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In Vitro Antioxidant Properties of Methanolic Leaf Extract of *Vernonia Amygdalina Del*

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Summary: Various methods employed in evaluating antioxidant activities of various samples gives varying results depending on the specificity of the free radical or oxidant used as a reactant. This study investigated the antioxidant /radical scavenging properties of the methanolic extract of *Vernonia amygdalina* (MEVA) leaves and studied the relationship between the assay methods. Antioxidant capacity of MEVA was evaluated by measuring the radical scavenging activity (RSA) of MEVA on 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\bullet}$), nitric oxide (NO) and hydrogen peroxide (HP), hydroxyl radical ($OH^{\bullet}$) scavenging activity (HRSA), lipid peroxidation inhibition activity (LPIA) against 2,2,-azobis(2-amidinopropane) hydrochloride (AAPH) and Trolox Equivalent Antioxidant Capacity (TEAC) of MEVA against 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{+}$) radicals as well as the reducing power (RP). Assay methods were subjected to regression analysis and their correlation coefficients calculated. Results were analysed using student's *t*-test and ANOVA. MEVA exhibited highest percentage RSA of 85.8% on HP, followed by $DPPH^{\bullet}$ (29.6%), $OH^{\bullet}$ (26.4%) and least on $NO^{\bullet}$ (21.8%). MEVA inhibited AAPH-induced lipid peroxidation by 30.0% and ABTS-induced radical by 1489% with a marked RP of 0.242±0.01. DPPH correlated excellently with RP (r^2 = 0.86), TEAC (r^2 = 0.94) and HRSA (r^2 = 0.89), the four having good relationship with each other, while LPIA correlated moderately with HP (r^2 = 0.48 and NO (r^2 = 0.34). MEVA exhibited significant free radical scavenging and antioxidant activities. The assay methods correlates very well and could therefore be employed for investigating and understanding antioxidant properties and scavenging activities of plant materials.

Keywords*:* Antioxidants, assay methods, correlation coefficient, free radicals, radical scavenging activity, *Vernonia amygdalina*

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INTRODUCTION

Free radicals and reactive species are widely believed to contribute to the development of several diseases by causing oxidative stress and ultimately oxidative damage (Halliwell, 2007; Ll-Bahr, 2013; Gan and Johnson, 2013; Aruoma *et al*., 2014; Wong and Duce, 2014; Vitetta and Linnane, 2014) which has been implicated in many pathological diseases (Lobo *et al*., 2010; Aprioku, 2013; Bhattacharyya *et al*., 2014; Urrutia *et al*., 2014).

Many years back, Halliwell (2001), alongside with other scientists (Liu *et al*., 2002a; Liu *et al*.,2003) came up with the fact that if oxidative damage could be found to be responsible for the ever increasing incidence of various pathological conditions, then timely actions that could decrease or prevent its occurrence would be therapeutically beneficial. It was then suggested that successful antioxidant treatment should be employed in the delay or prevention of onset of diseases induced by oxidative damage (Halliwell 2002a; 2002b; Galli *et al*., 2002; Steinberg and Witztum, 2002). Since then, knowledge regarding the chemical nature and mechanism of action of antioxidants, especially endogenous antioxidant and their important roles in disease prevention and treatment has been rapidly increasing (Neergheen *et al*., 2006; Halliwell, 2011; 2012; 2013; Choudhari et al., 2014). In view of this, much attention has been focused on the protective biochemical functions of naturally occurring antioxidants in biological systems, and the mechanisms of their actions.

The phenolic compounds, which are widely distributed in plants, were considered to play a very important role as dietary antioxidant component for the prevention of oxidative damage in living systems (Perron *et al*., 2008; Perron and Brumaghim, 2009; Albarracin *et al*., 2012; Aboul-Enein *et al*., 2013; Gao *et al*., 2014). Flavonoids are a large group of polyphenolic compounds abundantly present in

human diet (Gonzalez *et al*., 2011), first identified as plant pigment but later recognised as very potent antioxidant and immunomodulators (Middleton *et al*., 2000; Krifa *et al*., 2013). Flavonoids have been referred to as 'nature's biological modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergens, viruses and carcinogens. They show anti-allergic, anti-inflammatory, antimicrobial and anti-cancer activities (Yamamoto and Gaynor, 2001) which are attributable to the phenolic hydroxyl groups attached to the flavonoid structure (Divakaran *et al*., 2013; Pereira *et al*., 2013). In recent times, flavonoids have attracted tremendous interest as possible therapeutics against free radical mediated diseases (Middleton *et al*., 2000; Ishizawa, 2011; Grassi *et al*., 2013; Kamel *et al*., 2014; Peng *et al*., 2014; Liang *et al*., 2014).

Vernonia amygdalina (*Astereacea*) is a shrub or small tree of between 1 and 5m in height which grows throughout tropical Africa. The plant commonly known as bitter leaf due to the bitterness of its leaves is well cultivated and is common market merchandise in some African countries like Nigeria, Cameroon, Ethiopia and Zimbabwe. All parts of the plant have been found to be pharmacologically useful. In Nigeria, leaves of the plant are used as a green vegetable or spice in the popular bitter-leaf soup. The leaves could also be macerated and the water extract taken as appetizer or digestive tonic (Singha, 1972; Igile *et al*., 1995; Adesanoye and Farombi, 2010; Momoh *et al*., 2012). Many herbalists and naturopathic Doctors have recommended the aqueous extract for the treatment of ailments like diabetes, dysentery, gastrointestinal problems, nausea and appetite-induced abrosia. The root and the leaves are also used in ethnomedicine to treat fever, hiccups, kidney problems and stomach discomfort (Burkill, 1985; Hamowia and Saffaf, 1994; Ojiako and Nwanjo, 2006; Adesanoye *et al*., 2013; Yedjou *et al*., 2013). Various phytoconstituents have been discovered and isolated from *Vernonia amygdalina* (Farombi and Owoeye, 2011; Ijeh and Ejike, 2011; Toyang and Verpoorte , 2013). This study was designed to evaluate the antioxidant and radical scavenging activities of methanolic extract of *Vernonia amygdalina* (MEVA) *in vitro* and to study the correlation between the methods employed.

MATERIALS AND METHODS

Plant material

The leaves of *Vernonia amygdalina* were obtained from gardens of Forestry Research Institute of Nigeria (FRIN), Ibadan and were authenticated at the herbarium of the Institute. The powdered leaves were packed into soxhlet extractor and defatted with n-hexane. The defatted leaves was extracted in absolute methanol and the methanolic fraction evaporated in water bath at 40 - 50 ^{0}C to obtain a concentrated extract from which stock solutions were prepared.

Reducing power of MEVA

Reducing power of MEVA was determined according to the method of Oyiazu, (1986). Graded amounts of the extract (10-800 µg) in 1 ml of distilled water were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50^{o}C for 20 minutes. A portion (2.5 ml) of 10% TCA was added to the mixture which was then centrifuged at 3000 rpm for 10 minutes. 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% $FeCl_3$ and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

Scavenging effect of MEVA on Hydrogen peroxide

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch *et al*., (1989). Extracts doses in 4 ml distilled water (20-400µg) were added to hydrogen peroxide solution (0.6 ml). Absorption of hydrogen peroxide was determined 10 minutes later against a blank solution containing extract in phosphate buffer without hydrogen peroxide. Hydrogen peroxide concentration was determined spectrophotometrically from absorbance at 230 nm using the molar absorptivity of $81M^{-1}cm^{-1}$ (Beers and Sizer, 1952).

Determination of the effect of MEVA on DPPH radical

Effect of MEVA on 1,1-dipheny-2-picrylhydrazyl ($DPPH^{\cdot}$) radical was estimated according to the method of Hatano *et al*.,(1988). MEVA (25-500 µg) in 4 ml of distilled water was added to a methanolic solution of DPPH (1 mM, 1 ml). The mixture was shaken and left to stand at room temperature for 30 minutes. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Catechin (50 µg) was used as standard. The radical scavenging activity (RSA) was calculated as percentage of $DPPH^{\cdot}$ discolouration using the equation: % RSA = 100 x $(1-A_E/A_D)$ where A_E is the absorbance of the solution with extract, and A_D is the absorbance of the $DPPH^{\cdot}$ solution without extract.

Trolox equivalent antioxidant capacity (TEAC) of MEVA

TEAC of MEVA was carried out using an improved 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) decolourization assay (Re *et al*., 1999) as described by Neergheen *et al*., (2006). The $ABTS^+$ radical was generated by a reaction between ABTS (0.5 mM) and 1 mM potassium persulfate each in 100 ml of 0.1 M phosphate buffer. To 3 ml of the $ABTS^+$ solution, 0.5 ml of the extract was added and the decay in absorbance was followed for 6 minutes at 734 nm. Trolox was used as a

reference standard and TEAC values were expressed as µmol trolox equivalent.

Effect of MEVA on 2,2'-azobis (2-amidinopropane) hydrochloride (AAPH)-induced lipid peroxidation

The effect of MEVA on AAPH-induced lipid peroxidation was carried out according to the method described by Neergheen *et al*., (2006). An aliquot of 200 µl of post-mitochondrial fraction (PMF) of liver homogenate was diluted in 0.1 M potassium phosphate buffer, pH 7.5 (1 in 10 dilution). Then, 400 µl of extract (100-1000 µg) was added followed by 200 µl of AAPH (20 mM) to initiate peroxidation. The mixture was incubated at 37^{o}C for 1 hour with the solution gently shaken at 10 minutes interval. After incubation, 1.6 ml TCA-TBA-HCl stock solution (15% w/v TCA, 0.375% w/v TBA, 0.25 N HCl) was added to the solution and heated in a boiling water bath for 15 minutes. After cooling, the precipitate was removed by centrifugation and the absorbance of the resulting supernatant measured at 532 nm. Results were expressed as percentage inhibition of peroxidation with catechin used as standard.

Deoxyribose assay – hydroxyl radical scavenging activity of MEVA

The hydroxyl radical ($OH^{\cdot}$) scavenging potential of MEVA was determined using the deoxyribose assay (Halliwell *et al*., 1987; Aruoma, 1994a; 1994b) as described by Neergheen *et al*., 2006. About 200-1000 µg of extract in 100 µl of distilled water was added to a solution containing 200 µl KH_2PO_4 – KOH (100 mM), 200 µl deoxyribose (15 mM), 200 µl $FeCl_3$ (500 µM) and 100 µl EDTA (1 mM) in a test tube and allowed to mix. Then, 100 µl H_2O_2 (10 mM) and 100 µl ascorbic acid (1 mM) were added to initiate the reaction. The reaction mixture was incubated at 37^{o}C for 1 hour after which 1 ml of 1% $^{w}/_{v}$ TBA was added to each mixture followed by the addition of 1ml of 2.8% w/v TCA. The solution was heated in a water bath at 80^{o}C for 20 minutes to develop the pink coloured MDA-$(TBA)_2$ adduct. After cooling, the solution was centrifuged and the absorbance of the supernatant measured at 532 nm against distilled water as blank. Results were expressed as the percentage inhibition of deoxyribose degradation. % inhibition = (Abs control – Abs test/ Abs control) ×100.

Scavenging of nitric oxide radical by MEVA

The scavenging effect of MEVA on nitric oxide ($NO^{\cdot}$) radical was measured according to the method of Marcocci *et al*., 1994. About 10-400 µg of MEVA was added in the test tubes to 1 ml of sodium nitroprusside solution (25 mM) and the tubes incubated at 37^{o}C for 2 hours. An aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml of Griess reagent (1% sulphanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was immediately read at 570 nm against distilled water as blank with catechin (50 µg) used as standard. Results were expressed as percentage radical scavenging activity (%RSA) = (1 – ΔAbs of sample/ΔAbs of control) x 100.

RESULTS

The antioxidant ability of methanolic extract of *Vernonia amygdalina* (MEVA) to inhibit, scavenge and quench free radicals and reactive oxygen species or ameliorate their effects was examined in this study. Effect of MEVA on oxidative stress biomarkers was investigated using different method.

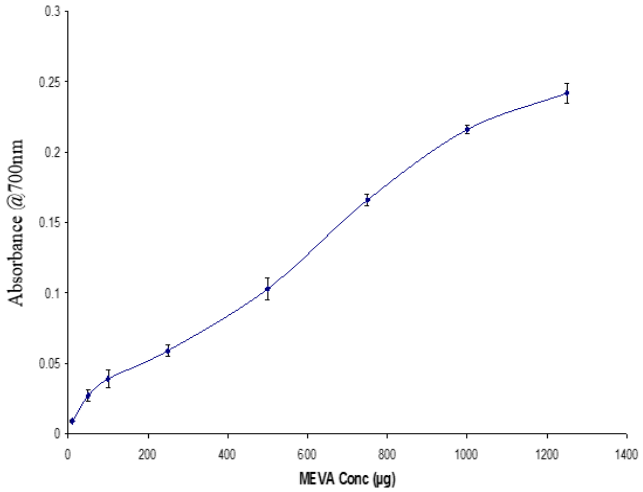

Figure 1: Reducing power of methanolic extract of *Vernonia amygdalina* (MEVA) using the Fe^{3+}/ferricyanide method. Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates.*p<0.05when compared with Control.

Table 1: Radical Scavenging activity (RSA) of methanolic extract of *Vernonia amygdalina* (MEVA) on $DPPH^{\cdot}$ radical

Concentration (µg)	Absorbance	% RSA
Control	1.699± 0.001	-
MEVA 40	1.371±0.060*	19.3
100	1.299±0.004*	23.5
250	1.284±0.016*	24.4
500	1.263±0.003*	25.7
750	1.241±0.032*	27.0
1000	1.196±0.037*	29.6
1500	1.206±0.018*	29.0
2000	1.212±0.013*	28.7
Catechin 50	1.216±0.029*	25.8
100	1.246±0.006*	26.7

Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates. *p<0.05 when compared with control.

The reducing power (RP) of MEVA is shown in Figure 1. MEVA showed significant (p<0.05) reducing power as indicated by the increase in absorbance value from 0.009±0.001 – 0.242±0.007 at

10 – 1250 µg. Table 1 shows the radical scavenging activity (RSA) of MEVA against DPPH$^{\bullet}$ radical exhibiting maximum RSA of 29.6% on DPPH$^{\bullet}$ radical ($p<0.05$) at 1000 µg. Possible inhibitory effect of MEVA on hydrogen peroxide (HP) is displayed in Table 2. MEVA showed highest percentage inhibition of 85.8% on HP at 20 µg (0.102±0.007) thereafter reducing to 24.2% (0.545± 0.021) at 500 µg. At 50 µg, MEVA (84.8%) compares very well with catechin (83.9%), showing similar scavenging activity. The antioxidant capacity of MEVA against ABTS^{+} radical was investigated in the TEAC (trolox equivalent antioxidant capacity) system as presented in Figure 2.

Table 2: Radical scavenging activity (RSA) of methanolic extract of *Vernonia amygdalina* (MEVA) on Hydrogen peroxides.

Concentration (µg)	Absorbance	% Inhibition
Control	0.719±0.007	-
MEVA 20	0.102±0.007*	85.8
50	0.109±0.008*	84.8
100	0.154±0.003*	78.6
200	0.237±0.006*	67.0
300	0.330±0.006*	54.1
400	0.447±0.004*	37.8
500	0.545±0.021*	24.2
Catechin 50	0.116±0.005*	83.9
100	0.161±0.006*	77.6

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

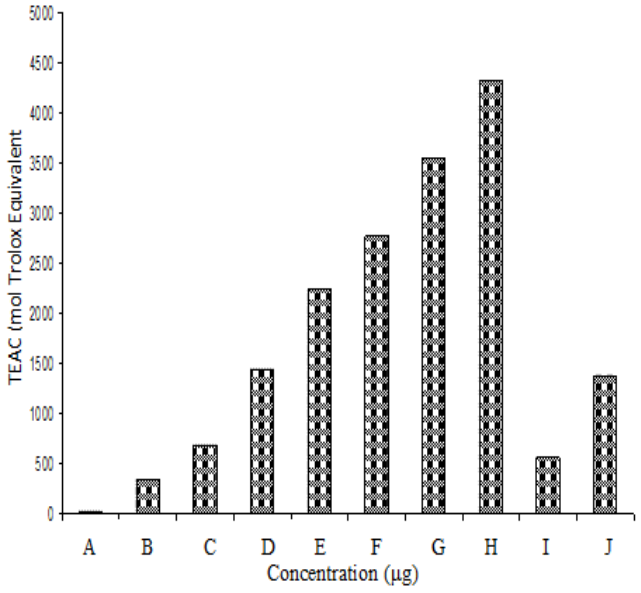

Figure 2: Trolox equivalent antioxidant capacity (TEAC) of methanolic extract of *Vernonia amygdalina* (MEVA). Absorbance values expressed as mean ± standard error of mean (SEM) of three replicates. A=Control, B=20µg MEVA, C=50µg MEVA, D=100µg MEVA, E=200µg MEVA, F=400µg MEVA, G=600µg MEVA, H=1000µg MEVA, I= 50µg catechin, J=100µg catechin.

Table 3: Scavenging effect of methanolic extract of *Vernonia amygdalina* (MEVA) on AAPH-induced lipid peroxidation

Concentration (µg)	Absorbance	% Inhibition
Control	0.087±0.004	-
MEVA 50	0.078± 0.004	10.3
100	0.062±0.003*	28.7
200	0.061±0.0005*	30.0
400	0.073± 0.0001*	16.1
800	0.076±0.001*	12.6
1000	0.081±0.001	6.9
Catechin 50	0.078±0.004	10.3
100	0.058±0.004*	33.3

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Table 4: Potential of methanolic extract of *Vernonia amygdalina* (MEVA) to inhibit deoxyribose degradation by Hydroxyl radical

Concentration (µg)	Absorbance	% Inhibition
Control	0.738±0.006	-
MEVA 50	0.607±0.023*	17.8
100	0.598±0.017*	19.0
200	0.562±0.028*	23.8
400	0.559±0.008*	24.3
600	0.543±0.021*	26.4
800	0.545±0.008*	26.2
1000	0.553±0.008*	25.1
Catechin 50	0.665± 0.015*	9.9
100	0.652±0.002*	11.7

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Table 5: Scavenging effect of methanolic extract of *Vernonia amygdalina* (MEVA) on Nitric Oxide radical

Concentration (µg)	Absorbance	% Inhibition
Control	0.229±0.01	-
MEVA 20	0.179± 0.003*	21.8
50	0.191±0.006*	16.9
100	0.211±0.002*	7.9
200	0.251± 0.002*	-9.6
300	0.287±0.0003*	-25.3
400	0.334±0.007*	-45.9
Catechin 50	0.192±0.001*	16.2
100	0.197±0.001*	14.0

Absorbance values expressed as mean ± SEM of three replicates. *p<0.05 when compared with control.

Likewise, MEVA was able to significantly ($p<0.05$) inhibit AAPH-induced lipid peroxidation by 28.7%, 30.0%, 16.1% and 12.6% at 100, 200, 400 and 800 µg respectively when compared with control (Table 3). Catechin showed greater ability at 100 µg by 33.3% when compared with MEVA (28.7%) at the same concentration. MEVA significantly ($p<0.05$) inhibited OH$^{\bullet}$-induced deoxyribose degradation from 50 – 1000 µg with the highest potency at 600 µg by 26.4% when compared with control as shown in Table 4. Similarly, MEVA exhibited scavenging effect on nitric oxide radical (NO$^{\bullet}$) but at relatively low concentration (Table 5).

Table 6: Correlation Coefficient between Assay methods

Assay methods	**Correlation coefficients (r^2)**
DPPH/RP	0.86
DPPH/LPIA	-0.25
DPPH/TEAC	0.94
DPPH/HP	-0.89
DPPH/HRSA	0.89
DPPH/NO	-0.93
RP/LPIA	-0.57
RP/TEAC	0.97
RP/HP	-0.99
RP/HRSA	0.71
RP/NO	-0.98
LPIA/TEAC	-0.50
LPIA/HP	0.63
LPIA/HRSA	-0.28
LPIA/NO	0.59
TEAC/HP	-0.98
TEAC/HRSA	0.85
TEAC/NO	-0.98
HP/HRSA	-0.76
HP/NO	0.99
HRSA/NO	-0.88

- Negative correlation coefficients

Table 7: Summary of the relationships between Assay methods

DPPH:	RP, TEAC, HRSA
RP:	DPPH, TEAC, HRSA
TEAC:	DPPH, RP, HRSA
HRSA:	DPPH, RP, TEAC
LPIA:	HP, NO
HP:	NO, LPIA
NO:	LPIA, HP

Very strong linear relationships (R^2) were observed between DPPH, RP, TEAC and HRSA while medium to low relationship was observed between LPIA, NO and HP. The level of correlation coefficients between the assays methods are shown in Table 6 while Table 7 summarised the relationship between the various methods.

DISCUSSION

Phytochemicals are diverse and complex in nature. As such, the antioxidant activities of plant extracts cannot be evaluated by only a single method. Different antioxidant components are resident in the antioxidant defence system of the body and the antioxidant capacity of these antioxidant components depends to a great extent on which free radical or oxidants are produced in the system (Choi *et al*., 2002; Akinmoladun *et al*., 2010; Jan *et al*., 2013; Tenore and Ciampaglia, 2013). Therefore, various methods used in evaluating the antioxidant activity of various samples can give varying results depending on the specificity of the free radical used as a reactant (Frankel and Meyer, 2001; Prakash, 2001; Aruoma, 2003; Akinmoladun *et al*., 2010; Patel *et al*., 2011; Ramasarma, 2012; Shabbir *et al*., 2013).

In the present study, MEVA was able to reduce the Fe^{3+}/ferricyanide complex to the ferrous form, Fe^{2+}, which was measured as the formation of Perl's Prussian blue colour at 700nm (Chung *et al*., 2002). MEVA at 250 µg (0.059±0.004) when compared with the reference antioxidant, catechin at 50 µg (0.051±0.003) indicates that the reducing power of MEVA is less than that of catechin, but nevertheless potent enough to function as a good electron and hydrogen-atom donor which can react with free radicals to convert them to more stable products and terminate radical chain reaction. Several studies have also reported the reducing power activity of several plants extracts (Rice-Evans *et al*., 1996; Amarowicz *et al*., 2004; Perez-Perez *et al*., 2006; Manian *et al*., 2008; Sen *et al*., 2013; Rahman *et al*., 2014), and this has been linked to the polyphenolic constituents of these extracts. The assay method used for measuring RP correlates excellently with other methods employed in this study, especially, DPPH, TEAC and HRSA at correlation coefficients (r^2) of 0.86, 0.97 and 0.71 respectively and as such is an excellent method for measuring the reductive ability of antioxidants. Amarowicz *et al*., (2004) in their findings also confirmed that there is a good relationship between RP and DPPH (Sen *et al*., 2013; Das *et al*., 2014).

DPPH radical has been used extensively as a free radical to test the reductive ability of extracts or compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts and foods (Porto *et al*., 2000; Manian *et al*., 2008). Antioxidants react with $DPPH^{\bullet}$ by providing electron or hydrogen atom, thus reducing it to 1,1-diphenyl-2-hydrazine (DPPH-H) or a substituted analogous hydrazine. The deep violet colour of DPPH at maximum absorption of 517 nm is changed to light yellow, colourless or bleached product, resulting in decrease in absorption (Miliauskas *et al*., 2004; Koksal *et al*., 2009). MEVA exhibiting significant scavenging effect on DPPH radical between 40 – 1000 µg (19.3% - 29.6%) compares well with catechin though showing less scavenging activity. Likewise, the assay method of DPPH scavenging activity correlates excellently with RP, TEAC and HRSA with correlation coefficients (r^2) of 0.86, 0.94 and 0.89 respectively. The scavenging effect of plant extracts on DPPH has been shown to be related to the phenolic concentration of the extracts (Manian *et al*., 2008; Akinmoladun *et al*., 2010; Sen *et al*., 2013; Das *et al*., 2014), which is believed to contribute to their electron transfer / hydrogen donating ability. It could therefore be suggested that MEVA contains flavonoids with hydroxyl groups that could stabilize free radicals or scavenge their activities and that the method employed is reliable.

The activity of hydrogen peroxide (HP) as an active oxygen species has been reported to come mainly from its potential to produce the highly reactive hydroxyl radical through the fenton reaction: $Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\bullet}$ (Namiki, 1990; Farombi *et al*., 2002; Koksal *et al*., 2009; Jeong *et al*., 2010; Ogasawara *et al*., 2014). Therefore, inhibition of HP formation will prevent further generation of radicals. In the present study, MEVA showed highest percentage inhibition of 85.8% on HP at 20 µg (0.102±0.007) thereafter reducing to 24.2% (0.545± 0.021) at 500 µg. At 50 µg, MEVA (84.8%) compares very well with catechin (83.9%), showing similar scavenging activity. The regression analysis of HP versus other methods in the study revealed a very good relationship between HP scavenging activity and nitric oxide scavenging activity (NO) with a correlation coefficient of 0.99, and a moderate relationship with lipid peroxidation inhibition activity with correlation coefficient of 0.35. Although HP on its own is not very reactive, but it could still be toxic both *in vitro* and *in vivo*; it could induce cell death *in vitro* and attack many cellular energy-producing systems *in vivo*. For instance, it has been reported that HP could deactivate the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (Hyslop *et al*., 1998; Koksal *et al*., 2009; Song *et al*., 2014).

$ABTS^+$ radical was generated by oxidation of ABTS with potassium persulfate (Re *et al*., 1999). MEVA increased TEAC values in a dose dependant manner from 50–600 µg with 339.4±155.3 – 4320.0±33.6 trolox equivalent and shows almost equal potency with catechin at 50 µg. $ABTS^+$, a more reactive radical (more reactive than $DPPH^{\bullet}$) is stabilized by antioxidants that can supply electrons, unlike H atom in $DPPH^{\bullet}$. MEVA was thus able to scavenge $ABTS^+$ radical-induced peroxodisulfate formation by electron transfer. The regression analysis for the assay methods employed in this study reveals that measurement of $ABTS^+$ scavenging activity as Trolox equivalent antioxidant capacity (TEAC) compares very well with other methods in showing strong linear correlation with DPPH, RP and HRSA with correlation coefficients (r^2) of 0.94, 0.97 and 0.85 respectively. This indicates that TEAC is a reliable method for measuring the total antioxidant activities of substances as reported by previous works (Koksal *et al*., 2009; Piljac-Zegarac *et al*., 2009; Chohan *et al*., 2012). Likewise, the report of Sasikumar *et al*., (2010) supported the relationship between TEAC and DPPH.

Lipid peroxidation is one of the consequent actions of ROS and free radicals. In this study, lipid peroxidation was induced with AAPH, a peroxy radical initiator. Thermal decomposition of AAPH produces peroxy radical which can attack polyunsaturated lipids initiating lipid peroxidation (Neergheen *et al*., 2006). MEVA was able to significantly ($p<0.05$) inhibit AAPH-induced lipid peroxidation by 28.7%, 30.0%, 16.1% and 12.6% at 100, 200, 400 and 800 µg respectively when compared with control. Catechin showed greater ability at 100 µg by 33.3% when compared with MEVA (28.7%) at the same concentration.

The decreasing lipid peroxidation product measured as thiobarbituric reactive substances (TBARS) showed the ability of MEVA to inhibit AAPH-induced lipid peroxidation by 10.3% – 30.0%. LPIA (Lipid peroxidation inhibitory activity of MEVA) correlates fairly well with HP and NO; the other methods employed in this study, with correlation coefficients (r^2) of 0.63 and 0.59 respectively and as such, LPIA is another reliable method of measuring the scavenging and inhibitory ability of antioxidants. In the report of Akinmoladun *et al*., (2010), very low to fair correlations were observed between LPIA and other methods, which is similar to what was observed in this study.

Furthermore, the antioxidant activity of MEVA was demonstrated against hydroxyl radical ($OH^{\bullet}$). $OH^{\bullet}$ has been reported to be the most reactive radical known; it attacks and damages almost every molecule it makes contact with (Halliwell, 1989; Aruoma, 1999; Manian *et al*., 2008; Ramasarma, 2012). $OH^{\bullet}$ rapidly reacts with any molecule, forming another radical species, thereby giving rise to chain peroxide formation. The deoxyribose method employed in this study is a simple assay to determine the rate constants of hydroxyl radical formation as reported by Halliwell *et al*., (1987). $OH^{\bullet}$ radical is generated when the mixture of $FeCl_3$-EDTA, H_2O_2 and ascorbate is incubated with deoxyribose in phosphate buffer (pH 7.4). $OH^{\bullet}$ attack on deoxyribose results ultimately in chain peroxidation which could be measured as TBARS. MEVA exhibited 17.8 – 26.4% inhibition of deoxyribose oxidation by $OH^{\bullet}$. The ability of MEVA to inhibit $OH^{\bullet}$ by preventing the oxidation of deoxyribose in the current *in vitro* model could be related to prevention of propagation of the process of lipid peroxidation *in vivo* as has been shown in our studies with CCl_4 (Adesanoye and Farombi, 2010). In this vein, Farombi *et al*., (2002) reported that the overall antioxidant effect of flavonoids on lipid peroxidation is the result of scavenging hydroxyl radicals and superoxide anions at the stage of initiation and termination of peroxyl radicals according to Hussain *et al*., (1987). In addition, MEVA showed better inhibition potential of 17.8% against 9.9% for catechin at 50 µg. The regression analysis in this study revealed a very high relationship between hydroxyl radical scavenging activity (HRSA) and other methods which is in line with some previous works (Perez-Perez *et al*., 2006; Sazikumar *et al*., 2010). HRSA correlated excellently

with DPPH, RP and TEAC, with correlation coefficients (r^2) of 0.89, 0.71 and 0.85 respectively. The relationship between these four methods is excellent as has been observed and reported by various scientists (Halliwell, 1989; Amarowicz *et al*., 2004; Manian *et al*., 2008; Piljac-Zegarac *et al*., 2009; Akinmoladun *et al*., 2010).

Similarly, MEVA exhibited scavenging effect on nitric oxide radical ($NO^{\bullet}$) but at relatively low concentration. $NO^{\bullet}$ shows dual function as both an antioxidant and pro-oxidant depending on the relative ratios of the reactants (Aruoma, 1996; Groß *et al*., 2013; Meenakshi and Agarwal, 2013). Antioxidant effects of $NO^{\bullet}$ occurs when $NO^{\bullet}$ reacts with alkoxy and peroxyl radical intermediates during lipid peroxidation thereby stabilizing the inhibition of LDL oxidation while the pro-oxidant reaction occurs when $NO^{\bullet}$ reacts with $O_2^{\bullet}$ to yield peroxynitrite ($ONOO^{\bullet}$) (Zielonka et al., 2010). This dual effect could explain why the scavenging effect on $NO^{\bullet}$ decreased with increasing concentration of the extract and became pro-oxidant at concentrations greater than 100 µg. MEVA exhibited similar scavenging effect with catechin. At 50 µg, MEVA elicited 16.9% inhibition while catechin showed 16.2% inhibition potential. The ability of MEVA to scavenge $NO^{\bullet}$ and especially its deleterious metabolite, ($ONOO^{\bullet}$) will be highly beneficial in biological system as $ONOO^{\bullet}$ and some other $NO^{\bullet}$ metabolites have been implicated in various pathological conditions such as malaria, cardiovascular diseases, inflammation, cancer and diabetes (Aruoma, 1996; Groß et al., 2013; Meenakshi and Agarwal, 2013). NO correlated with LPIA and HP with correlation coefficients (r^2) of 0.59 and 0.99 respectively.

CONCLUSION

In conclusion, this study demonstrated that MEVA is a potent antioxidant and scavenger of reactive species *in vitro*. The observation concerning the diversity and complexity of the phytochemicals in plant extracts with different mechanisms of reaction for specific antioxidant species applies very well to the present study. The antioxidant capacity observed by all the methods assayed in this work appears to be reliable for measuring antioxidant and scavenging capacity of substances. From various experimental results (Dasgupta and De, 2004; Perez-Perez *et al*., 2006; Akinmoladun *et al*., 2010; Rao *et al*., 2010; Sasikumar *et al*., 2010; Patel *et al*., 2011; Ramasarma, 2012; Shabbir *et al*., 2013), a linear correlation with high correlation coefficients has been observed between total phenolic/flavonoid content and various antioxidant and free radical scavenging activities of various plants indicating that the various activities are based on the flavonoid and phenolic contents of the plant materials. It could therefore be inferred that the activities of MEVA in this study is due to its phenolic content, especially the flavonoids. Therefore, MEVA could be recommended as a potential antioxidant in ameliorating oxidative and free radical-induced pathologies.

REFERENCES

Aboul-Enein, H.Y., Berczyńsk, P. and Kruk, I. (2013). Phenolic compounds: the role of redox regulation in neurodegenerative disease and cancer. Mini Rev. Med. Chem. 13(3): 385-98.

Adesanoye, O.A., Adekunle, A.E., Adewale, O.B., Mbagwu, A.E., Delima, A.A., Adefegha, S.A., Molehin, O.R. and Farombi, E.O. (2013). Chemoprotective effect of Vernonia amygdalina Del. (Astereacea) against 2-acetylaminofluorene-induced hepatotoxicity in rats. Toxicol. And Ind. Health. 2013 Sep 10. [Epub ahead of print]

Adesanoye, O.A. and Farombi, E.O. (2010). Hepatoprotective effects of Vernonia amygdalina (astereaceae) in rats treated with carbon tetrachloride. Exp. and Toxicol. Path. 62: 197-206.

Akinmoladun, A.C., Obuotor, E.M. and Farombi, E.O. (2010). Evaluation of Antioxidant and Free radical scavenging capacities of some Nigerian Indigenous medicinal plants. J. Med. Food. 13(2): 1-8.

Albarracin, S.L., Stab, B., Casas, Z., Sutachan, J.J., Samudio, I., Gonzalez, J., Gonzalo, L., Capani, F., Morales, L. and Barreto, G.E. (2012). Effects of natural antioxidants in neurodegenerative disease. Nutr. Neurosci. 15(1): 1-9.

Amarowicz, R., Pegg, R.B., Rahimi – Moghaddam, P., Barl, B. and Weil, J.A. (2004). Free radical scavenging capacity and antioxidant activity of selected plant species from the Canadian praires. Food Chemistry. 84: 551-562.

Aprioku, J.S. (2013). Pharmacology of Free Radicals and the Impact of Reactive Oxygen Species on the Testis. J. Reprod. Infertil. 14(4): 158-172.

Aruoma, O.I. (1994a). Nutrition and health aspects of free radicals and antioxidants. Food Chem. Toxicol. 32: 671-683.

Aruoma, O.I. (1994b). Deoxyribose assay for detecting hydroxyl radicals. Methods Enzymol. 233: 57-66.

Aruoma, O.I. (1996). Characterization of drugs as antioxidant prophylactics. Free Rad. Biol. Med. 20: 6775-6705.

Aruoma, O.I. (1999). Free radicals, antioxidants and international nutrition. Asia Pacific J. Clin. Nutr. 8(1): 53-63.

Aruoma, O.I. (2003). Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutt. Res. 523-524: 9-20.

Aruoma, O.I., Somanah, J., Bourdon, E., Rondeau, P. and Bahorun, T. (2014). Diabetes as a risk factor

to cancer: Functional role of fermented papaya preparation as phytonutraceutical adjunct in the treatment of diabetes and cancer. Mutat. Res. S0027-107(14)000736.doi:10.1016/j.mrfmmm.2014.04.007. [Epub ahead of print].

Beers, R.F. and Sizer, I.W. (1952). A spectrofluorimetric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. 195: 133-140.

Bhattacharyya, A., Chattopadhyay, R., Mitra, S. and Crowe, S.E. (2014). Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. Physiol. Rev. 94(2): 329-354.

Burkill, H.M. (1985). The useful plants of West Tropical Africa. 1985;Vol 1.

Chohan, M., Naughton, D.P., Jones, L. and Opara, E.I. (2012). An investigation of the relationship between the anti-inflammatory activity, polyphenolic content, and antioxidant activities of cooked and in vitro digested culinary herbs. Oxid. Med. Cell Longev. 2012: 627843. doi: 10.1155/2012/627843. Epub 2012 May 21.

Choi, C.W., Kim, S.C., Hwang, S.S., Choi, B.K., Ahn, H.J., Lee, M.Y., Park S.H. and Kim S.K. (2002). Antioxidant and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 163: 1161-1168.

Choudhari, S.K., Chaudhary, M., Gadbail, A.R., Sharma, A. and Tekade, S. (2014). Oxidative and antioxidative mechanisms in oral cancer and precancer: a review. Oral Oncol. 50(1): 10-18.

Chung, Y-C., Chang, C-T., Chao, W-W., Lin, C-F. and Chou, S-T. (2002). Antioxidant activity and safety of the 50% ethanolic extract from red bean fermented by bacillus subtilis IMR-NKI. J. Agric. Food Chem. 50: 2454-2458.

Das, N., Islam, M.E., Jahan, N., Islam, M.S., Khan, A., Islam, M.R. and Parvin, M.S. (2014). Antioxidant activities of ethanol extracts and fractions of Crescentia cujete leaves and stem bark and the involvement of phenolic compounds. BMC Complement Altern. Med. 14: 45. doi: 10.1186/1472-6882-14-45.

Dasgupta, N. and De, B. (2004). Antioxidant activity of Piper betle L. leaf extract In vitro. Food Chem. 88: 219-224.

Divakaran, S.A., Hema, P.S., Nair, M.S. and Nair, C.K.K. (2013). Antioxidant capacity and radioprotective properties of the flavonoids galangin and Kaempferide isolated from Alpinia galangal L. (Zingiberaceae) against radiation induced cellular DNA damage. Inter. J. Radiat. Res. 11(2): 81-89.

Farombi, E.O., Akanni, O.O. and Emerole, G.O. (2002). Antioxidant and Scavenging activities of flavonoid extract (kolaviron) of Garcinia kola seeds. Pharmaceutical Biol. 40(2): 107–116.

Farombi, E.O. and Owoeye, O. (2011). Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. Int. J. Environ. Res. Public Health. 8(6): 2533-2555.

Frankel, E.N. and Meyer, A.S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J. Science of Food and Agric. 80: 1925-1941.

Galli, R.L., Shukitt-Hale, B., Youdim, K.A. and Joseph, J.A. (2002). Fruit polyphenolics and brain aging: nutritional interventions targeting age-related neuronal and behavioural defects. Ann. NY. Acad. Sci, 959: 128-132.

Gan, L. and Johnson, J.A. (2013). Oxidative damage and the Nrf2-ARE pathway in neurodegenerative diseases. Biochim. Biophys. Acta. S0925-4439(13)00366-9. doi: 10.1016/j.bbadis.2013.12.011. [Epub ahead of print]

Gao, C.Y., Tian, C.R., Zhou, R., Zhang, R.G. and Lu, Y.H. (2014). Phenolic composition, DNA damage protective activity and hepatoprotective effect of free phenolic extract from Sphallerocarpus gracilis seeds. Int. Immunopharmacol. 20(1): 238-247.

Gonzalez, R., Ballester, I., Lopez-Posadas, R., Suarez, M.D., Zarzuelo, A., Martinez-Augustin, O. and Anchez De Medina, F.S. (2011). Effects of Flavonoids and other Polyphenols on Inflammation. Critical Reviews in Food Science and Nutrition. 51: 331–362.

Grassi, D., Desideri, G., Di Giosia, P., De Feo, M., Fellini, E., Cheli, P., Ferri, L. and Ferri, C. (2013). Tea, flavonoids, and cardiovascular health: endothelial protection. Am. J. Clin. Nutr. 98(6Suppl): 1660S-1666S.

Groß, F., Durner, J. and Gaupels, F. (2013). Nitric oxide, antioxidants and prooxidants in plant defence responses. Front. Plant Sci. 4: 419.

Halliwell, B. (1989). Current Status Review: Free radicals, reactive oxygen species and human disease: a critical evaluation with special reference to atherosclerosis. Br. J. Exp. Path. 70: 737-757.

Halliwell, B. (2001). Role of Free radicals in the neurodegenerative diseases, therapeutic implications for antioxidant treatment. Drugs Aging. 18: 685-716.

Halliwell, B. (2002a). Vitamin E and the treatment and prevention of diabetes: a case for controlled clinical trial. Singapore Med. J. 43: 479-484.

Halliwell, B. (2002b). Effect of diet on cancer development: Is oxidative DNA damage a biomarker? Free Radic. Biol. Med. 32: 968-974.

Halliwell, B. (2007). Biochemistry of oxidative stress. Biochem. Soc. Trans. 35: 1147- 1150.

Halliwell, B. (2011). Free radicals and antioxidants - quo vadis? Trends Pharmacol Sci. 32(3): 125-130.

Halliwell, B. (2012). Free radicals and antioxidants: updating a personal view. Nutr. Rev. 70(5): 257-265.

Halliwell, B. (2013). The antioxidant paradox: less paradoxical now? Br. J. Clin. Pharmacol. 75(3): 637-644.

Halliwell, B., Gutteridge, J.M.C. and Aruoma, O.I. (1987). The deoxyribose method: a simple "test tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem. 165: 215-219.

Hamowia, A.M. and Saffaf, A.M. (1994). Pharmacological studies on Vernonia amygdalina (Del) and Tithonia diversifolia (Gray). Vet. Med. J. Giza. 2: 91-97.

Hatano, T., Kagawa, H, Yashuhara, T. and Okuda, T. (1988). Two new flavonoids and other constituents in licorice root: their relative astringency and radical scavenging effects. Chem. Pharm. Bul. 36: 2090-2097.

Hussain, S.R., Cillard, J. and Cillard, P. (1987). Hydroxyl radical scavenging activity of flavonoids. Phytochemistry. 26: 2489-2491.

Hyslop, P.A., Hinshaw, D.B., Hakey, W.A., Schraufstatter, I.U., Sauerheber, R.D., Spragg, R.G. and Cochrane C.G. (1988). Mechanism of oxidant-mediated cell injury. The glycolytic and mitochondrial pathways of ADP phosphorylation are major intracellular targets inactivated by hydrogen peroxide. J. Biol. Chem. 263: 1665-1675.

Igile, G.O., Oleszek, W., Burda, S. and Jurzysta, M. (1995). Nutritional assessment of *Vernonia amygdalina* leaves in growing mice. J. Agr. Food Chem. 43: 2162-2166.

Ijeh, I.I. and Ejike, C.E.C.C. (2011). Current perspectives on the medicinal potentials of Vernonia amygdalina Del. J. Med. Plant. Res. 5(7): 1051-1061.

Ishizawa, K. Drug discovery for improvement of chronic kidney disease and cardiovascular disease. Yakugaku Zasshi. 131(9): 1347-1352.

Jan, S., Khan, M.R., Rashid, U. and Bokhari, J. (2013). Assessment of antioxidant potential, total phenolics and flavonoids of different solvent fractions of monotheca buxifolia fruit. Osong Public Health Res. Perspect. 4(5): 246-254.

Jeong, J.B., De Lumen, B.O. and Jeong, H.J. (2010). Lunasin peptide purified from Solanum nigrum L. protects DNA from oxidative damage by suppressing the generation of hydroxyl radical via blocking fenton reaction. Cancer Lett. 293(1): 58-64.

Kamel, K.M., Abd El-Raouf, O.M., Metwally, S.A., Abd El-Latif, H.A. and El-Sayed, M.E. (2014). Hesperidin and Rutin, Antioxidant Citrus Flavonoids, Attenuate Cisplatin-Induced Nephrotoxicity in Rats. J. Biochem. Mol. Toxicol. May 13. doi: 10.1002/jbt.21567. [Epub ahead of print]

Koksal, E., Gulcin, I., Beyza, S., Sarikaya, O. and Bursal, E. (2009). In vitro antioxidant activity of silymarin. J. Enz. Inh. Med. Chem. 24(2): 395-404.

Krifa, M., Bouhlel, I., Ghedira-Chekir, L. and Ghedira, K. (2013). Immunomodulatory and cellular anti-oxidant activities of an aqueous extract of Limoniastrum guyonianum gall. J. Ethnopharmacol. 146(1): 243-249.

Lee, C.K., Allison, D.B., Brand, J., Weindruch, R. and Prolla, T.A. (2002). Transcriptional profiles associated with aging and middle age-onset Cabric restriction in mouse hearts. Proc. Natl. Acad. Sci. U.S.A. 99: 14988-14993.

Liang, C.P., Chang, C.H., Liang, C.C., Hung, K.Y. and Hsieh, C.W. (2014). In vitro antioxidant activities, free radical scavenging capacity, and tyrosinase inhibitory of flavonoid compounds and ferulic acid from Spiranthes sinensis (Pers.) Ames. Molecules. 19(4): 4681-4694.

Liu, Q., Raina, A.K., Smith, M.A., Sayre, L.M. and Perry, G. (2003). Hydroxynonenal toxic carbonyls and Alzheimer disease. Mol. Aspects Med. 24: 305-313.

Ll-Bahr, S.M. (2013). Biochemistry of Free Radicals and Oxidative Stress. Science International. 1(5): 111-117.

Lobo, V., Patil, A., Phatak, A. and Chandra, N. (2010). Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn. Rev. 4(8): 118-126.

Manian, R., Anusuya, N., Siddhuraju, P. and Manian, S. (2008). The antioxidant activity and free radical scavenging potential of two different solvent extracts of Camellia sinensis (L.) O. Kuntz, Ficus bengalensis L. and Ficus racemosa L. Food Chem. 107: 1000-1007.

Marcocci, L., Marguire, J.J., Droy-Lefaiz, M.T. and Packer, L. (1994). The nitric oxide – scavenging properties of Ginkgo biloba extract EGB 761. Biochem. & Biophys. Res. Comm. 201: 748-755.

Meenakshi, S.R. and Agarwal, R. (2013). Nitric oxide levels in patients with chronic renal disease. J. Clin. Diagn. Res. 7(7): 1288-1290.

Middleton, E. Jr., Kandaswami, C., Theoharides, T.C., Aherne, S.A. and O'Brien, N.M. (2000). The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol. Rev. 52: 673–751.

Miliauskas, G., Venskutonis, P.R. and Van Beek, T.A. (2004). Screening of radical scavenging

activity of some medicinal and aromatic plant extracts. Food Chemistry. 85: 231-237.

Momoh, M., Muhamed, U., Agboke, A., Akpabio, E. and Uduma, E.O. (2012). Immunological effect of aqueous extract of Vernonia amygdalina and a known immune booster called immunace(®) and their admixtures on HIV/AIDS clients: a comparative study. Asian Pac. J. Trop. Biomed. 2(3): 181-184.

Namiki, M. (1990). Antioxidant/antimutagens in foods. CRC Critical Reviews in food Science and Nutrition. 29: 273-300.

Neergheen, V.S., Soobrattee, M.A., Bahorun, T. and Aruoma, O.I. (2006). Characterization of the phenolic constituents in Mauritian endemic plants as determinants of their antioxidant activities in vitro. Journal of Plant Physiology. 163(8): 787-799.

Ogasawara, Y., Imase, M., Oda, H., Wakabayashi, H. and Ishii, K. (2014). Lactoferrin directly scavenges hydroxyl radicals and undergoes oxidative self-degradation: a possible role in protection against oxidative DNA damage. Int. J. Mol. Sci. 15(1): 1003-1013.

Ojiako, O.A. and Nwanjo, H.U. (2006). Is Vernonia amygdalina hepatotoxic or hepatoprotective? Response from biochemical and toxicity studies in rats. Afr. J. Biotech. 5(18): 1648-1651

Oyiazu, M. (1986) Studies on products of browning reaction: Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn. J. Nutr. 44: 307-315.

Patel, D., Kumar, R., Prasad, S. and Hemalatha, S. (2011). Pedalium murex Linn (Pedaliaceae) fruits: a comparative antioxidant activity of its different fractions. Asian Pac. J. Trop. Biomed. 1(5): 395-400.

Peng, C., Wang, X., Chen, J., Jiao, R., Wang, L., Li, Y.M., Zuo, Y., Liu, Y., Lei, L., Ma, K.Y., Huang, Y. and Chen, Z.Y. (2014). Biology of Ageing and Role of Dietary Antioxidants. Biomed. Res. Int. 2014: 831-841.

Pereira, R.B., Sousa, C., Costa, A., Andrade, P.B. and Valentao, P. (2013). Glutathione and the Antioxidant Potential of Binary Mixtures with Flavonoids: Synergisms and Antagonisms. Molecules. 18: 8858-8872.

Perez-Perez, E.M., Rodriguez-Malaver, A.J., Padilla, N., Medina-Ramirez, G. and Davila, L. (2006). Antioxidant capacity of Crude extracts from clones of Banana and Plane species. J. Med. Food. 9(4): 517-523.

Perron, N.R. and Brumaghim, J.L. (2009). A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem. Biophys. 53(2): 75-100.

Perron, N.R., Hodges, J.N., Jenkins, M. and Brumaghim, J.L. (2008). Predicting how polyphenol antioxidants prevent DNA damage by binding to iron. Inorg. Chem. 47(14): 6153-6161.

Piljac, Z.J., Belscak, A. and Piljac, A. (2009). Antioxidant capacity and Polyphenolic content of Blueberry (Vaccinium corymbosum L.) Leaf Infusion. J. Med. Food. 12(3): 608-614.

Porto, C.D., Calligaris, S., Celloti, E. and Nicoli, M.C. (2000). Antiradical properties of commercial cognacs assessed by the DPPH• test. J. Agric. Food Chemistry. 48: 4241-4245.

Prakash, A. (2001). Antioxidant activity. Anal. Prog. 19: 1-6.

Rahman, M.M., Habib, M.R., Hasan, M.A., Al Amin, M., Saha, A. and Mannan, A. (2014). Comparative assessment on in vitro antioxidant activities of ethanol extracts of Averrhoa bilimbi, Gymnema sylvestre and Capsicum frutescens. Pharmacognosy Res. 2014 6(1): 36-41.

Ramasarma, T. (2012). Emergence of oxyl radicals as selective oxidants. Indian J. Biochem. Biophys. 49(5): 295-305.

Rao, A.S., Reddy, S.G., Babu, P.P. and Reddy, A.R. (2010). The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. BMC. Comp. Alt. Med. 10(4): 1-9.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C.A. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radic. Biol. Med. 26: 1231-1237.

Rice-Evans, C.A., Miller, N.J. and Paganga, G. (1996). Structure–antioxidant activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20: 933-956.

Ruch, K.J., Cheng, S.J. and Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechin isolated from Chinese green tea. Carcinogenesis. 10: 1003-1008.

Sasikumar, J.M., Maheshu, V., Aseervatham, G.S.B. and Darsini, D.T.P. (2010). In vitro antioxidant activity of Hedyotis corymbosa (L.) Lam. aerial parts. Ind. J. of Biochem. and Biophy. 47: 49-52.

Sen, S., De, B., Devanna, N. and Chakraborty, R. (2013). Total phenolic, total flavonoid content, and antioxidant capacity of the leaves of Meyna spinosa Roxb., an Indian medicinal plant. Chin J. Nat. Med. 11(2): 149-157.

Shabbir, M., Khan, M.R. and Saeed, N. (2013). Assessment of phytochemicals, antioxidant, anti-lipid peroxidation and anti-hemolytic activity of extract and various fractions of Maytenus royleanus leaves. BMC Complement Altern. Med. 13:143. doi: 10.1186/1472-6882-13-143.

Singha, A.K. (1972). Colorimetric assay of catalase. Anal. Biochem. 47: 389-394.

Song, J.L., Choi, J.H., Seo, J.H., Kil, J.H. and Park, K.Y. (2014). Antioxidative effects of fermented sesame sauce against hydrogen peroxide-induced oxidative damage in LLC-PK1 porcine renal tubule cells. Nutr. Res. Pract. 8(2): 138-145.

Steinberg, D. and Witztum, J.L. (2002). Is the oxidative modification hypothesis relevant to human artherosclerosis? Do the antioxidant trials conducted to date refute the hypothesis? Circulation. 105: 2107-2111.

Tenore, G.C. and Ciampaglia, R. (2012). Antioxidant profile of selected Mediterranean red wines. Nat. Prod. Res. 27(10): 855-861.

Toyang, N.J. and Verpoorte, R. (2013). A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae). J. Ethnopharmacol. 146(3): 681-723.

Urrutia, P.J., Mena, N.P. and Núñez, M.T. (2014). The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. Front. Pharmacol. 10; 5:38.

Vitetta, L. and Linnane, A.W. (2014). Endocellular regulation by free radicals and hydrogen peroxide: key determinants of the inflammatory response. Inflammopharmacology. 22(2): 69-72.

Wong, B.X. and Duce, J.A. (2014). The iron regulatory capability of the major protein participants in prevalent neurodegenerative disorders. Front. Pharmacol. 5(81): doi: 10.3389/fphar.2014.00081

Yamamoto, Y. and Gaynor, R.B. (2001). Therapeutic potential of inhibition of the NF-κB pathway in the treatment of inflammation and cancer. J. Clinic. Inv. 107(2): 135-142.

Yedjou, C.G., Izevbigie, E.B. and Tchounwou, P.B. (2013). Vernonia amygdalina-Induced Growth Arrest and Apoptosis of Breast Cancer (MCF-7) Cells. Pharmacol. Pharm. 2013 Jan 1; 4(1). doi: 10.4236/pp.2013.41013.

Zielonka, J., Sikora, A., Joseph, J. and Kalyanaraman, B. (2010). Peroxynitrite is the major species formed from different flux ratios of co-generated nitric oxide and superoxide: direct reaction with boronate-based fluorescent probe. J. Biol. Chem. 285(19): 14210-14216.