NISEB Journal Vol. 16, No. 3, September, 2016 1595-6938/2016
Printed in Nigeria (2016) Society for Experimental Biology of Nigeria
http://www.nisebjournal.org

In vitro antioxidative studies on *Murraya koenigii* leaves

Usunobun Usunomena[1] and Ewere G. Efosa[2]*
[1]Department of Basic Sciences (Biochemistry unit), Faculty of Basic and Applied sciences, Benson Idahosa University, P.M.B 1100, Benin City, Edo State, Nigeria
[2]Biochemistry Department, Faculty of Basic Medical sciences, Uyo, Akwa Ibom state, Nigeria

Abstract
Murraya koenigii known as curry leaf is used for flavouring foodstuffs and is a treasure in traditional system of medicine. The study thus provides in vitro information of Murraya koenigii leaves. The leaves of Murraya koenigii were examined for their phytochemicals, mineral constituents and for their in vitro antioxidant activities using 2, 2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging and reducing power assays. Higher antioxidant potential of Murraya koenigii and the standard, Vitamin C was observed in both DPPH radical scavenging assay and reducing activity assays at higher concentrations. Phytochemicals present includes flavonoids, tannins, steroids, saponins, alkaloids. The leaves had higher concentration in calcium (320.60mg/kg) and least concentration in chromium (0.58mg/100g). These findings suggest that Murraya koenigii leaves possess potent antioxidant property, which may be responsible for some of its reported effective traditional use and pharmacological actions.
Keywords: 2, 2-diphenyl-1-picryl hydrazyl, Minerals, Phytochemicals, Reducing power

Introduction
Current researches are largely plant-based looking for new leads to develop better drugs against free radical caused inflammations, microbial infections and diseases. In rural areas of developing countries, herbal materials continue to be used as the primary source of medicines [1]. About 80% of the people in developing countries use traditional medicines for their primary health care [2]. One of such plants with use for health maintenance is *Murraya koenigii.* It belongs to the family Rutaceae and commonly called curry leaf, a popular spice herb with high value due to its characteristic aroma and medicinal value. The bark and leaf extracts of *Murraya koenigii* are therapeutically being used in folk and traditional medicine to control diabetes, dysentery, jaundice, pancreatitis, asthma. Pharmacological activities including vasodilatory, hypo-cholesterolemic, anti-ulcer, anti-diarrheal, phagocytic, analgesic ,antinociceptive and wound healing of *Murraya koenigii* leaves have been reported. Application of curry leaves paste helps in treating bruises and burns as well as rashes and insect bites. Regular consumption of curry leaves strengthens hair, cures dandruff and prevents premature graying of hair. Curry leaves are useful in eye problems such as cataract as they protect the eyes, keeping the retina healthy and preventing vision loss. The curry leaves have been known to promote appetite and digestion and have been popularly used as stomachic, purgative, antiemetic, anti-inflammatory and carminative [3], hence aptly known as cure leaf [4]. This study is aimed at determining phytochemicals and minerals as well as *in vitro* antioxidant potentials of *Murraya koenigii* leaves.

Materials and Methods
Collection, Identification and Preparation of Plant materials: The fresh leaves of *Murraya koenigii* were collected from a local farm in South Eastern part of Nigeria. Identification and authentication were carried out by a Botanist in the Department of Basic Sciences, Benson Idahosa University after which the leaves were washed and air dried at room temperature for three weeks. They were grounded into fine powder using an electric blender and stored in a cool dry container until use for analysis.
Phytochemical analysis: Qualitative phytochemical screening using standard methods as described [5-9].
Mineral analysis: Mineral analysis was carried out using Atomic Absorption Spectrophotometer (AAS) as previously done by Usunobun and Okolie [10-11].
Determination of reducing power ability: The reducing power activity of *Murraya koenigii* leaves was carried out using the reducing power method as described by Aiyegoro and Okoh [12]. A mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of stock *Murraya koenigii* leaves filtrate (0.2–1.0 mg/ml) prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10% w/v) and centrifugation at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was measured at 700 nm against reagent blank sample. Increased absorbance of the reaction mixture indicates higher reducing power of *Murraya koenigii* leaves.
2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability: The DPPH method according to Liyana-Pathiana and Shahidi [13] was used for the determination of DPPH free radical scavenging activity of the

**Corresponding Author's Email: uusunobun@biu.edu.ng*

Murraya koenigii leaves as follows: DPPH (1 ml, 0.135 mM) prepared in methanol was mixed with 1.0 ml of stock *Murraya koenigii* leaves filtrate ranging in concentration from 0.2 to 1.0 mg/ml. The reaction mixture was then vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. The scavenging ability was calculated using the equation: DPPH scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$,
Where: $Abs_{control}$ is the absorbance of DPPH + methanol and Abs_{sample} is the absorbance of DPPH radical + sample (sample or standard).
Statistical analysis: Data obtained from this study were expressed as mean value ± standard deviation.

Results The phytochemical screening of *Murraya koenigii* leaves as shown in table 1 revealed the presence of saponins, flavonoids, alkaloids, glycosides, tannins, and steroids while anthraquinones and phlobatannins were absent.

Table 1: Phytochemical screening of *Murraya koenigii* leaves

Phytochemicals	*Murraya koenigii* leaves
Saponins	Positive
Alkaloids	Positive
Flavonoids	Positive
Tannins	Positive
Glycosides	Positive
Steroids	Positive
Anthraquinones	Negative
Phlobatannins	Negative

The result of mineral analysis in *Murraya koenigii* leaves in mg/100g revealed the plant to have a high concentration of calcium, phosphate and potassium as well as a very low concentration of chromium, copper and manganese as shown in table 2.

Table 2: Minerals present in *Murraya koenigii* leaves (mg/100g)

Minerals	*Murraya koenigii* (mg/100g)
Calcium	320.60±2.04
Magnesium	93.30±1.01
Potassium	112.80±1.62
Sodium	39.60±2.03
Phosphate	143.35±1.07
Iron	73.65±1.21
Zinc	3.57±1.89
Copper	1.28±1.76
Manganese	0.81±0.09
Chromium	0.58±0.11

Values are means ± SD for 2 determinations

The result of the reducing power ability of *Murraya koenigii* leaves as displayed in figure 1 shows the reducing power activity to be concentration dependent i.e. the reducing power activity increases as the concentration increases from 0.2 to 1.0mg/ml.

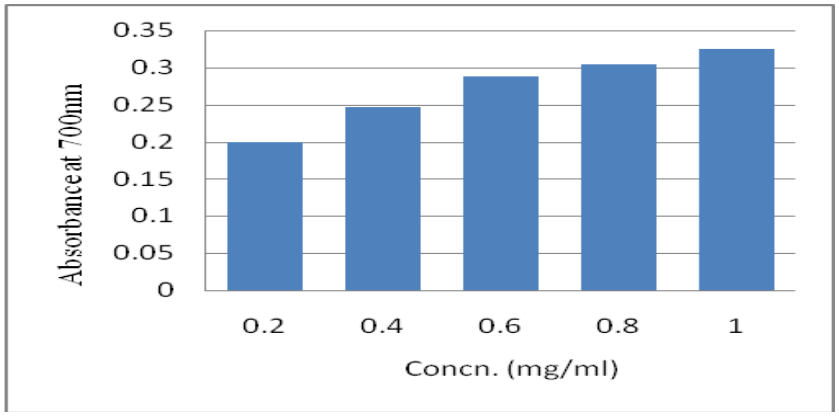

Figure 1: Reducing power ability of *Murraya koenigii* leaves

Figure 2 shows the DPPH radical scavenging effect of *Murraya koenigii* leaves and the standard compound, Vitamin C. The scavenging effect of *Murraya koenigii* and Vitamin C produced a marked scavenging effect on DPPH radical in a dose dependent manner with the highest percentage of (90.58) and (91.99%) respectively observed for the highest concentration (1.0mg/ml).

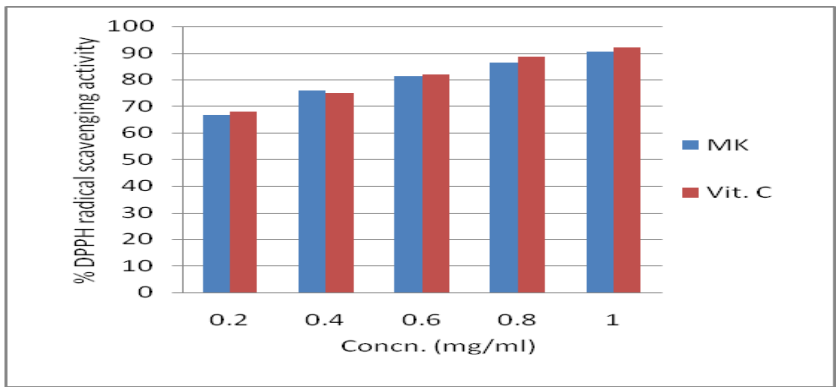

Figure 2: DPPH radical scavenging activity of *Murraya koenigii* (MK) leaves

Discussion

The phytochemical analysis conducted on *Murraya koenigii* leaves showed the presence of tannins, flavonoids, alkaloids, glycosides, steroids and saponins. Studies have shown that plants containing tannins are useful in treatment of inflamed or ulcerated tissues and they have remarkable activity in cancer prevention and anticancer [14]. Thus, *Murraya koenigii* containing tannins may serve as a potential source of bioactive compounds in the treatment of inflammation. Flavonoids have been shown to exhibit their actions through effects on membrane permeability, and by inhibition of membrane-bound enzymes such as the ATPase and phospholipase A_2 [15]. *Murraya koenigii* leaves was revealed to also contain saponins, known to produce inhibitory effect on inflammation [16] justifying the use of *Murraya koenigii* leaves in traditional medicine. The plant was also positive for steroids which are very important compounds especially due to their relationship with compounds such as sex hormone [17].

Reducing power assay is often used to evaluate the ability of an antioxidant to donate an electron [18]. In this study, the presence of antioxidants in *Murraya koenigii* leaves resulted in reduction of the ferric cyanide complex (Fe^{3+}) to the ferrous cyanide form (Fe^{2+}). In reducing power assay, antioxidants cause the reduction of the Fe^{3+} into Fe^{2+}, thereby changing the solution into various shades from green to blue, depending on the reducing power of the compounds [19]. The higher the absorbance of the reaction mixture, the higher would be the reducing power. Reducing power activity increased with increased concentration of *Murraya koenigii* leaves. It has been reported that the reducing power of substances is probably because of their hydrogen donating ability [20]. *Murraya koenigii* leaves, therefore, contain high amount of reductones which can act as electron donors and could react with free radicals to convert them into more stable products and then terminate the free radical chain reactions.

DPPH analysis is one of the best-known, accurate, and frequently employed methods for evaluating antioxidant activity [21]. It is a stable free radical because of its spare electron delocalization over the whole molecule. In the present study, *Murraya koenigii* leaves exhibited DPPH free radical scavenging ability in a dose-dependent manner. The Scavenging of DPPH radical in this study indicates the potency of the plant in donating hydrogen

proton to the lone pair electron of the radicals. The increased free radical scavenging property at higher concentration of *Murraya koenigii* leaves may be due to the presence of phytochemicals such as flavonoids.
Minerals such as calcium, sodium, magnesium, iron etc posesses biochemical and physiological functions as well as play essential roles in maintaining human health. Magnesium serve as cofactor in over 300 enzyme reactions, particularly those involving metabolism of food components. Magnesium content of *Murraya koenigii* (93.30mg/100g) is low when compared to 122.50mg/100g of *Celosia argentea* [22], 961.9mg/100g of *Annona muricata* and 681.36mg/100g of *Vernonia amygdalina* [10-11]. Calcium plays an important role in stimulus–response coupling involving signal transduction pathways, as its release from intracellular pools activates various protein kinases, phosphatases, or phospholipases, whose target molecules subsequently regulate many cellular functions [23]. Calcium content of *Murraya koenigii* (320.60mg/100g) compared favorably with 295mg/100g of *Celosia argentea* but low when compared to 1118.30mg/100g of *Annona muricata* and 1264.18mg/100g of *Vernonia amygdalina* [10-11]. Zinc content of *Murraya koenigii* (3.57mg/100g) is low compared to 5.42mg/100g of *Celosia argentea* [22] but high compared to 0.83mg/100g of *Annona muricata* and 1.42mg/100g of *Vernonia amygdalina* [10-11]. Sodium content of *Murraya koenigii* (39.60mg/100mg) compared favourably with 48.31mg/100g of *Vernonia amygdalina* [11] but low compared to 69.49mg/100g of *Annona muricata* Usunobun and Okolie [10] and 71.32mg/100g of *Celosia argentea* [22]. Potassium content of *Murraya koenigii* (112.80mg/100g) compared favorably with 128.33mg/100g of *Celosia argentea* [22] but high when compared to 36.31mg/100g of *Annona muricata* and 62.79mg/100g *of Vernonia amygdalina* [10-11]. Copper content of *Murraya koenigii* (1.28mg/100mg) compared favourably with 1.95mg/100g of *Vernonia amygdalina* and 1.42mg/100g of *Annona muricata* reported by Usunobun & Okolie [10-11] but low compared to 2.18mg/100g of *Celosia argentea* [22].
This study thus gives credence to health benefits of *Murraya koenigii* leaves. Further studies are currently on going to clarify the *in vivo* potential of this plant in the management of human diseases resulting from oxidative stress.

References

[1] Chitme HR, Chandra R, Kaushik S. Studies on anti-diarrheal activity of *Caltropis gigantean* R. In experimental animals. J. Phar. Pharma. Sci. 7: 70-75. 2003
[2] Kim HS. Do not put too much value on conventional medicines. Journal of Ethnopharma. 100: 37-39. 2005
[3] Gupta P, Nahata A, Dixit VK. An update on *Murraya koenigii* spreng: a multifunctional Ayurvedic herb. Zhong Xi Yi Jie He Xue Bao 9: 824-833. 2011.
[4] Bhandari PR. Curry leaf or cure leaf: review of its curative properties. Journal of medical nutrition and nutraceuticals 1: 92-97. 2012
[5] Sofowora LA. Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, pp55-71. 1993
[6] Trease GE, Evans WC. Pharmacognosy 11th Ed., Tindall Ltd, London, pp60-75. 1985
[7] Harbone JB. Methods of extraction and isolation. In: Phy-tochemical Methods. Chapman and Hall, London; Pp60-66. 1998
[8] Usunobun U, Okolie NP, Anyanwu OG. Adegbegi AJ, Eghare-vba ME. Phytochemical screening and proximate composition of *Annona muricata* leaves. Euro. J. Bot, Plant Sci.Phytol. 2(1): 18-28. 2015
[9] Usunobun U, Okolie PN. Phytochemical analysis and proximate composition of *Vernonia amygdalina.* Int. J. Scient. World 4 (1): 11-14. 2016
[10] Usunobun U, Okolie NP. Phytochemical analysis and mineral composition of *Annona muricata* leaves. Int. J. Res. Curr. Devel. 1(1): 38-42. 2015
[11] Usunobun U, Okolie NP. Phytochemical, trace and mineral composition of *Vernonia amygdalina* leaves. Int. J. Biol. Pharma. Res.. 6(5): 393-399. 2015
[12] Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Compl. Alterna. Med. 10: 21-32. 2010
[13] Liyana-Pathiana CM, Shahidi F. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivium* L) as affected by gastric pH conditions. J. Agric. Food Chem. 53: 2433–40. 2005.
[14] Motar MLR, Thomas G, Barbosa FJM. Effects of *Anacardium occidentale* stem bark extract on *in vivo* inflammatory models**.** J. Ethnopharma. 95(2-3): 139-142. 1985
[15] Li H, Wang Z,. Liu Y. Review in the studies on tannins activity of cancer prevention and anticancer**.** Zhong-Yao-Cai 26(6): 444- 448. 2003
[16] Just MJ, Recio MC, Giner RM, Cuellar MJ, Manez S, Bilia AR, Rios JL. Anti-Inflammatory activity of unusual lupine saponins from**.** Bupleurum fruticescens 64: 404-407. 1998.
[17] Okwu DE. Evaluation of the chemical composition of medicinal plants belonging to Euphorbiaceae**.** Pak. Veter. J. 14: 160-162. 2001.

[18] Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF, Bilaloglu V. Comparison of antioxidant and antimicrobial activities of Tilia (*Tilia argentea* Desf ex DC), sage (*Salvia triloba* L.), and Black tea (*Camellia sinensis*) extracts. J. Agric. Food Chem. 48(10): 5030-5034. 2000
[19] Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: individual cap and stipe activity," Food Chem. 100(4): 1511-1516. 2007
[20] Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the soybean oil in cyclodextrin emulsion," J. Agric. Food Chem. 40(6): 945-948. 1992
[21] Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation," *LWT*—Food Sci. Tech. 37(7): 717–721. 2004.
[22] Usunobun U., Ekpemupolo IS. Phytochemical analysis, mineral composition and *in vitro* antioxidant activities of *Celosia argentea* leaves. Int. J. Scient. World 4 (1): 19-22. 2016
[23] Bush DS. Calcium regulation in plant cells and its role in signaling. Ann Rev. Plant Physio. Plant Mole. Biol. 46: 95–122. 1995.