golden yellow. The change in colour from deep violet to light yellow was measured with spectrophotometer at 517nm. The 50% inhibitory concentration value (IC₅₀) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

Ferric Reducing Antioxidant Power (FRAP) Assay

The Ferric Reducing Antioxidant Power (FRAP) assay was carried out using a modified method of Benzie and Strain (1996). The assay is based on the ability of antioxidant to reduce Fe³⁺ to Fe²⁺ in the presence of 2, 4, 6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe²⁺-TPTZ complex with an absorption maximum at 593 nm. FeSO₄ was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)/g of the extract) for the extracts were then extrapolated from the standard curve.

Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were estimated according to the method of Ohkawa *et al.* (1979). Malondialdehyde (MDA), a secondary end product of the oxidation of poly unsaturated fatty acids reacts to yield a pinkish red chromogen with an absorbance maximum at 532nm.

Estimation of Reducing Power

The reducing power of extract was determined according to the method described by Lai *et al.* (2001). Fe (III) reduction is often used as an indication of electron donating activity, which is an important mechanism of phenolic antioxidant action.

Statistical Analysis

The experimental results were expressed as mean ± Standard Error of Mean (SEM) of three replicates. Where applicable, the data were subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by Duncan's multiple range tests using Graph Pad Prism 5 Statistical package. P values < 0.05 were regarded as significant. IC₅₀ was calculated using EZ enzyme kinetics software.

RESULTS

Qualitative Phytochemical Constituents of *M. barteri* and *M. cecropioides* Methanol Extracts

Result of the qualitative phytochemical screening (Table 1) showed that there was the presence of flavonoids, tannins, cardiac glycosides, saponins, alkaloids, coumarins, phenols, and terpenoids in the methanol extracts of both plants. However, anthroquinones and phlobatannins were not detected in both plants.

Table 1: Qualitative phytochemical constituents of methanol extracts of *M. cercropioides* and *M. barteri*

PHYTOCHEMICALS	<i>Musanga cercropioides</i>	<i>Maesobotrya barteri</i>
Flavonoids	+	++
Tannins	+	+
Cardiac glycosides	+++	+
Saponins	+++	++
Steroids	++	+++
Alkaloids	++	+
Reducing sugars	+	+
Carbohydrates	+	+
Anthroquinone	-	-
Coumarin	+	++
Phlobatannins	-	-
Phenols	++	+
Terpenoids	+++	+++

Not detected= '-'; moderately detected= '+'; Detected= '++'; Highly detected= '+++'

Quantitative Phytochemical Constituents of Dried Leaves of *M. cercropioides* and *M. barteri*

The total phenolic content, proanthocyanidin content, total tannin content and total flavonoid content of *M. barteri* and *M. cercropioides* leaves are presented in Table 2. The

results indicate that *M. cercropioides* was significantly higher total phenol, total tannins and total proanthocyanidins ($p < 0.05$). However, *M. barteri* had higher total flavonoids contents.

Table 2: Quantitative phytochemical constituents of methanol extracts of *M. cercropioides* and *M. barteri*

Phytochemicals	<i>M. cercropioides</i>	<i>M. barteri</i>
Total Phenol (mg gallic acid eq/g of extract)	51.7 ± 1.33 ^a	16.13 ± 1.22 ^b
Total Flavonoids (mg quercetin eq/g of extract)	9.92 ± 1.31 ^a	70.17 ± 0.42 ^b
Total Tannin (mg tannic acid eq/g of extract)	56.1 ± 43.67 ^a	14.7.33 ± 0.23 ^b
Proanthocyanidin (mg ascorbic acid eq/g of extract)	53.9 ± 0.63 ^a	35.31 ± 0.24 ^b

All values are expressed as mean ± SEM (n = 3). Different lower case letters represent significant difference between means at $p < 0.05$.

In Vitro Antioxidant Activity of *M. barteri* and *M. cercropioides*

Ferric Reducing Antioxidant Potential (FRAP)

The Ferric Reducing Action Potential (FRAP) of the two plants (811.95 ± 11.27 and 626.94 ± 4.57 μmole Fe(II)/g of extracts of *M. cercropioides* and *M. barteri*, respectively)

were significantly lower than that recorded for standard ascorbic acid (960.47 ± 12.12 μmole Fe(II)/g of extract) (Figure 1).

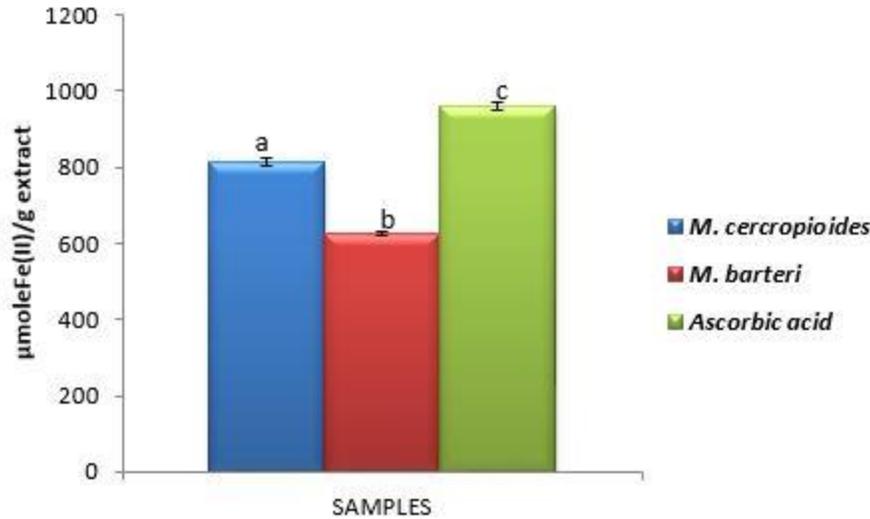


Figure 1: Ferric Reducing Action Potential (FRAP) of *M. cercropioides* and *M. barteri*
 All values are expressed as mean ± SEM (n = 3). Different lower case letters represent significant difference between means at $p < 0.05$

Reducing Power Assay

There was no significant difference ($p > 0.05$) in the reducing power of *M. cercropioides* when compared to the

standard ascorbic acid at concentrations of 0.1 - 1.0 mg/mL (Figure 2). However, the reducing power of *M. barteri* extracts was significantly lower ($p < 0.05$) than that of the standard ascorbic acid and *M. cercropioides*.

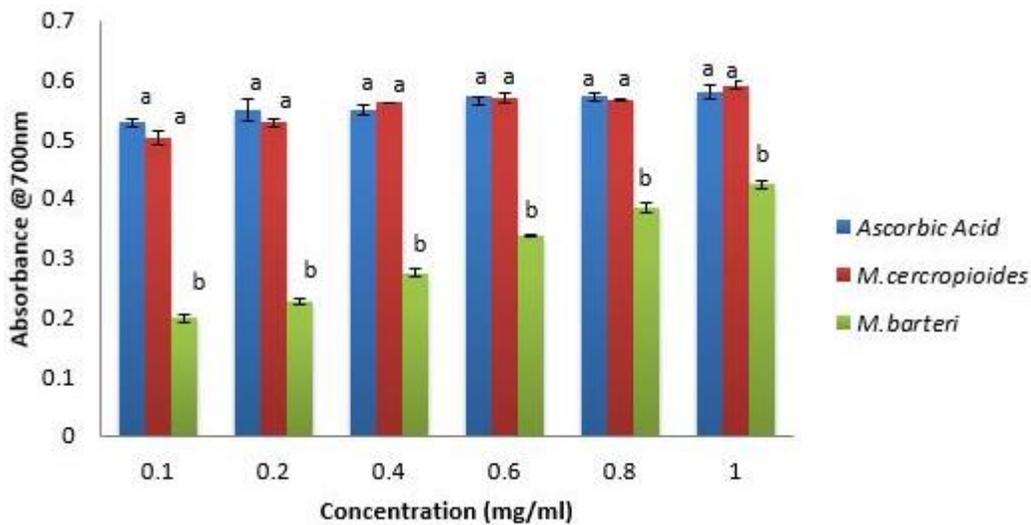


Figure 2: Reducing power of *M. cercropioides* and *M. barteri*
 All values are expressed as mean ± SEM (n = 3). Different lower case letters represent significant difference between means at $p < 0.05$.

***In vitro* Inhibition of Lipid Peroxidation**

Figure 3 shows the inhibition of lipid peroxidation as estimated by the thiobarbituric acid reactive substances (TBARS) test. Inhibition of lipid peroxidation was highest

in *M. cercropioides* extract ($76.46 \pm 3.53\%$) and lowest *M. barteri* extracts ($39.88 \pm 2.09\%$).

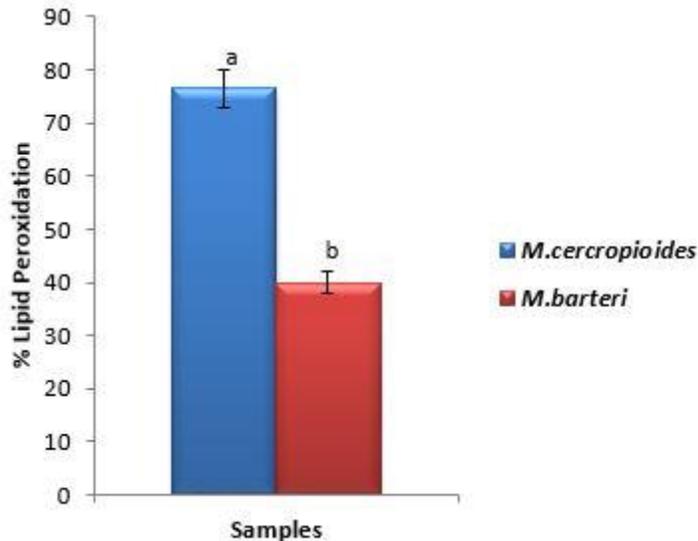


Figure 3: *In vitro* lipid peroxidation of *M. cercropioides* and *M. barteri*

All values are expressed as mean \pm SEM (n = 3). Different lower case letters represent significant difference between means at $p < 0.05$.

DPPH Scavenging Activity

The DPPH radical scavenging activities of the extracts are presented in Figure 4. DPPH radical scavenging activity assay showed that *M. cercropioides* was best inhibitor of the DPPH radical at higher concentrations (20 – 200

$\mu\text{g/mL}$) of the extracts when compared to *M. barteri*. IC_{50} values for the two extracts were found to be significantly higher than that of the reference antioxidant, ascorbic acid ($0.15 \pm 0.003 \text{ mg/mL}$) (Table 3).

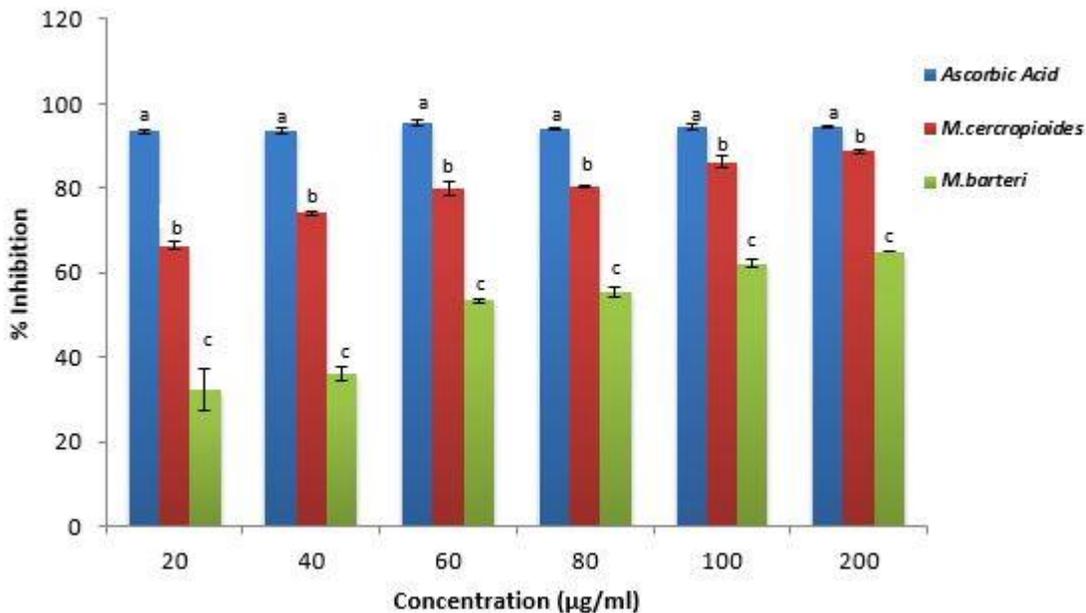


Figure 4: DPPH radical scavenging activity of *M. cercropioides* and *M. barteri* extracts

All values are expressed as mean \pm SEM (n = 3). Different lower case letters represent significant difference between means at $p < 0.05$.

Table 3: DPPH IC₅₀ values for Ascorbic acid, *M. cercropioides* and *M. barteri*

Sample	mg/mL
Ascorbic acid	0.15 ± 0.003 ^a
<i>M. cercropioides</i>	0.243 ± 0.046 ^b
<i>M. barteri</i>	1.418 ± 0.023 ^c

All values are expressed as mean ± SEM (n = 3). Different lower case letters represent significant difference between means at $p < 0.05$.

DISCUSSION

Screening plant materials for their phytochemicals is usually the first step in research protocol aimed at the isolation and purification of natural compounds with bioactivities (Omoriegie and Oikeh, 2015). It allows the researcher to see at a glance the phytochemicals present in a plant material and, hence, gives an idea of the potential use of the plant material (Oikeh *et al.*, 2013). In this study, apart from phlobatannins and anthraquinones that were not detected in both plants' methanol extracts, the extracts contained flavonoids, tannins, cardiac glycosides, saponins, steroids, alkaloids, coumarins, phenols and terpenoids. These phytochemicals or phyto-constituents are reported to confer protective effect on plants against microbial infections or infestation by pests (Doughari, 2012). The results from this study revealed a higher total phenolic content in *M. cercropioides* extract. Phenolic compounds in plants have been shown to possess good antioxidative activities (Ayoola *et al.*, 2008). The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group(s) (-OH), which is bond directly to an aromatic hydrocarbon (phenyl ring). This makes them donate electrons easily to electron seeking free radicals, thus curbing their effects in living cells (Uyoh *et al.*, 2013). The antioxidant potential of phenols is believed to be conferred on them by their hydroxyl group(s) (-OH), which is bond directly to an aromatic hydrocarbon (phenyl ring). This makes them donate electrons easily to electron seeking free radicals, thus curbing their effects in living cells (Uyoh *et al.*, 2013). Several studies have demonstrated a direct relationship between total phenol content and antioxidant activity in different plants (Ayoola *et al.*, 2008; Ghasemi *et al.*, 2009; Hegazy and Ibrahim, 2012). The results of this study showed that the total phenolic content of *M. cercropioides* was significantly higher than that of *M. barteri*. flavonoids represent the most common and widely distributed groups of plant phenolics (Omoriegie *et al.*, 2014). They are water soluble super antioxidants that function in scavenging free radicals, inhibition of lipid peroxidation and chelating transition metals. This study showed that the flavonoid content of *M. barteri* was significantly higher than that of *M. cercropioides*. It can be suggested that the high phenolic content recorded in *M. cercropioides* may be of a different origin. Proanthocyanidins are condensed tannins formed as a result of the coupling reactions between flavanyl units (Prakash and Gupta, 2009). The proanthocyanidins are believed to possess potent antioxidant activities and exert several

protective effects on humans (Gu *et al.*, 2003). The present study showed that *M. cercropioides* had higher proanthocyanidin when compared to *M. barteri*.

In this study, it was found that *M. cercropioides* had a significant higher level of tannins than *M. barteri*. Tannins are phenolic compounds of high molecular weight. They are used as antiseptic and this activity is due to the presence of phenolic group (Doughari, 2012). Tannin-rich medicinal plants are used as healing agents in a number of diseases. For instance, in Ayurveda practices, formulations based on tannins-rich plants have been used for the treatment of diseases like leucorrhoea and diarrhea (Doughari, 2012). Reactive oxygen species (ROS) are important players in biological environment and in the pathogenesis of several diseases. They are generated *in vivo* during several physiological processes (Omoriegie *et al.*, 2014). Antioxidants act as free radical scavengers and thus prevent tissue damages caused by ROS. Results from this study showed that *M. cercropioides* extract inhibited lipid peroxidation more than the *M. barteri* extract. Lipid peroxidation occurs as a result of oxidative deterioration of polyunsaturated lipids and it involves ROS and transition metals (Oikeh *et al.*, 2014). It is a molecular mechanism of cell injury that yields a wide range of cytotoxic products, most of which are aldehyde-like malondialdehyde (MDA), 4-hydroxynonenal (HNE) (Badarinath *et al.*, 2010). *In vitro* inhibition of lipid peroxidation can thus be used as index of evaluating the antioxidant capacity of a plant extract.

The ferric reducing antioxidant potential (FRAP) and reducing power (RP) values of methanol extract of *M. cercropioides* observed in this study compared favourably with the standard antioxidant used, ascorbic acid. FRAP assay is a simple, fast and precise assay that measures the total antioxidant power of biological fluids (Aljadi and Kamaruddin, 2004). FRAP assay is reproducible and linearly related to molar concentration of the antioxidant present in the biological sample (Katalinic *et al.*, 2006). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. This is because the presence of reductants such as antioxidant substances in the sample causes the reduction of the Fe³⁺ ferricyanide complex to the ferrous form (Rahman *et al.*, 2013).

In this study, the significantly higher ($p < 0.05$) reducing potential, ferric reducing antioxidant potential, % inhibition of lipid peroxidation, nitric oxide scavenging activity and the DPPH radical scavenging activity observed in the *M. cercropioides* extracts may be ascribed to the antioxidant

activity of phenolic compounds present. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. This is because the presence of reductants such as antioxidant substances in the sample causes the reduction of the Fe^{3+} ferricyanide complex to the ferrous form (Rahman *et al.*, 2013).

Conclusion

The findings from this study revealed that *M. cercropioides* leaf extract had higher phytochemical constituents when compared with *M. barteri*. The methanol extract of *M. cercropioides* showed better antioxidative activities than that of *M. barteri*. This may be attributed to the higher amount of some important phytochemicals in *M. cercropioides* extract. Therefore, both plants may be employed in the management of some degenerative diseases caused by oxidative stress. Extensive toxicity studies of both plants need to be done to ascertain their safety levels in man. Further studies on *in vivo* antioxidant activities should be investigated in future.

Conflict of Interest: The authors declare no conflict of interest.

Authors Contribution: U.B Shemishere A. A. Turaki and J. E. Taiwo prepared the plants extracts and well as executed the *In vitro* antioxidant activity assays, while A. Bello and D.A Anyebe did the phytochemical analyses. Prof E.S Omoregie designed the experiments as well as supervised the successful completion of the work.

REFERENCES

- Ak, T. and Gulcin, I. (2008). Antioxidant and radical scavenging properties of curcumin. *Chemico-Biological Interactions*, 174: 27–37.
- Aljadi, A.M. and Kamaruddin, M.Y. (2004). Evaluation of the phenolic contents and antioxidant capacities of two Malaysian floral honeys. *Food Chemistry*, 85: 513–518.
- Ayinde, B.A., Omogbai, E.K.I. and Onwukaeme, D.N. (2003). Pharmacognostic characteristics and hypotensive effects of the stem bark of *Musanga cercropioides*. *West African Journal of Pharmacology and Drug Research*, 19(2): 37-41.
- Ayoola, G. A., Folawewo, A. D., Adesegun, S. A., Abioro, O. O., Adepoju-Bello, A. A. and Coker, H.A.B. (2008). Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. *African Journal of Plant Science*, 2(9):124-128.
- Badarinath, A., Mallikarjuna, V. K., Madhu, C. R., Sudhana, C.C., Ramkanth, S., Rajan, T.V.S. and K.Gnanaprakash, K. (2010). A Review on *In-vitro* Antioxidant Methods: Comparisons, Correlations and Considerations. *International Journal of Pharmtech Research*. 2(2): 1276-1285.
- Benzie, I.F.F., and Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *Analytical Biochemistry*, 239: 70-76.
- Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *Lebensmittel Wissenschaft und Technologie*, 28:25-30.
- Cicco, N, M.T. Lanorte, M. Paraggio, M. Viggiano, and Lattanzio, V. (2009). A reproducible, rapid and inexpensive Folin-Ciocalteu micromethod in determining phenolics of plant methanol extracts. *Microchemical Journal*. 91: 107 – 110.
- Cekic, D., Cetinkaya, A., Avan, A. and Apak, R. (2013). Correlation of total antioxidant capacity with reactive oxygen species (ROS) consumption measured by oxidative conversion. *Journal of Agriculture and Food Chemistry*, 61: 5260–5270.
- Dongmo, A.B., Kamanyi, A. and Bopelet, M (1996). Saponins from the leaves of *Musanga cercropioides* (Cecropiaceae) constitute a possible source of potent hypotensive principles. *Phytotherapy. Research*, 10: 23–27.
- Doughari, J.H. (2012). Phytochemicals: Extraction methods, basic structures and mode of action as potential chemotherapeutic agents. In: V Rao (Ed). *Phytochemicals - A Global Perspective of Their Role in Nutrition and Health*. Croatia: In Tech Europe. Pp. 1 – 32.
- Ghasemi, K., Ghasemi, Y. and Ebrahimzadeh, M.A. (2009). Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. *Pakistan Journal of Pharmaceutical Science*, 22(3): 277-281.
- Govindappa, M., Bharath N., Shruthi H. B., Sadananda, T. S and Sharanappa, P (2011). Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity and phytochemical screening of *Crotalaria pallida* Aiton. *African Journal of Pharmacy and Pharmacology*. 5(21): 2359-2371.
- Gu, L., Kelm, M.A., Hammerstone, J.F., Beecher, G., Holden, J., Haytowitz, D., Gebhardt, S. and Prior, R.L. (2003). Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *The Journal of Nutrition*, 134: 613 – 617.
- Halliwell, B. (1991) Reactive oxygen species in living systems: Source, biochemistry, and role in human disease. *The American Journal of Medicine*, 91: S14–S22.
- Harbone, J.B. (1998). *Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis*. 3rd Ed. New York: Chapman and Hall. Pp. 149-188.
- Hegazy, A.E. and Ibrahim, M.I. (2012). Antioxidant activities of orange peel extracts. *World Applied Sciences Journal*, 18(5): 684-688.
- Kamanyi, A., Bopelet M., Aloamaka, C.P., Obeifuna, P.C.M and Ebeigbe A.B. (1991). Endothelium – dependent rat aortic relaxation to the aqueous leaf extract of *Musanga cercropioides*. *Journal of Ethnopharmacology*. 34: 283-286.

- Kamanyi, A., Bopelet, M. and Tatchum, T.R (1992). Contractile effects of some extracts from the leaves of *Musanga cercropioides* (Cecropiaceae) on uterine smooth muscle of the rat. *Phytotherapy Research*, 6:165-167.
- Kamanyi, A., Bopelete M., Lontsi, D. and Noamesi, B. K. (1996). Hypotensive effects of some extracts of *Musanga cercropioides* R. Brown (Cecropiaceae) studies in the cat and the rat. *Phytomedicine*. 2: 209-212.
- Kamanyi A, Watcho A, Wansi S, Nguetefack T, and Nguetigum, T F (2000). Further studies on *Musanga cercropioides* Cecropiaceae). A possible source of active antiglycaemic principles. *Phytomedicine*. 7(S2): 4.
- Kasangana, P.B., Haddad, P.S. and Stevanovic, T. (2015). Study of Polyphenol content and Antioxidant capacity of *Myrianthus arboreus* (Cecropiaceae) Root bark. *Antioxidants*, 4: 410-426
- Katalinic, V., M. Milos, T. Kulisic and M. Jukic. (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. *Food Chemistry*. 94: 550–557.
- Lai, L.S., Chou, S.T and Chao, W. W. (2001). Studies on the antioxidant of Hsian-tsoa (*Mesona procumbens* Heml) leaf gum. *Journal of Agriculture and Food Chemistry*, 49: 963-968.
- Lopez-Alarcon, C. and Denicola, A. (2013). Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays. *Analytica Chimica Acta*, 763, 1–10.
- Martin-Sanchez, A., Cherif, S., Ben-Abda, J., Barber-Valles, X., Perez-Alvarez, J. and Sayas-Barbera, E. (2014). Phytochemicals in date co-products and their antioxidant activity. *Food Chemistry*, 158, 513–520.
- Miliauskas, G., Vensketonis, P.R. and Van Beck, T.A. (2004). Screening of radical scavenging of some medicinal and aromatic plant extracts. *Food Chemistry*, 85: 231 – 237.
- Ogbuagu, M.N. and Agu, B. (2008). Fruit nutritive composition of *Maesobotrya barteri*, an underexploited tropical African tree. *Fruits*, 63: 357-361.
- Ohkowa, H., Ohisi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Analytical Biochemistry*, 95: 351-358.
- Oikeh, E.I., Oriakhi, K. and Omoregie, E.S. (2013). Proximate analysis and phytochemical screening of *Citrus sinensis* fruit wastes. *The Bioscientist*, 1(2):164-170.
- Oikeh, E.I, Oriakhi, K. and Omoregie, E.S. (2014). Phenolic content and *in vitro* antioxidant activities of sweet orange (*Citrus sinensis* L.) fruit wastes. *Archive of Basic and Applied Medicine*, 2(1) 119 – 126.
- Ojemekole O, Irabor, F., Ebohon, O. and Omoregie, E.S. (2017). A Comparative Study on the Phytochemical Screening and *in vitro* Antioxidant Activity of Methanol Leaf Extracts of *Chrysophyllum albidum* and *Irvingia gabonensis*. *Saudi Journal of Life Sciences*, 2(3):58-64.
- Omoriege, E.S. and Oikeh, E.I. (2015). Comparative studies on the phytochemical composition, phenolic content and antioxidant activities of methanol leaf extracts of *Spondias mombin* and *Polyathia longifolia*. *Jordan Journal of Biological Science*, 8(2): 145-149.
- Omoriege, E.S., Oriakhi, K., Oikeh, E.I., Okugbo, O.T. and Akpobire, D. (2014). Comparative study of phenolic content and antioxidant activity of leaf extracts of *Alstonia boonei* and *Eupatorium odoratum*. *Nigerian Journal of Basic and Applied Sciences*, 22(3-4): 91-97.
- Oriakhi, K., Oikeh, E.I., Ezeugwu, N., Anoliefo, O., Aguebor, O. and Omoriege, E.S. (2014). *In vitro* antioxidant activities of extracts of *Vernonia amygdalina* and *Ocimum gratissimum* leaves. *Journal of Pharmacy and Bioresources*, 11 (2): 58-65.
- Polshettiwar, S. A., Ganjwale, R. O. Wadher, S. J and Yeole, P.G. (2007). Spectrophotometric estimation of total tannins in some Ayurvedic eye drops. *Indian Journal of Pharmaceutical Sciences*, 547-577.
- Prakash, D. and Gupta, K.R. (2009). The antioxidant phytochemicals of nutraceutical importance. *Open Nutraceuticals Journal*, 2:20-35.
- Rahman, M.A., T.B. Imran and S. Islam. (2013). Antioxidative, antimicrobial and cytotoxic effects of the phenolics of *Leea indica* leaf extract. *Saudi Journal Biological Sciences*, 20: 213–225.
- Royer, M., Diouf, P. and Stevanovic, T. (2011). Polyphenols contents and radical scavenging capacities of red maple (*Acer rubrum* L.) extracts. *Food and Chemical Toxicology*, 49: 2180–2188.
- Saurai, P. (2015). Silymarin as a natural antioxidant: An overview of the current evidence and perspectives. *Antioxidants* 4: 204–247.
- Sofowora A. (1993). Medicinal Plants and Traditional Medicine in Africa, 2nd ed. Ibadan, Spectrum Books Limited. Pp. 134–156.
- Sowemimo A., Okwuchuku E, Samuel F. M., Ayoola O., and Mutiat I. (2015). *Musanga cercropioides* leaf extract exhibits anti-inflammatory and anti-nociceptive activities in animal models. *Revista Brasileira de Farmacognosia* 25: 506–512.
- Stevanovic, T., Diouf, N. and Garcia-Perez, M. (2009). Bioactive polyphenols from healthy diets and forest biomass. *Current Nutrition and Food Science*, 5: 264–295.

- Sun, B., Ricardo-daSilva, J. M., and Spranger, I. (1998). Critical factors of Vanillin assay for catechins and proanthocyanidins. *Journal of Agriculture and Food Chemistry*, 46: 4267-4274.
- Trease, G. E. and Evans, W.C. (1989). *A Textbook of Pharmacognosy*, 13th ed. London: BailliereTyndall Ltd. Pp. 345- 356.
- Uwah, A.F., Otitoju, O., Ndem, J.I. and Peter, A.I., (2013). Chemical composition and antimicrobial activities of adventitious root sap of *Musanga cecropioides*. *Der Pharmacia Lettre* 5, 13–16.
- Uyoh, E. A., Chukwurah, P. N., David, I. A., and Bassey, A. C. (2013). Evaluation of Antioxidant Capacity of Two *Ocimum* Species Consumed Locally as Spices in Nigeria as a Justification for Increased Domestication. *American Journal of Plant Science*, 4: 222-230.