Journal of
Food and Pharmaceutical Sciences

Research Article

Phytochemical Composition, Mineral Content and Antioxidant Activities of the Methanol Extract of *Curcuma longa* and *Viscum album*

Sarah Onyenibe Nwozo[1]*, and Magdalene Eno Effiong[1]

[1]University of Ibadan, Ibadan, Nigeria

[2]Nutritional/industrial Biochemistry Unit, Biochemistry Dept, FBMS, College of Medicine, University of Ibadan, Ibadan

*Corresponding author: Sarah Onyenibe Nwozo | Email: onyenibe.nwozo@mail.ui.eu.ng

Received: 10 March 2019; Revised: 28 March 2019; Accepted 29 April 2019; Published: 30 April 2019

ABSTRACT: The medicinal value of plants have assumed a more important dimension in the past few decades owing largely to the discovery that extracts from plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potentials. However, there is dearth of knowledge on the medicinal uses and nutritional benefits of *Curcuma longa* and *Viscum album*. This study was designed to focus on the phytochemical and mineral composition in addition to the antioxidant activities of the methanol extracts of *C. longa* and *V. album*. Results indicated that the micro and macro mineral analysis exhibited significant presence of Fe, Zn, Mn, Ca and Mg while Cd, Pb, Ni and Cr were present in minute quantities. Phytochemical screening unravelled the presence of alkaloids, cardiac glycosides, steroids, phenols, tannins, saponins and flavonoids that were also proved by the quantitative analysis. There was a high correlation between the flavonoid content and antioxidant activities of the plant extracts. The antioxidant activities of the extracts were found to be concentration dependent. The results suggest that both plant extracts have good medicinal potentials and can serves as good sources of macro minerals.

Keywords: *Curcuma longa*; *Viscum album*; antioxidant activity; DPPH; flavonoids, phytochemical

1. INTRODUCTION

Antioxidants have generated much attention as it inhibits and protects damage due to free radicals and reactive oxygen species leading to the pathophysiology of human diseases such as neurodegenerative disorders, inflammation, viral infections, autoimmune disorders and digestive system disorders [1]. Humans possess the ability to oxidize oxygen with the benefit of metabolizing fats, proteins and carbohydrates for energy, however, with a concomitant production of damaging molecules called free radicals as a result of the very high potential of oxygen to become an active metabolite when attacked by leaked electrons from electron transport chain [2].

Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function. These cells damaged by free radicals appears to be a major contributing factor to aging and degenerative diseases such as cancer [3], ischemia reperfusion disease, cardiovascular disease, cataracts, immune system decline and brain dysfunction [4].

Antioxidants are capable of stabilizing or deactivating free radicals before they attack cells [5]. They are absolutely crucial for maintaining optimals cellular and systemic health and well-being [1]. Organisms are endowed with endogenous antioxidant defence systems which control free radical formation [6]. However, the generation of free radicals beyond its antioxidant capacity leads to oxidative stress, a fundamental mechanism underlying a number of disorders [7]. The need for antioxidants becomes even more critical with high levels of pollution, cigarette, smoke, drugs, illness, stress and even exercise which increases the production of free radicals [5].

Many folk plants are recognised as sources of natural antioxidants like phenolics acids, tannins, flavonoids and other metabolites which governs their medicinal properties [8]. These antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, antibacterial and antiviral activities. *C. longa* is a perennial herb and member of the Zingiberaceae (ginger) family and is cultivated extensively in India and China The rhizome, the portion of the plant used medicinally, yields a yellow powder. Dried *C. longa* is the source of turmeric which possesses numerous pharmacological properties such as anti-inflammatory [9], antioxidant, hepatoprotective [10], anti-carcinogenic [11], anti-diabetic, antimicrobial, antidepressant properties [10]. It is used for the treatment of acne, wounds, boils, bruises, blistering, ulcers, eczema, insect bites, parasitic infections, haemorrhages and skin diseases like herpes zoster and pemphigus [12].

V. album is a specie of mistletoe in the family of Santalaceace. It is a hemiparasite in several species of trees, from which it draws water and nutrients. It has radical scavenging activity and protective effect against hydroperoxide generation [13]. It has been reported to possess a number of therapeutic applications in folk medicines in curing or managing a wide range of diseases [6] such as diabetes mellitus [14,15], chronic cramps, stroke, stomach problems, heart palpitations, to lower blood pressure, alleviate difficulties in breathing and hot flushes [16].

The focus of the study is to elucidate the phytochemical constituents, mineral elements and antioxidant activity of the methanolic extracts of *C. longa* and *V. album* in order to obtain readily available and safe antioxidants for subsequent prevention and treatment of diseases arising from oxidative stress.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Collection of plant materials

The fresh leaves V. album and tubers of C. longa were purchased from Ojoo market, Ibadan, Oyo state and Main market, Jos, Plateau state, Nigeria. They were identified at the Department of Botany, University of Ibadan, Oyo state, Nigeria.

2.2. Methods

2.2.1. Sample preparation and extraction

The plant materials were hand-picked to remove debris, cut into small pieces and air dried at room temperature with adequate ventilation and pulverized samples were extracted with methanol by reflux. Exactly 50g of the powdered samples were weighed into 400 ml of methanol in a reflux flask and refluxed for 2 h. The extracts were filtered hot using a muslin cloth and subsequently concentrated to dryness using a rotary evaporator. The extracts were scraped using spatula, placed in sterile sample bottles, weighed and stored in the refrigerator until required for use.

2.3. Phytochemical screening

2.3.1. Qualitative phytochemical screening

The qualitative phytochemical screening for the presences of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins, terpenoids and steroids were carried out suing standard procedures described by Harborne, 2008 [17].

2.3.2. Quantitative phytochemical determination

a). Flavonoid determination

The total flavonoid content (TFC) content of C. longa and V. album was determined by using method of Zhishen et al., (1999) [18]. Briefly, to 10 g of each powdered was added 100 mL of 80% methanol. 0.2 mL was taken and added to 4 mL double-distilled water and 0.3 mL of 5% NaNO2 to the flask. The samples were shaken for 5 min, and 0.3 mL of 10% AlCl3 was added. After 6 min, 2 mL NaOH was added to and it was made up to 10 mL using distilled water. The absorbance was measured at 510 nm. TFC was calculated using a calibration curve of quercetin equivalents.

b). Phenol determination

The total phenol content of the extracts were determined using the method reported by Singleton et al., (1999) [19]. Appropriate dilutions of the extracts (0.5 mL) was oxidized with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by adding 0.2mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45oC and the absorbance measured at 765 nm. The total phenol contents were subsequently calculated using gallic acid as standard.

c). Alkaloid determination

Five grams of each dried powdered samples of Curcuma longa and Viscum album were weighed into a 250 mL beaker and 200 mL of 10% acetic acid in ethanol were added, covered and allowed to stand for 4 h. This was filtered, concentrated on a water bath to one-quarter of the original volume and concentrated ammonium hydroxide solution was added drop wise to the extract until precipitation was completed. The precipitate was removed, washed with dilute ammonium hydroxide and used as described by Harborne, (2008) [17].

d). Saponin determination

Exactly 0.5 g of the extracts were added to 20 mL of 1N HCl and boiled for 4 h. It was allowed to cool, filtered and 50 ml of petroleum ether was added to the filtrate to obtain ether layer [20].

e). Tannin determination

5 g of powdered C. longa and V. album samples were weighed into a 50 mL plastic bottle. 50 mL of distilled water were added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL volumetric flask and made up to the mark. To 5mL of the filtrate in a test tube was added with 2 mL of 0.1N HCl solution and 0.008 M potassium ferricyanide. The absorbance was measured at 120 nm [21].

2.4. Mineral analysis

The determination of the mineral content of the samples were carried out using atomic

absorption spectrometry (AAS) as described by AOAC (2010) [22].

2.5. Total flavonoid assay

Total flavonoid content was measured using aluminium chloride colorimetric assay. 1mL of extract or standard solution of catechin (300μg) were added to 10 mL volumetric flask containing 4 mL of distilled water. To the above mixture, 0.3 mL of 5% NaNO2 was added, after 5 minutes, 0.3 mL of 10% AlCl3 was added. At the 6th minute, 2 mL of 1M NaOH was added and it was made up to mark with distilled water. The solution was mixed thoroughly and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid contents were expressed as percentage of catechin equivalent per 100 g of extract [18].

2.6. Free radical scavenging activity

The free radical scavenging ability of the samples against DPPH (1,1′-diphenyl-2 picrylhydrazyl) free radical were evaluated. Briefly, appropriate dilution of the extracts (0 – 1.0 mL) was mixed with 1 mL of 0.4 mM methanol DPPH radical solution. The mixture was left in the dark for 30 min and the absorbance was taken at 516 nm [23].

2.7. Determination of reducing property

The reducing power was determined by assessing the ability of the sample extracts to reduce $FeCl_3$ solution as described by Pulido *et al*., (2002) [24]. Briefly, appropriate dilutions (0 – 1.0 mL) of samples were mixed with 2.5 mL 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixtures were incubated at 50ºC for 20 min. Thereafter, 2.5 mL of 10% Trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min. Then 5 mL of the resulting supernatant was mixed with equal volume of water and 1mL of 0.1% ferric chloride. The absorbance was taken at 700 nm against a reagent blank.

2.8. Fe^{2+} chelation assay

The ability of the sample extracts to chelate Fe2+ was determined using a modified method of Minotti and Aust (1987) with a slight modification [25]. Briefly 150 μL of freshly prepared 500 μM FeSO4 was added to a reaction mixture containing 168 μL of 0.1 M Tris-HCl (pH 7.4), 218 μL normal saline and the methanol leaf extracts (0 – 500 μL). The reaction mixture was incubated for 5 min, before the addition of 13 μL of 0.25% 1.10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in the spectrophotometer.

2.9. Statistical analysis

Statistical analysis were carried out using one-way analysis of variance (ANOVA) to compare the experimental groups followed by Bonferroni's test to identify significantly different groups (SPSS for Windows, version 17, SPSS Inc., Chicago, IL) and ϱ values less than 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1. Phytochemical constituents of C. longa and V. album

Table 1 shows the phytochemical constituents of *C. longa* and *V. album***.** The plant materials were found to contain a wide array of phytochemicals. The qualitative results indicated the

presence of alkaloids, cardiac glycosides, flavonoids, phenols, saponins, tannins and steroids. Terpenoids was found only in the *C. longa* and phlobatanins was absent in both plants.

Table 1. Qualitative Phytochemical screening result of *Curcuma longa* and *Viscum album*

Phytochemicals	Plants	
	Curcuma longa	***Viscum album***
Alkaloids	++	++
Cardiac glycosides	+++	+++
Flavonoids	+++	++
Phenols	++	++
Saponin	++	++
Tannin	++	++
Terpenoids	++	-
Phlobatanins	-	-
Steroids	+	+

Key: +++ (Highly present), ++ (Moderately present), + (Present), - (Absent).

Figure 1 shows the quantitative phytochemical contents of C. longa and V. album. This revealed the presence of phenols (366.66 ± 4.90; 499.76 ± 1.80 mg/g); Tannins (191.00 ± 0.10; 466.23 ± 6.20 mg/g); Flavonoids (134.16 ± 4.79; 122.88 ± 0.10 mg/g); Alkaloids (84.63 ± 2.19; 209.50 ± 0.20 mg/g) and Saponins (9.45 ± 1.69; 106.00 ± 4.60 mg/g) in C. longa and V. album respectively.

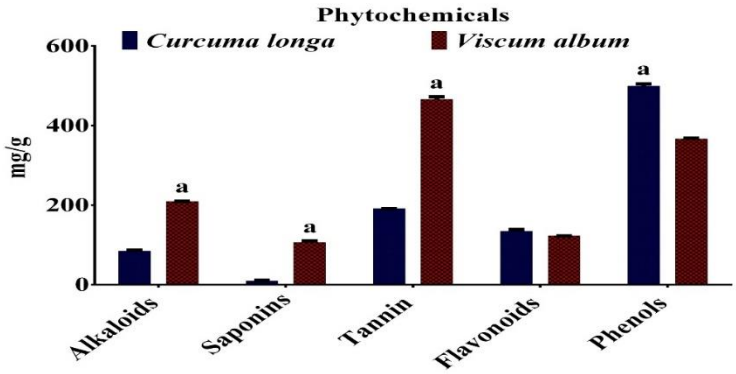

Figure 1. Quantitative phytochemical screening of Curcuma longa and Viscum album. The data are expressed as mean ± S.D. for triplicates (n=3). a = Values of the Curcuma longa extract differ significantly ($p < 0.05$) from Viscum album, indicating the higher value.

3.2. Mineral content of the methanol extracts of C. longa and V. album

The mineral content analysis showed significant variability among the different macro and micro elements (Fig. 2). This revealed the presence of Fe (209.13 ± 4.78; 194.24 ± 2.49 μg/L) and

Zn (196.58 ± 5.38; 181.69 ± 3.69 µg/L) in large amounts, Ca (11.01 ± 0.11; 10.22 ± 0.24 µg/L), Mg (10.04 ± 0.40; 9.25 ± 0.26 µg/L) and Mn (14.32 ± 0.32; 13.30 ± 0.31 µg/L) in moderate amounts and Cd (2.20 ± 0.30; 2.20 ± 0.04 µg/L), Pb (0.70 ± 0.10; 0.70 ± 0.30 µg/L), Ni (1.10 ± 0.02; 1.00 ± 0.06 µg/L) and Cr (4.00 ± 0.03; 0.40 ± 0.01 µg/L) in minute quantities in the methanolic extract of *C. longa* and *V. album* respectively.

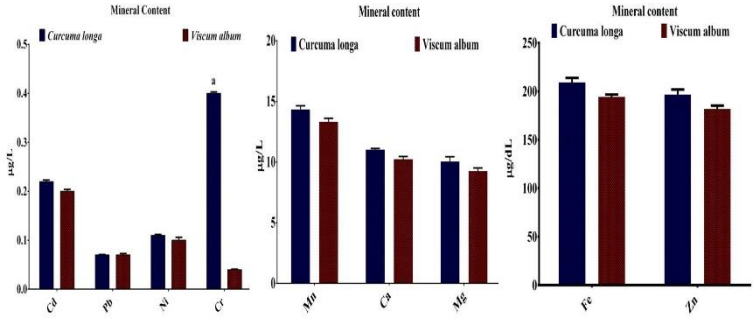

Figure 2. Mineral analysis of Curcuma longa and Viscum album. The data are expressed as mean ± S.D. for triplicates (n=3). a = Values of the Curcuma longa extract differ significantly from Viscum album, indicating the higher value.

3.3. Antioxidant content and activity of C. longa and V. album

Figure 3 shows the DPPH scavenging activity, flavonoid content, reducing power and ferric ion chelatting activity of the methanol extracts of C. longa and V. album at various concentrations.

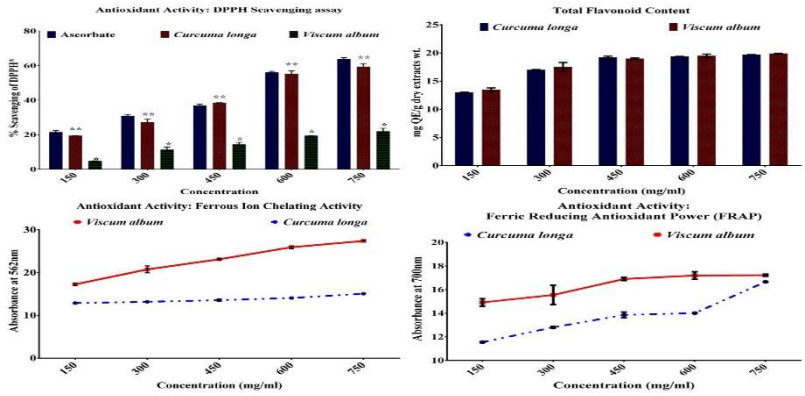

Figure 3. The Antioxidant content and activity of Curcuma longa and Viscum album. The data are expressed as mean ± S.D. for triplicates (n=3). *: Values of the Curcuma longa extract differ significantly from the standard, ascorbate; ($p < 0.05$)**: Values of the Curcuma longa extract differ significantly from Viscum album ($p < 0.05$), indicating the higher value

The flavonoid content of the extracts of *C. longa* and *V. album* were non-significantly different at all concentrations. The DPPH scavenging activity of *C. longa* was found to be similar to ascorbate standard but significantly higher than *V. album*. The scavenging activities were concentration dependent. The reducing power and ferric ion chelating activity of the plant extracts revealed that *V. album* had a significantly higher activity compared to *C. longa* at all concentrations. Their activities were concentration dependent.

Phytochemicals are natural bioactive compounds found in plants, such as vegetables, fruits, medicinal plants, flowers, leaves and roots. These plant bioactive compounds that work with

nutrients and fibers to act as a defence system against diseases [7]. They exhibit extensive potential as antioxidants [26], antimicrobial agents [27], anticancer [28] and immune boosters in the body system [29]. In the present study, the preliminary screening of phyto-constituents (Table 1) revealed a significant presence of alkaloids, flavonoids, cardiac glycosides, phenols, saponins, steroids and tannins in both plant extracts. The quantitative analysis, (Fig. 1) showed that the pesence of phenols and tannins were the highest, alkaloids and flavonoids were in moderate concentration while saponin was the least present. Overall, *V. album* had a higher phytochemical content compared to *C. longa*.

Mineral are required for normal growth activities of muscles, skeletal development, regulation of acid-base balance, chemical reaction in the body, intestinal absorption, cellular activity, oxygen transport, fluid balance and nerve transmission etc. There is scarcity of data on the mineral composition of the extracts of *C. longa* and *V. album*. The trend was similar to the reports of Gala and Gujar (2014) [30]. The study showed that both extracts contain iron and zinc in very high concentration; calcium, manganese and magnesium in moderate concentration and Cadmium, Lead, nickel and Chromium had negligible concentration levels. It has been reported that for many plant species Cr proved to be toxic at 5 mg/L and Pb at 2.60 mg/L [29]. In this regard, both plant extracts have very low concentration of Cr and Pb as compared to the reported levels of toxicity [31]. This indicates that *C. longa* and *V. album* are good sources of iron and zinc, enabling them to function as enhancers of blood formation, digestion and absorption [32]. The findings are similar to the reports of Fakankun *et al*., (2013) and Ayoola (2012) and have low levels of toxic metals [33,34].

Free radicals especially reactive oxygen species (ROS) had been implicated in a lot of degenerative diseases such as Parkinson and Alzhiemer diseases [35]. Overproduction of ROS can directly attack the polyunsaturated fatty acids, cell membranes and induce lipid peroxidation [36]. Antioxidants carry out their protective properties on cells either by preventing the production of free radicals or by neutralizing/scavenging free radicals produced in the body [35,37]. The antioxidant activity of *C. longa* and *V. album* could be attributed to the presence of phytochemicals (Table 1). The antioxidant activity of phenolics is mainly because of their redox properties which allows them to act as reducing agents, hydrogen donors, free radical scavengers, singlet oxygen quenchers and metal chelators [9]. The flavonoid content of *C. longa* and *V. album* (Fig. 3) increased with increasing concentration. Similar flavonoid content was noted in both plants.

DPPH radical scavenging activity has been extensively used for screening antioxidants capacity in fruits, cereals, vegetables, juices, decoctions as well as extracts [34]. The radical scavenging activity (RSA) of *C. longa* and *V. album* were tested using the methanolic solution of the free radical, DPPH. The DPPH solution shows a deep purple coloration with an absorption maximum at 517 nm. The purple colour disappears in the presence of an antioxidant. Thus, antioxidant molecules can quench DPPH free radicals either by providing hydrogen atoms or by donating electrons through a free radical attack on the DPPH molecule, thereby eliciting a colourless product 2,2′-diphenyl-1-hydrazine or a substituted analogous hydrazine. *C. longa* was found to be a good DPPH radical scavenger with a higher percent inhibition than *V. album* (Fig. 3).

Reducing ability is a measure of the ability test item to reduce Fe^{3+} to Fe^{2+} and this reflects their antioxidant properties. Antioxidants are strong reducing agents principally based on the redox properties of the hydroxyl groups and the structural relationships between varying parts of the chemical moiety [37]. The concentration dependent ferric reducing antioxidant power exhibited by

the extracts indicates their high antioxidant property and *V. album* exhibited significantly higher reducing ability compared to *C. longa.* This could be as a result of the harvest time, the nature of the host tree of *V. album* as well as soil characteristics as parasitic plants are known to derive additional pharmacologically active compounds from the host and this could increase its antioxidant capacity [13].

Metal chelators form complex ions and co-ordination with metals by occupying all metal co-ordination sites and preventing metal redox cycling [38]. Metal chelators convert metal ions into insoluble metal complexes or generate steric hindrance which inhibits the interactions of between metals and lipid. Fe^{2+} is necessary in relatively large amounts for haemoglobin, myoglobin and cytochrome production but xanthine oxidase and other Fe proteins require small amounts of Fe for their metabolic functions [37]. However, Free Fe in the mitochondira can cause considerable oxidative damage via Fenton reaction. The hydroxyl radical generated causes the oxidation of lipids, proteins, DNA. It can directly attack poly unsaturated fatty acids of cell membranes and induce lipid peroxidation [39]. Overall, *V. album* showed a stronger Fe chelating capability than *C. longa*.

4. CONCLUSION

The plants extract present good nutritional sources of macronutrients and micronutrients, exhibiting least toxic risks regarding the heavy metals content. The phytochemical composition revealed the presence of considerable levels of phenols, tannins, flavonoids, alkaloids and saponins amongst others as well as free radical scavenging/antioxidant properties.

Acknowledgment: We acknowledge University of Ibadan Senate Research Grant.

Conflict of interest: The authors declare that there are no conflicts of interest.

Reference

1. Aruoma OI. Methodological Considerations for Characterizing Potential Antioxidant Actions of Bioactive Components in Plant Foods. Mutat Res, **2003**, 523(524): 9-20, DOI: 10.1016/s0027-5107(02)00317-2.
2. Akinmoladun AC, Ibukun EO, Afor E, Akinrinlola BL. Onibon TR, Akinboboye AO, Obuotor EM, Farombi EO. Chemical Constituents and Antioxidant Activity of *Alstonia boonei*. African Journal of Biotechnology. **2007, 6**(10): 1197-1201.
3. Jayaprakasha GK, Singh RP, Sakariah KK. Antioxidant Activity of Grape Seed (*Vitrus vinifera*) Extracts on Peroxidation Models in Vitro. Food Chemistry. **2001,** 73: 285-290, DOI: 10.1016/S0308-8146(00)00298-3.
4. Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI. On the In Vitro Antioxidant Properties of Melatonin. Journal of Pineal Research. **2002,** 33: 167-171, DOI: 10.1034/j.1600-079x.2002.20926x.
5. Aggarwal BB, Van Kuiken ME, Iyer LH, Harikumar KB, Sung B. Molecular Targets of Nutraceuticals derived from Dietary Spices: Potential Role in Suppression of Inflammation and Tumorgenesis. Expt Biol Med. **2009,** 234: 825-849, DOI: 10.3181/0902-MR-78.
6. Sun J, Chu Y, Wu X, Liu R. Antioxidant and Antiproliferative Activities of Common Fruits. Journal of Agricultural Food and Chemistry. **2002,** 50: 7449-7454.
7. Berma SD, Singh P, Verma D, Soni AG and Nema R. *Boerhaavia diffusa*: An Important Medicinal Plant and Their Phytochemistry (Review). CMBT Journal of Sciences and Technology. **2013,** 1(1): 1-4.
8. Omale J and Okafor PN. Comparative Antioxidant Capacity, Membrane Stabilization, Polyphenol Composition and Cytotoxicity of the Leaf and Stem of *Cissus multistriata.* African Journal of Biotechnology. **2008,** 7(17): 3129-3133.

9. Aggarwal BB, Kumar A, Aggarwal MS, Shishodia S. In : Bagchi D, Preus HG (Eds) Phytopharmaceuticals in Cancer Chemoprevention, CRC Press: Boca Raton. 2004, pp349-387.
10. Sawant RS, Godghate AG. Qualitative Phytochemical Screening of Rhizomes of *Curcuma longa* Linn. International Journal of Science, Environment and Technology. **2013,** 2(4): 634-641.
11. Trinidad PT, Rosario SS, Marco PL, Aida CM, Melissa PB. *ZIngiber officinale* and *Curcuma longa* as Potential Functional Foods/Ingredients. Food and Public Health. **2012,** 2(2): 1-4, DOI: 10.5923/j.fph.20120202.01.
12. Gopinathan NM, Sai HS, Chitra K, Uma MRC. In Vitro Antiplatelet Activity Ethanolic Extract of Rhizome of *Curcuma longa* Linn. IJBR. **2011,** 2(2): 138-142.
13. Onay-Ucar E, Karagoz A, Arda N. Antioxidant Activity of *Viscum album* ssp. Fitoterapia. **2006,** 77: 556-560, DOI: 10.1016/j.fitote.2006.08.001.
14. Onunogbo CC, Ohaeri OC, Eleazu CO. Effect of Mistletoe (*Viscum album*) Extract on the Blood Glucose, Liver Enzymes and Electrolytes Balance in Alloxan-induced Diabetic Rats. American Journal of Biochemistry and Molecular Biology. **2012,** 1-8.
15. Orhan DD, Aslan M, Sendogdu N, Ergun F, Yesilada E. Evaluation of the Hypoglycaemic Effect and Antioxidant Activity of the Three *Viscum album* subspecies (European mistletoe) in Streptozotocin Diabetic Rats. Journal Ethnopharmacology. **2005,** 98(1-2): 95-102, DOI: 10.1016/j.jep.2004.12.03.
16. Ohiri FC, Esimone CO, Nwafor SV, Okoli OO. Hypoglycemic Properties of *Viscum album* (mistletoe) in Alloxan-induced Diabetic Animals. Pharmaceutical Biology. **2003,** 41(3): 184-187, DOI: 10.1076/phbi.41.3.184.15098.
17. Harborne JB. Phytochemical Methods: a Guide to Modern Techniques of Plant Analysis (Third Ed). Springer Pvt Ltd, New Delhi, 2008.
18. Zhishen J, Mengcheng T, Jianming W. The Determination of Flavonoid Contents in Mulberry and Their Scavenging Effects on Superoxide Radicals. Food Chemistry. **1999,** 64(4): 555-559, DOI: 10.1016/S0308-8146(98)00102-2.
19. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Cioalteau Reagent. Method Enzymol. **1999,** 299: 152-178, DOI: 10.1016/S0076-6879(99)99017-1.
20. Kobaisy M, Tellez MR, Webber CL. Phytotoxic and Fungitoxic Activities of the Essential Oil of Kenaf (*Hibiscus cannabinus* L) Leaves and Its Composition. Journal of Agricultural Food and Chemistry. **2001,** 49(8): 3768-3771, DOI: 10.1021/jf0101455.
21. Sarker SD and Nahar L. Chemistry for Pharmacy Students. General, Organic. John Wiley and Sons Ltd.
22. Association of Official Analytical Chemists. Official Methods of Analysis of AOAC International (18th Ed). Association of Official Analytical Chemists, Washington DC, 2010.
23. Ursini F, Maiorino M, Morazzoni P, Roveri A, Pifferi G. A Novel Antioxidant (IdB 1031) Affecting Molecular Mechanisms of Cellular. Free Radical Biol Med. **1994,** 16: 547-553, DOI: 10.1016/0891-5849(94)90054-X.
24. Pulido R, Bravo L, Saura-Calixto F. Antioxidant Activity of Dietary Polyphenols as Determined by Modified Ferric Reducing/Antioxidant Power Assay. J Agric Food Chem. **2000,** 48: 3396-3402, DOI: 10.1021/jf9913458
25. Minotti G and Aus SD. An Investigation into the Mechanism of Citrate Fe^{2+} Dependent Lipid Peroxidation. Free Radical Biol Med. **1987,** 3: 379-387, DOI: 10.1016/0891-5849(87)90016-5.

26. Khan MS, Ansari IA, Ahmad S, Akhtar F, Hashim A and Srivastava AK. Chemotherapeutic Potential of *Boerhaaavia diffusa* Linn (Review). Journal of Applied Pharmaceutical Sciences. **2013,** 3(1): 133-139, DOI: 10.7324/JAPS.2013.30126.
27. Das S. Antimicrobial Activity Study of Ethanolic Extract of *Boerhaavia diffusa* Whole Plant. International Journal of Pharmacy and Life Sciences. **2012,** 3(10): 2006-2009.
28. Amadi B, Onuoha N, Amadi C, Ugbogu A and Duru M. Elemental, Amino Acid and Phytochemical Constitute of Fruit of Three Different Species of Eggplant. International Journal of Medicinal and Aromatic Plants. **2013,** 3(2): 200-203.
29. Asia A, Ghulam M, Munir AS, Muhammad S, Hang X. The Biochemical Significances of the Proximate, Mineral and Phytochemical Composition of Selected Vegetables from Pakistan. Mat Sc Pharm. **2017,** 1(1): 6-9, DOI: 10.26480/msp.01.2017.06.09.
30. Gala BV and Gujar V. Product Development, Biochemical, Antimicrobial and Organoleptic Analysis on (*Trigonella foenum graecum*) Fenugreek Seeds and Leaves. Plant Sciences Feed. **2014,** 4(2): 15-18.
31. Gbolahan D. Lesson Note on Medical Importance of Trace Elements. Centre for Natural Health Studies.
32. Akinyele IO and Shokunbi OS. Concentrations of Mn, Fe, Cu, Zn, Cr, Cd, Pb, Ni in Selected Nigerian Tubers, Legumes and Cereals and Estimates of the Adult Daily Intakes. Food Chemistry. **2015,** 173: 702-708, DOI: 10.1016/j.foodchem.2014.10.098
33. Fakankun OA, Babayemi JO and Utiaruk JJ. Variations in the Mineral Composition and Heavy Metals Content of *Moringa oleifera*. African Journal of Environmental Scinece and Technology. **2013,** 7(6): 372-279, DOI: 10.5897/AJEST12.228.
34. Ayoola PB. Trace Elements and Major Minerals of *Persea Americana, Magnifera indica* and *Cocos nucifera* Shell Charcoal. World Journal of Engineering, Pure and Applied Science. **2012,** 2(2): 81-86.
35. Oboh G. Antioxidant Properties of Some Commonly Consumed and Underutilized Tropical Legumes. European Food Research amd Technology. **2006,** 224: 61-65, DOI: 10.1007/s00217-006-0289-x.
36. Elmegeed GA, Ahmed HA, Hussein JS. Novel Synthesized Aminosteroidal Heterocycles Intervention for Inhibiting Iron-induced Oxidative Stress. European Journal of Medicinal Chemistry. **2005,** 40(12): 1283-1294.
37. Oboh G and Rocha JBT. Polyphenols in Red Pepper (*Capsicum annuum* var. aviculare (Tepin)) and Their Protective Effect on Some Pro-Oxindants induced Lipid Peroxidation in Brain and Liver In Vitro. European Food Research and Technology. **2007,** 225: 2, DOI: 10.1007/s00217-006-0410-1.
38. Livana-Pathiana CM and Shahidi F. Antioxidant Activity of Commercial Soft and Hard Wheat (*Triticum aestiiyium* L) as affected by Gastric pH conditions. Journal of Agricultural Food and Chemistry. **2005,** 53: 2433-2440, DOI: 10.1021/jf049320i.
39. Fraga CG and Oteiza PI. Iron Toxicity and Antioxidant Nutrients. Toxicology. **2002,** 180: 23-32, DOI: 10.1016/s0300-483x(02)00379-7.