Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(4); 551-557

ISSN: 0975-4873

Research Article

In Vitro and *In Vivo* Antioxidant Activity of Aqueous and Ethanol Leaf Extracts *of Murraya Koenigii*

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Available Online: 13th March, 2016

ABSTRACT

Reactive oxygen species generated from certain cellular processes cause tissue and cell damage and may alter the structure and functioning of proteins and nucleic acids. Endogenous antioxidant defence mechanisms involving certain enzymes as well as exogenous antioxidant from plants are capable of preventing such complications. This study investigated the in vitro and in vivo antioxidant activity of *Murraya koenigii* (curry leaf) extracts. Water and ethanol extracts of the leaf were obtained by soxhlet extraction. *In vitro* antioxidant activity was by the ferric reducing power and hydrogen peroxide scavenging assays and the total flavonoid and flavonol levels determined. The in vivo activity was assessed using albino wistar rats which were treated with 100mg/kg of aqueous and ethanol leaf extracts and ascorbic acid as positive control for six consecutive days after which a single dose (2.5mL/kg body) of CCl_4 was administered except for the normal control group 24hrs later. The animals were sacrificed and the liver homogenate was used to assess the catalase activity and lipidic peroxidation. Aqueous and ethanol extracts showed ferric reducing activity and were concentration dependent. The hydrogen peroxide scavenging potential was very high at various concentrations for both extracts (between 81.2 to 99.9%) comparable to ascorbic acid. Molondialdehyde (MDA) level (lipidic peroxidation) was significantly lower ($p<0.05$) in group 2 animals which received aqueous extract compared to group 1 (normal control) and group 4 (negative control). There was a significant difference ($p<0.05$) in the catalase activity of group 2 animals which received aqueous extract compared to the normal control. Acute toxicity was observed in group 5 animals administered ethanol extract. Flavonoid and flavonol were greater in the ethanol extract (625.33 and 835.04 g/g of rutin) than in the aqueous extract (449.33 and 100.33 g/g of rutin) respectively. These results suggest that *Murraya koenigii* (curry leaf) aqueous and ethanol extracts possess antioxidant activity due to high amounts of flavonoid and flavonol. This property may partly be responsible for the medicinal application of the plant.

Keywords: oxidative stress, *Murraya koenigii* extracts, catalase, lipidic peroxidation, flavonoid, flavonol, CCl_4

INTRODUCTION

Oxidative stress, an unhealthy condition is widely known to significantly contribute to several human diseases such as inflammatory diseases, ischemic diseases, acquired immunodeficiency syndrome, gastric ulcers, hypertension, neurological disorders, diabetes, multiple sclerosis etc.[1,2]. Oxidative stress is also known to be one of the major sources of carcinogenesis, the top-ranked cause of human mortality world-wide[3]. Defined by an overwhelmed level of pro-oxidants out numbering the antioxidant capacity in cells, oxidative stress is as a result of overproduction of free radicals or decrease in antioxidant defence systems[4,5]. Free radicals are usually molecular species capable of independent existence such as hydroxyl radical, superoxide anion radical, hydrogen peroxide, oxygen singlet, hypochlorite, nitric oxide radical, and peroxynitrite radical etc.[6,7]. They contain an unpaired electron in an atomic orbital which are unstable and highly reactive species, capable of attacking the nucleus and membranes of cells and damaging biologically relevant molecules such as DNA, proteins, carbohydrates and lipids[8]. They can cause substantial damage to these macromolecules through the generation of adducts, destruction of unsaturated C-C bonds, and oxidation of disulfides[9]. Free radicals are derived either from normal essential metabolic processes in the human body or from external sources such as exposure to X-rays, ozone, cigarette smoking, air pollutants, and industrial chemicals[10,11.] Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non enzymatic reactions. On the other hands, antioxidants which are molecules stable enough to donate an electron to a rampaging free radical, can remove or neutralize it, thus reducing its capacity to damage[12]. Hence, free radicals which are responsible for oxidative stress can be removed or regulated by antioxidants. These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged[13]. Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist, and metal-chelating agents. Both enzymatic and non enzymatic

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Figure 1: Experimental design chart

25 Albino wistar rats					
Randomization	5	5	5	5	5
Groups	Group 1 Normal control	Group 2 Experimental Group	Group 3 Positive control	Group 4 Negative control	Group 5 Experimental Group
Day 1-6	Distilled water	Aqueous extract	Ascorbic acid	Distilled water	Ethanol extract
Day 7	Distilled water	CCl_4	CCl_4	CCl_4	CCl_4
Day 8	Animals sacrificed and liver collected for catalase activity and lipidic peroxidation determination				

antioxidants exist in the intracellular and extracellular environment to detoxify reactive oxygen species (ROS)[14]. The enzymatic detoxification processes is initiated firstly by superoxide dismutase (SOD) which catalyse the breakdown of superoxide released during oxidative phosphorylation to hydrogen peroxide and oxygen[15,16]. Catalase and glutathione peroxidase remove hydrogen peroxide by decomposing it to water and oxygen[17-19]. Some of the non enzymatic antioxidants which are produced during normal metabolism in the body include glutathione, ubiquinol, and uric acid[20]. Other lighter antioxidants cannot be manufactured by the body so they are supplied by the dietary sources[21] or medicinal plants. These include vitamin E (α-tocopherol), vitamin C (ascorbic acid), and Β-carotene, polyphenols, flavonoids, flavonol etc. *Murraya koenigii* belonging to the family *Rutaceae* is native to India and is usually found in tropical and subtropical regions. It is cultivated in other countries such as China, Australia, Nigeria and Ceylon[22]. Locally known in Nigeria as curry leaf, is a natural flavouring agent with a number of important health benefits, which makes our food both healthy and tasty along with pleasing aroma. They are known for various medicinal properties to control diarrhoea, gastrointestinal problems such as indigestion, excessive acid secretion, peptic ulcers, dysentery, diabetes and an unhealthy cholesterol balance[23]. They are also believed to have cancer fighting properties and are known to help protect the liver[24]. *Murraya koenigii* is rich in nutrients such as carbohydrates, energy, fiber, calcium, phosphorous, iron, magnesium, copper and minerals. It also contains various vitamins like nicotinic acid and vitamin C, vitamin A, vitamin B, vitamin E, plant sterols, amino acids, glycosides and flavonoids[25]. The various medicinal applications of *Murraya koenigii* and presence of vitamins and flavonoids which are known to have antioxidant properties prompted this study.

MATERIALS AND METHODS

Collection of plant leaves

Fresh curry leaves identified botanically by as *Murraya koenigii* were purchased from Abakpa market, Enugu East local government area of Enugu State Nigeria. The leaves of *Murraya koenigii* were air dried at room temperature in the chemistry laboratory of Godfrey Okoye University for 7 days.

Preparation of plant extract

The dried leaves were ground into powdery form using an electric blender. 60g each of plant material was extracted using 500ml of ethanol and 1L of distilled water in a soxhlet extractor. The extracted solution containing the plant extract was evaporated at 45^0C for 48 hours to obtain a yield of 7.5g and 22.5g respectively.

In vitro antioxidant activity

Determination of reducing power

The reducing power of the extract was evaluated according to the method of Yen and Chen[26]. A volume of 1.0 ml of the aqueous and ethanol extracts and ascorbic acid (0.125 - 1.0 mg/ml) each were mixed individually to a mixture containing 2.5ml of 0.2 M phosphate buffer (pH 7.0) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$) (1%w/v). The resulting mixture was incubated at 50°C for 20 minutes, followed by the addition of 2.5ml of trichloroacetic acid (10% w/v), which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferrous chloride (0.1% w/v). The absorbance was measured at 700nm against a blank sample. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide by the plant extract was estimated using the method of Ruch, Cheng and Klaunig[27]. A volume of 4ml of aqueous and ethanol extracts and ascorbic acid at various concentration (0.125 - 1.0 mg/ml) each was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.0) and incubated for 10 min. The absorbance (Abs) of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation:

H_2O_2 radical scavenging activity = {(Abs control – Abs sample)/ (Abs control)} x 100

Where; Abs control is the absorbance of H_2O_2 radical + solvent; Abs sample is the absorbance of H_2O_2 radical + sample extract or standard.

In vivo antioxidant activity

Animals and handling

Adult wistar albino rats weighing between 120g and 200g were obtained from the animal house of Faculty of Biological Sciences, University of Nigeria, Nsukka and transported to the animal house of Godfrey Okoye University Enugu, Enugu State of Nigeria. The animals

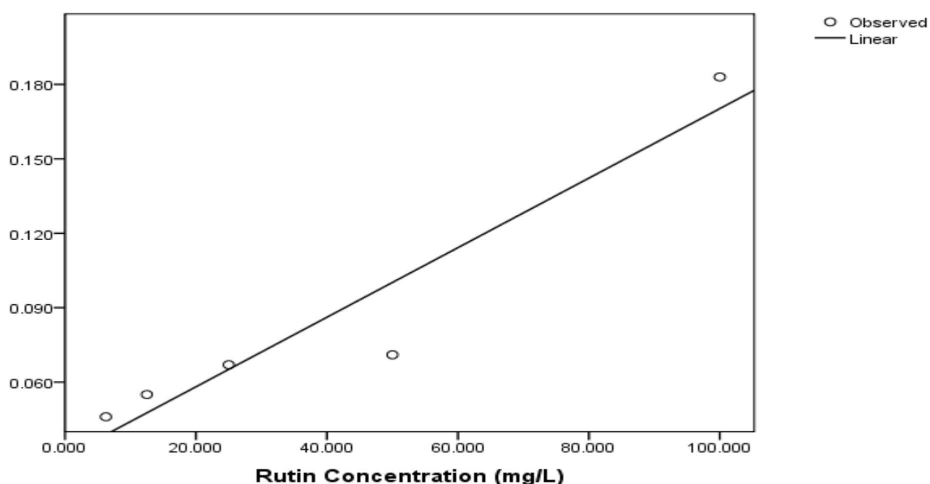

Figure 2: Total flavonoid calibration curve of Rutin

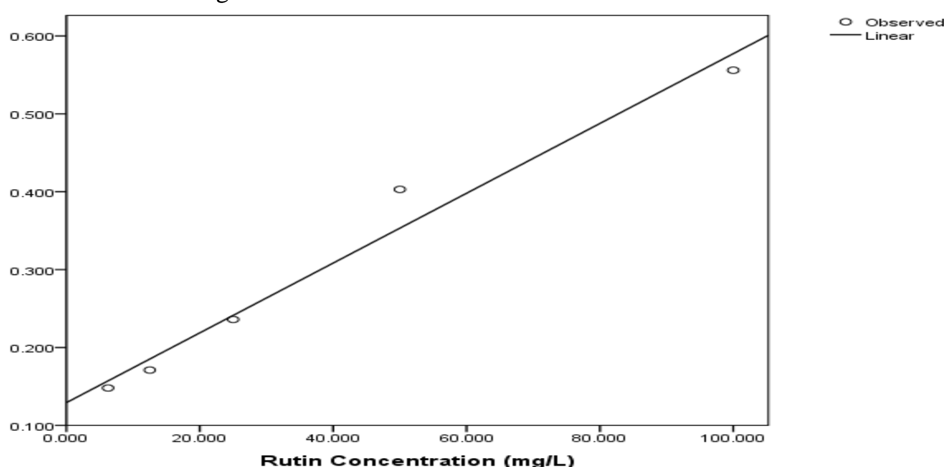

Figure 3: Total flavonol calibration curve of Rutin

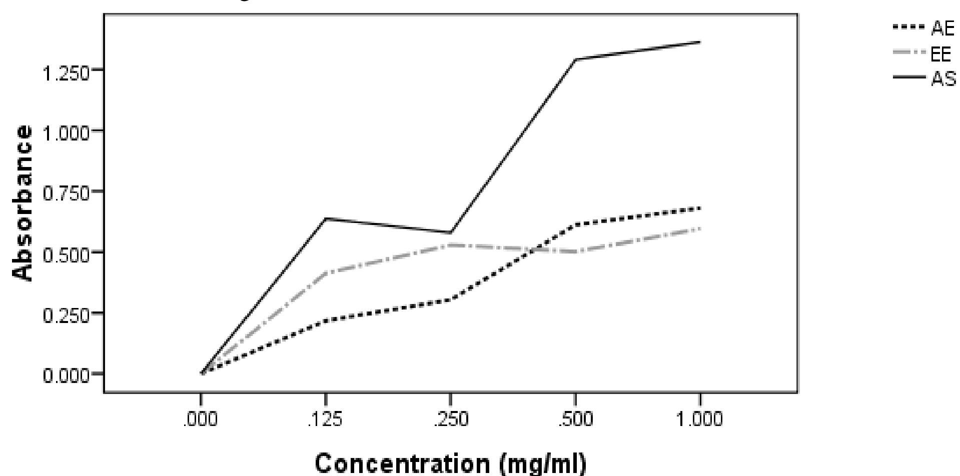

Figure 4: Reducing power activity of plant extract compared to standard

were acclimatised for a duration of 14 days under standard environmental conditions with a 12 hour light/dark phase and were allowed access to food (top feeds, growers mash) and water *ad libitum* twice daily.

Animal grouping and treatment

A total of 25 rats were randomized into five groups consisting of five animals each and orally administered 0.5 ml of treatment daily for seven days. Group 1 and 4; the normal and negative controls respectively were given distilled water, group 3; the positive control received

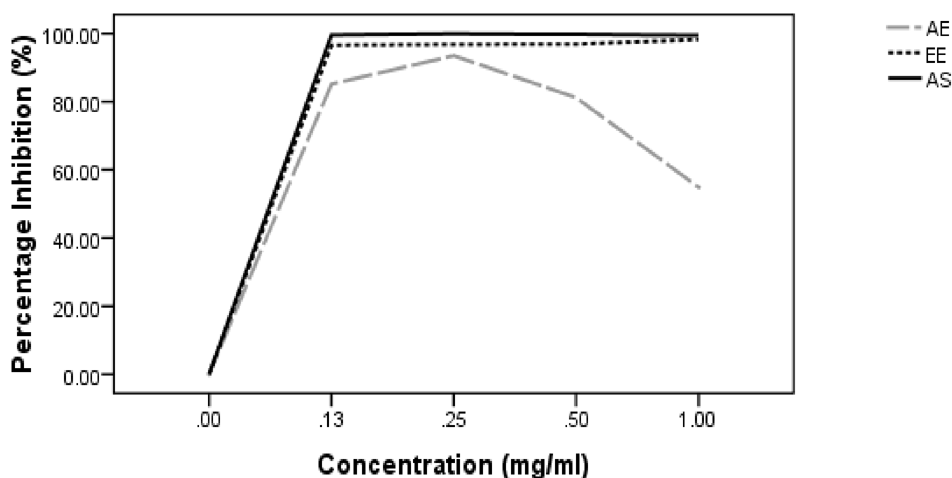

Figure 5: Hydrogen peroxide scavenging potential of plant extract compared to standard

ascorbic acid (vitamin C) while group 2 and 5; the experimental groups were given 100mg/kg body weight aqueous and ethanol extracts respectively.

Inducement of liver damage

On the seventh day about 6 hours after treatment administration, groups 2 to 5 were treated intraperitoneally with 0.5ml of a single dose of 2.5mL/kg body weight of Carbon tetrachloride (CCl_4) and olive oil (1:1). Group 1 was given distilled water/olive oil (1:1).

Collection of Samples from Animals

Following an overnight fasting for 24 hours, the animals were sacrificed by cardiac puncture under mild anaesthesia using chloroform and the liver from each animal was excised and rinsed with normal saline. 10% liver homogenate was prepared using 0.25M sucrose in phosphate buffer (0.2 M, pH 7.0) and centrifuged at 12,000rpm for 5minutes. The supernatant obtained was used for the estimation of catalase and lipid peroxidation. The experimental design chart is shown in Figure 1.

Determination of catalase activity

The activity of catalase was assayed following the method described by Pari and Latha[28]. The percentage inhibition was evaluated following decrease in absorbance at 620nm. The liver was homogenized in 0.01M phosphate buffer (pH 7.0) and centrifuge at 5000rpm. The reaction mixture consisted of 0.4ml of hydrogen peroxide (0.2 M), 1ml of 0.01 M phosphate buffer (pH 7.0) and 0.1ml of liver homogenate (10% w/v). The reaction of the mixture was stopped by adding 2ml of dichromate-acetic acid reagent (5% $K_2Cr_2O_7$ prepared in glacial acetic acid). The changes in the absorbance was measured at 620nm and recorded. Percentage inhibition was calculated using the equation:

% catalase inhibition = [(normal activity – inhibited activity) / (normal activity)] x 100%

Where;

Normal activity = hydrogen peroxide + phosphate buffer;

Inhibited activity = hydrogen peroxide+ phosphate buffer + liver homogenate

Estimation of lipid peroxidation

Lipid peroxidation in the liver was estimated colorimetrically by thiobarbituric acid reactive substances (TBARS) using the modification method of Niehius and Samuelsson[29]. In brief, 0.1 ml of liver homogenate (10% w/v) was treated with 2 ml of (1:1:1 ratio) TBA- TCA-HCl reagent (thiobarbituric acid 0.37%, 15% trichloroacetic acid and 0.25 N HCl). All the tubes were placed in a boiling water bath for 30 min and cooled. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 535nm against reference blank. Concentration of MDA was calculated using the equation:

$$C = \frac{A}{E \times L}$$

Where; A is the absorbance of the sample, E is the extinction coefficient (1.56×10^5 M^{-1} cm^{-}) and L is the length of the light path (1cm)

Estimation of polyphenol compounds

Total flavonoids

The method of Ordon Ez *et al.* was used to estimate total flavonoids contents of the extract solution based on the formation of a complex flavonoids-aluminium[30]. A volume of 0.5ml of 2% $AlCl_3$ ethanol solution was added to 0.5ml of extract solution. After one hour of incubation at room temperature, the absorbance was measured at 420nm using UV-VIS spectrophotometer. All determinations were done in triplicate and values were calculated from the calibration curve of rutin (Figure 2) using the equation:

$$Y = 0.001X + 0.030, R^2 = 0.910$$

$$X = \frac{Y - 0.030}{0.001}$$

Where; Y = absorbance, X = concentration of rutin (mg/g)

Total flavonols

Total flavonol content was determined by adopting the procedure described by Kumaran and Karunakaran[31]. The reacting mixture consisted of 2.0 ml of the sample, 2.0 ml of $AlCl_3$ prepared in ethanol and 3.0 ml of (50 g/l) sodium acetate solution. The absorption at 440 nm was read after 2.5 h at 20°C. Total flavonol content was calculated as rutin (mg/g) equivalent from the calibration curve of rutin (Figure 3) using the equation:

$$Y = 0.004X + 0.129, R^2 = 0.973$$

$$X = \frac{Y - 0.129}{0.004}$$

Where; Y = absorbance, X = concentration of rutin (mg/g)

Statistical Analysis

Table 1: Catalase activity and lipidic peroxidation in various treated animal groups

	Group 1	Group 2	Group 3	Group 4	P-value
Catalase (% Inhibition)	27.83±4.35	26.68±2.20[a]	31.93±2.59	30.29±3.13	0.580
Lipidic peroxidation (µM)	3.67±0.33	3.25±0.48[a]	4.40±0.51	4.50±0.65	0.323

Table 2: Total flavonoid and flavonol content in plant extract

	Concentration (mg/g Rutin)		
	Aqueous extract	Ethanol extract	p-value
Flavonoid	476	686	
	464	608	0.009
	408	582	
Mean	449.33±20.96	625.33±31.25	
Flavonol	91	771.75	
	101.5	868	0.000
	108.5	865.38	
Mean	100.33±8.81	835.04±54.88	

The data obtained were analyzed using statistical package for social sciences (SPSS) version 16.0 and the results expressed as mean ± standard error. Significant differences were established by one way analysis of variance (ANOVA) and the acceptance level of significance was P≤ 0.05.

RESULTS

In vitro antioxidant activity

The extracts of *Murraya koenigii* (aqueous and ethanol extracts) showed ferric reducing power though not as high as that of ascorbic acid. For the various concentrations, the absorbance at 700nm for aqueous extract ranged from 0.218 to 0.681, the ethanol extract was from 0.413 to 0.596 and ascorbic acid from 0.637 to 1.363. The ferric reducing power for both extracts as well as ascorbic acid increased with increasing concentration, thus was dose dependent (Figure 4).

Legend: AE: Aqueous extract EE: Ethanol Extract AS: Ascorbic acid Both the aqueous and ethanol extracts of *Murraya koenigii* showed very high hydrogen peroxide scavenging activity comparable to that of ascorbic acid. The ethanol extract had a higher percentage inhibition of hydrogen peroxide than the aqueous extract for the various concentrations. The hydrogen peroxide scavenging activity was very high for both extracts at a very low concentration of 0.125mg/mL but the aqueous extract showed the lowest activity (54.9%) at a concentration of 1mg/mL (Figure 5).

In-vivo antioxidant activity

Following the administration of plant extracts to the various animal groups, acute toxicity was observed in all animals of group 5 administered ethanol extract and also in a few animals in group 4 (negative control) after CCl_4 administration. Catalase activity was observed in all the animal groups and was highest (31.93±2.59%) in group 3 (positive control). The catalase free radical percentage inhibition in group 2 animals administered aqueous extract though low (26.68±2.20), was comparable to that of the normal control (27.83±4.35) and significantly different ($p<0.05$) from the negative control (30.29±3.13). The quantity of malondialdehyde formed was used to determine the degree of lipidic peroxidation. The malondialdehyde level (µM) was lowest (3.25±0.48) in group 2 animals administered aqueous extract comparable to the normal control (3.67±0.33) and significantly different ($p<0.05$) from the negative control (4.50±0.65). (Table 1). Legend: Group 1: Normal control, Group 2: Aqueous extract, Group 3: Positive control, Group 4: Negative control, S.E: Standard Error, a superscript indicates significant difference Total flavonoids and flavonols were present in different amounts in the aqueous and ethanol extracts. The concentration of flavonoid was significantly higher ($p <0.05$) in the ethanol extract (625.33±31.25) than in the aqueous extract (449.33±20.96). Also, the flavonol content was significantly higher ($p<0.05$) in the ethanol extract (835.04±54.88) than in the aqueous extract (100.33±8.81). (Table 2).

DISCUSSION

In view of increasing risk factors of human to various deadly diseases, there has been a global trend toward the use of natural substances present in medicinal and dietary plants as therapeutic antioxidants[21]. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers[32]. The Leaf of *Murraya koenigii* has been shown to have various medicinal properties[33]. Apart from its medicinal value, the leaf of this plant known as curry leaf in Nigeria is a local spice for various dishes and widely used as a flavouring agent because of its pleasing aroma and taste. Because of its wide consumption, a potential antioxidant activity will be of great importance to the masses. Several *in vitro* techniques have been used to determine the antioxidant activity in order to allow rapid screening of substances with high antioxidant activity since substances that have low antioxidant activity *in vitro*, will probably show little activity *in vivo*. In this study, the ferric reducing power and hydrogen peroxide scavenging activity were assessed. Antioxidants have the ability to scavenge free radicals in vitro by reducing Fe^{3+} to Fe^{2+} through electron transfer ability[34]. This serves as an indicator of the antioxidant activity. The findings showed the aqueous and ethanol extracts of *Murraya koenigii* to possess in vitro ferric reducing activity though not as high as that of

ascorbic acid. The reducing power activity increased with increasing concentrations thus was concentration dependent. Hydrogen peroxide is a highly important reactive oxygen species because of its ability to penetrate biological membranes and damage them[35]. Antioxidants can remove hydrogen peroxides by converting them to water[36]. This study showed the ability of *Murraya koenigii* plant extracts to scavenge hydrogen peroxide in the reaction mixture. The percentage inhibition of both the aqueous and ethanol extracts was similar to that of ascorbic acid which is a known or reference antioxidant. This could be due to the presence of phenolic compounds that donate electron to H_2O_2 and thus neutralizing it to water[37]. *In vivo* antioxidant assays are usually important to confirm the in vitro activity of certain antioxidant. To assess this *in vivo* antioxidant activity in animal models, a state of oxidative stress needs to be induced after the administration of plant extract to evaluate the ability of the plant extract to scavenge free radicals and prevent liver damage. Carbon tetrachloride (CCl_4) is a substance that can cause oxidative stress by generating free radicals and thus one of the most commonly used hepatotoxins in experimental studies to cause liver damage[38]. The hepatotoxic effects of this chemical is mostly based on membrane lipid peroxidation. Consequently, leads to the induction of trichloromethyl radical that results into severe cell damage[39]. In this present study, the rats treated with single dose of CCl_4 showed hepatic damage and lipidic peroxidation by an increase in the Malondialdehyde (MDA) level in group 4 animals (negative control) compared to group 1 (normal control). The MDA level was significantly lowest ($p<0.05$) in group 2 animals administered aqueous extract of *Murraya koenigii* which suggest that *Murraya koenigii* plant extract can prevent lipidic peroxidation and liver damage. Hence, has an antioxidant as well as a hepatic protective activity. Catalase is another antioxidant enzyme widely distributed in the animal tissues. It decomposes hydrogen peroxide to water and protects the tissues from highly reactive hydroxyl radicals[18]. The reduction of activity of this enzyme may lead to deleterious effects as a result of hydrogen peroxide assimilation[17]. In the present study, the percentage inhibition of catalase was significantly lower ($p<0.05$) compared to the negative control. Thus, the plant extract was shown to promote the production of catalase or enhance its activity.

Acute toxicity was observed in the animals administered ethanol extract as they all died during the course of treatment. This may imply that ethanol may extract certain phytochemicals that are toxic to the animals. Also, a few casualties were observed in animals of the negative control group which were not treated before induction of liver damage with CCl_4. Polyphenols are the major plant compounds with high level of antioxidant activity[40]. This activity could be due to their ability to absorb, neutralize and to quench free radicals[41]. Their ability as free radical scavenger could also be attributed to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation[42]. Flavonoid and flavonol are plant-based compounds with powerful antioxidant properties found in many fruits and vegetables[43]. In this present study, the leaf extracts of *Murraya koenigii* showed high level of flavonoids and flavonols. The ethanol extract showed a higher level of total flavonoids and flavonols compared to the aqueous extract which suggests that the type of solvent used is an important factor in the extraction of phytochemicals. In conclusion, *Murraya koenigii* aqueous and ethanol extracts possess both *in vitro* and *in vivo* antioxidant ability in scavenging free radicals which may be due to the presence of high levels of flavonoids and flavonols. This activity may partly be due to the presence of high levels of flavonoids and flavonols. The ability of this plant to prevent liver damage may be of interest for further studies to evaluate its potentials in the management of liver diseases.

ACKNOWLEDGEMENT

We are grateful to the management of Godfrey Okoye University Nigeria especially the Vice Chancellor, Rev. Fr. Prof. Christian Anieke for financial assistance.

REFERENCES

1. Patel VP, Chu CT. Nuclear transport, oxidative stress, and neurodegeneration. *Int J Clin Exp Pathol.* 2011; 4(3): 215–29.
2. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options *Curr Neuropharmacol,* 2009*; 7:* 65-74
3. Toyokuni S. Molecular Mechanisms of Oxidative Stress-induced *Carcinogenesis. Department of Pathology and Biology of Diseases*, Japan; 2008.
4. Kohen R, Nyska A. Oxidation of Biological Systems: Oxidative Stress Phenomena, Antioxidants, Redox Reactions, and Methods for Their Quantication *Toxicol Pathol*, 2002; 30 (6): 620–650,
5. Sies H. Oxidative stress: Oxidants and antioxidants. *Exp Physiol.* 1997; 82: 291–295.
6. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. *Nature Chem Biol* 2008; 4:278-286. .
7. Kunwar A, Priyadarsini KI. Free radicals, oxidative stress and importance of antioxidants in human health *J Med Applied Sci* 2011; 1(2):53-60
8. Young IS, Woodside JV. Antioxidant in health and diseases. *J Clin Pathol*, 2001. 54:176–186
9. Valko M, Leibfritz D, Moncola J, Cronin MD. Free radicals and antioxidants in normal physiological functions and human disease. Review. *Int. J Biochem Cell Biol.* 2007; 39:44-84
10. Lien Ai Pham-Huy, Hua He and Chuong Pham-Huy, Free Radicals, antioxidants in disease and health. *Int J Biomed Sci*. 2008; 4(2):89-96.
11. Droge W. Free radicals in the physiological control of cell function. Review. *Physiol. Rev.* 2002; 82:47-95.
12. Poljsak B, Dahmane R. Free Radicals and Extrinsic Skin Aging. *Dermatol Res* Practice 2012: 1–4
13. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: A new pharmacological approach

in shock, in• ammation, and ischemia/reperfusion injury. *Pharmacol Rev* 2001; 53: 135–159.
14. Maritim AC, Sanders RA, and Watkins III JB. Diabetes, Oxidative Stress, and Antioxidants: A Review *J Biochem Mol Toxicol* 2003; 17(1) : 24–38
15. Johnson F, Giulivi, C. Superoxide dismutases and their impact upon human health. *Mol Aspects Med.* 2005; 26: 340–352.
16. Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radic Biol Med.* 2002; 33:337–349.
17. Ferraris A, Rolfo M, Mangerini R, Arena S, Kirkman H. Predominant role of catalase in the disposal of hydrogen peroxide within human erythrocytes. *Blood*, 1996; 87:1595–9.
18. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative Stress and Antioxidant Defense *World Allergy Org J* 2012; 5:9–19)
19. Hayes J, Flanagan J, Jowsey I. Glutathione transferases. *Ann Rev Pharmacol Toxicol.* 2005; 45: 51–88.
20. Masella R, Di Benedetto R, Vari R, Filesi C, Giovannini C. Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes. *J Nutr Biochem.* 2005; 16:577–586.
21. Seifried HE, Anderson DE, Fishera I, *et al.* A review of the interaction among dietary antioxidants and reactive oxygen species. *J Nut Biochem* 2007; 18:567-579.
22. Salikutty J, Peter KV. Curry leaf (*Murraya koenigii*), perennial, nutritious, leafy vegetable. *Economic Bot.* 1985; 39(1): 68-73
23. Ajay S, Rahul S, Sumit G, Paras M, Mishra A, Gaurav A. Comprehensive review: Murraya koenigii Linn. *Asian J Pharm Lif Sci.* 2011; 1(4) 2231 – 4423.
24. Farhath-Khanum KR, Sudarshana-Krishna KR, Viswanathan KR, and Santhanam K. Anticarcinogenic effects of curry leaves in dimethylhydrazine treated rats. *Plant Food Human Nutri.* 2000; 55: 347-355
25. Sinha P, Akhtar J, Batra N, Jain H, Bhardwaj A. Curry Leaves – A Medicinal Herb. *Asian J. Pharm. Res.* 2012; 2(2):51-53
26. Yen G, Chen H Antioxidant activity of various tea extract in relation to their antimutagenicity. *J. Agric. Food Chem. 1995; 43:7-32.*
27. Ruch RJ, Cheng SJ, Klaunig JE Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from chinese green tea. Carcinogen. 1989; 10: 1003-1008.
28. Pari L, Latha M. Protective role of *Scoparia dulcis* plant extract on brain antioxidant status and lipid peroxidation in STZ diabetic male Wistar rats. *BMC Complem. Altern. Med.* 2004; 4: 16; doi:10.1186/1472-6882-4-16.
29. Niehius WG, Samuelson B. Formation of malondialdehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem.* 1968; 6: 126-130.
30. Ordon Ez AL, Gomez JD, Vattuone MA, Isla MI. Antioxidant activities of *Sechium edule* (Jacq). *Food Chem.* 2006; 97: 452-458.
31. Kumaran A and Karunakaran RJ. *In vitro* antioxidant activities of methanol extracts of *Phyllantus* species from India. *Lebens-Wisstechnologie.* 2007; 40: 344-352.
32. Walton NJ, Brown DE (1999) Chemicals from Plants: Perspectives on Plant Secondary Products. Imperial College press, London, UK
33. Chevallier A. *The encyclopedia of medicinal plants.* Darling Kindersley, London, UK; 1996.
34. Jiao Y, Wilkinson J, Christine Pietsch E, *et al.* Iron chelation in the biological activity of curcumin. *Free Radic Biol Med* 2006; 40:1152-1160.
35. Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. *FEBS Lett* 2000; 486: 10–13.
36. Mates JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicol.* 2000; 153:83-104.
37. Mathew S, Abraham TE. In vitro antioxidant activities and scavenging effects of Cinnamomum verum leaf extract assayed by different methodologies. *J Food Chem Toxicol*. 2006; 44: 198-206.
38. Liu KX, Kato Y, Yamazaki M, Higuchi O, Nakamura T, Sugiyama Y. Decrease in the hepatic clearance of hepatocyte growth factor in carbon tetrachloride-intoxicated rats. *Hepatol*. 1993; 17(4): 651–60.
39. Johnson DE, Kroening C. Mechanism of early carbon tetrachloride toxicity in cultured rat hepatocytes. *Pharmacol Toxicol.* 1998; 83: 231-239.
40. Manach C, Scalbert A, Morand C, *et al*. Polyphenols: food sources and bioavailability. *Am J Clin Nutr* 2004; 79:727-747.
41. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: Antioxidants and beyond. *Am J Clin Nutri.* 2005; 81 (I), 2 15s-2 17s.
42. Galati G, Sabzevari O, Wilson JX, *et al.* Prooxidant activity and cellular effects of the phenoxyl radicals of dietary flavonoids and other polyphenolics. *Toxicol* 2002; 177:91–104.
43. Rice-Evans CA, Miller NJ, and Paganga G. Structure–antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 1996; 20: 933–956.