COMPARATIVE *IN VITRO* ANTIOXIDANT ACTIVITY AND TOTAL PHENOLIC CONTENTS OF DIFFERENT PARTS OF *Vernonia amygdalina*

Supported by tetfund TERTIARY EDUCATION TRUST FUND

I. O. Okoro
Department of Biochemistry, Delta State University, Abraka, Nigeria
israelik@yahoo.com

Received: November 20, 2018 **Accepted:** May 21, 2019

Abstract: A comparative *in vitro* assessment of the leaves, stem and roots of *Vernonia amygdalina* was done in this study using acetone and methanol extracts by 1, 1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene bleaching, reducing power and ferric reducing antioxidant power (FRAP) assays. Additionally, the total phenolic content (TPC) and total flavonoids contents (TFC) of the extracts were determined. Results showed that *V. amygdalina* leaves acetone extract had the highest phenol (116.36 ± 5.03 mg GAE/g of extract) and flavonoids (78.38 ± 2.18 mg QE/g of extract) contents with correlated antioxidant potential. The extracts generally displayed potent DPPH, β-carotene bleaching, FRAP and reducing power in concentration dependent manner. Comparison of the two solvent used revealed that the extract of both solvents produced similar concentration dependent antioxidant activities. However, the acetone leave extract showed significantly ($p < 0.05$) greater antioxidant activity compared to all other extracts studied. Thus, this study has shown clearly that the extracts of *V. amygdalina* possessed antioxidant activity and could be a valuable therapeutic agent against oxidative stress.

Keywords: *In-vitro* antioxidant activity, total flavonoids, totals phenolic, *Vernonia amygdalina*

Introduction

A free radical is said to be an atom or molecule having unpaired electrons. Reactive oxygen species like superoxide anion (O2), peroxyl (ROO•), hydroxyl (OH•), hydroperoxyl (OOH•) and alkoxyl (RO•) are oxygen derivative free radicals (Halliwell and Gutteridge, 1999), which are made by our body and are used to stabilize natural function of the body, but surplus amount can result to cell or tissue damage (Sen *et al.,* 2010). Also, it could cause oxidative impairment to DNA, lipids, proteins and cause chronic diseases like diabetes, cancer, aging and further human degenerative diseases (Aiyegoro and Okoh, 2010).

An antioxidant refers to, any substance that inhibits or interrupts oxidative damage to a molecule. The specific mark of an antioxidant is the capability of scavenging free radicals as a result of their singlet oxygen quencher and redox hydrogen donators (Anokwuru *et al.,* 2011). Natural and synthetic antioxidants are capable of scavenging free radicals. An example of natural antioxidant is plants, while the synthetic ones include butylated hydroxyl toluene, tetra butyl hydro quinone and butylated hydroxyl anisol (Mbaebe *et al.,* 2012). However there are limitations to the usages of the common synthetic antioxidants due to side effects associated with them, hence the urgent need to find natural antioxidants as replacement for the synthetic antioxidants (Meenakshi *et al.,* 2011).

The plant kingdom has been the major source of natural antioxidants, but there is still not sufficient and adequate data and knowledge regarding the usefulness of these plants in practical terms. Secondary plant metabolites, phenolics, and flavonoids are usually found in numerous vegetables, fruits and herbs and have been revealed to be protective against oxidizing agents and free radicals induced oxidative stress (Sarikurkcu *et al*., 2009). Polyphenols (phenolic derivatives and flavonoids) are similarly known for their capability to inhibit fatty acids oxidative decay and further offer extra value to plants used, as food ingredients (Fecka *et al*., 2007).

Vernonia amygdalina, commonly known as bitter leaf, is of the Compositae family. It is a plant with reported great medicinal properties and it is also a main vegetable occupying an important position in the diet of numerous ethnic groups in Nigeria (Oriakhi *et al*., 2014). The medicinal uses of *Vernonia amygdalina* in Africa include treatment of diseases like malaria, diabetes, infertility, sexually transmitted diseases and gastrointestinal problems (Farombi and Owoeye, 2011). Also, Izevbigie *et al*. (2004) have reported the anthelmintic, antitumourigenic and antibacteria properties of *V. amygdalina* extracts. The plant also is used to help wound healing (Adetutu *et al.,* 2011). Additionally, the root is frequently used as chewing stick in Nigeria due to its positive effects on dental caries. The key bioactive constituents reported for the leaves are sesquiterpene lactones (Aregheore *et al*., 1998).

Although, the uses and medicinal values of several parts of the plant are well known and an ethno-botanical survey in parts of Nigeria of the herbs used by traditional medical practitioners showed that some of the respondents agreed to using the root rather than leaves extract of the plant as antidiabetic agent (Abo *et al*., 2007). However, available reports in the literature are majorly focused on the leaves, neglecting the other parts (the stem and roots) of the plant.

There are various extraction techniques for recovery of antioxidants from plants, like maceration, subcritical water extraction, Soxhlet extraction and ultrasound assisted extraction. Nevertheless, extraction yield and also antioxidant activity are said to depend on not only the extraction technique, but on the solvent used for extraction (Turkmen *et al.,* 2006). Therefore, this work was aimed to comparatively evaluate the total phenols content and *in vitro* antioxidant activities of *V. amygdalina* leaves, roots and stem, using acetone and methanol extracts by DPPH, β-carotene/linoleic acid and reducing power assays. Additionally, total flavonoids contents of the extracts were determined.

Materials and Methods

Collection, identification and preparation of plant

The leaves, stems and roots of *Vernonia amygdalina* were obtained from campus II of the Delta State University, Abraka and were identified at the Plant Science Department of the University of Benin, Edo State. They were washed and air dried for two weeks. Thereafter, they were pulverized into powder using mortar and pestle. The powdered sample (50 g) was extracted by maceration with 200 mL of methanol for 24 h along with intermittent shaking. This was followed with filtration and then concentration, using rotary evaporator under vacuum at 45°C to obtain the crude methanol extract used for the assays. The procedures above were repeated with acetone to obtain the acetone extract.

Total phenolic content determination

The total phenolic content (TPC) of the extracts of the different parts of the plant was determined according to the method of Singleton *et al*. (1999). The TPC result was stated as gallic acid equivalent (GAE).

Total flavonoid content determination

The determination of total flavonoid content (TFC) of the extracts of *V. amygdalina* was done by the method of Miliauskas *et al.* (2004), with the TFC calculated as mg quercetin equivalent /g of extract.

DPPH radical scavenging activity assay

The technique for DPPH Radical scavenging activity determination as reported by Brand-Williams *et al.* (1995) was used for this assay, using ascorbic acid as the standard.

β-carotene-linoleate bleaching assay

The antioxidant activity of *V. amygdalina* extracts was estimated using the procedure of β-carotene bleaching as described by Norhaizan *et al.* (2011).

Reducing power assay

The method of Oyaizu (1986) was used for evaluating the Reducing power of the two extracts, with ascorbic acid (AA) being used as standard.

Ferric reducing antioxidant power (FRAP) assay

The procedure as described by Benzie and Strain (1996) for the determination of FRAP was followed.

Statistical analysis

All tests were done in three replicates and the results presented as mean ± standard deviation (SD). The data obtained were statistically analyzed by one-way ANOVA with the level of statistical significance set at $p < 0.05$.

Results and Discussion

Numerous investigations have shown differences in the biological actions of extracts done with diverse extraction techniques. Thus, in trying to find the right extraction method and the solvent, factors such as sample matrix properties, matrix-analyte interaction and the chemical properties of analytes efficiency are often taken into consideration (Hayouni *et al*., 2007; Ishida *et al*., 2001). Previously, acetone, methanol, ethyl acetate and ethanol have been used for extracting phenolic compounds from plant materials (Alothman *et al*., 2009; Lafka *et al*., 2007).

In this study, total phenol content was determined in the leaves, roots and stem of *V. amygdalina* by the use of methanol and acetone. The total phenol content (TPC) of the extracts is shown in Fig. 1. The TPC values for the two extracts range from 88.37 mg GAE/g of methanol stem extract to 116.36 mg GAE/g of acetone leaves extract, and were found to decrease in the following order: Acetone-L > Acetone-R > Methanol-R> Acetone-S > Methanol-L > Methanol-S. Though the TPC of the acetone extracts were higher than their corresponding methanol extracts, but the level of TPC for the acetone extract were only significant ($p<0.05$) when Acetone-L vs. Acetone-S; Acetone-S vs. Acetone-R and Acetone-R vs. Methanol-L were compared. The results are in concord with the report of Michiels *et al*. (2012) in which they observed that acetone-based mixtures were more active for phenolic extraction yields than methanol-based mixtures from fruits and vegetables.

Similarly, Gonzalez-Montelongo *et al.* (2010) reported mixtures of acetone and water to be more active for extraction of total phenolic and for production of extracts from lyophilized banana peel with a corresponding high antioxidant capacity. However, the methanolic extract of *B. vahlii* leaves was observed by Sowndhararajan and Kang (2013) to show stronger antioxidant potential than those of chloroform, acetone and water extracts.

The Total flavonoids (TFC) of the methanol and acetone extracts for the leaves, stem and roots are reported in Fig. 2. The highest level of TFC was seen in the Acetone-L extract while the lowest level was seen in the Acetone-S extract. Similar to the TPC, a decrease in the following order was observed: Acetone-L > Acetone-R > Methanol-L > Methanol-R> Methanol-S > Acetone-S. Effect of the solvents on TFC was similar to what was seen in the TPC. The highest TFC was obtained in the acetone-L extract, followed by the Acetone-R extract. Thus, from the results of both TPC and TFC, the extracting solvents (acetone and methanol) were seen to significantly ($P < 0.05$) affect the measured contents of polyphenol (phenol and flavonoids).

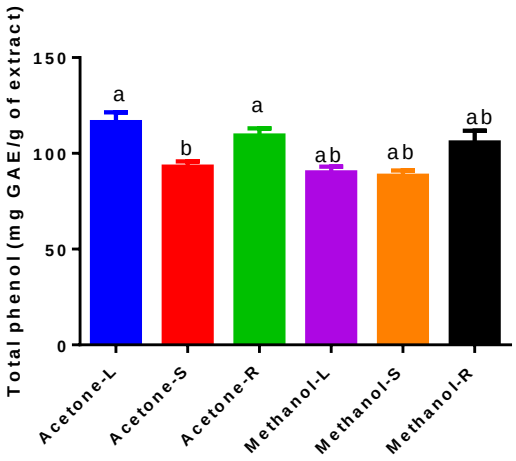

Fig. 1: Total phenol content of methanolic and acetone extracts of *V. amygdalina*

*Values are as mean ±SD of three determinations. Bars with different letters differ significantly ($p< 0.05$).**Acetone-L= Acetone leaves extract; Acetone-S=Acetone stem bark extract; Acetone-R-Acetone roots extract; Methanol-L= Methanol leaves extract; Methanol-S= Methanol stem bark extract; Methanol-R= Methanol roots extract; GAE= Gallic acid equivalent.

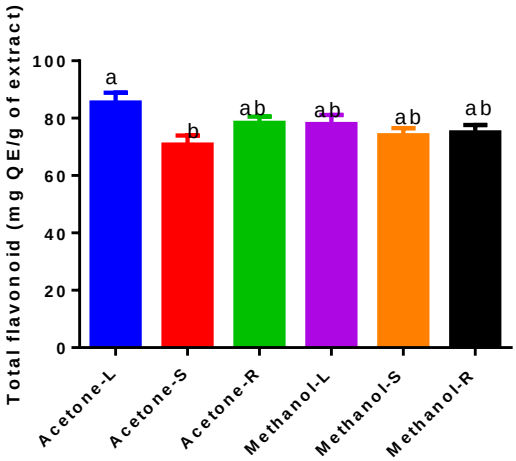

Fig. 2: Total flavonoid content of methanolic and acetone extracts of *V. amygdalinaI*

*Values are as mean ±SD of three determinations. Bars with different letters differ significantly ($p< 0.05$) **Acetone-L= Acetone leaves extract; Acetone-S=Acetone stem bark extract; Acetone-R-Acetone roots extract; Methanol-L= Methanol leaves extract; Methanol-S= Methanol stem bark extract; Methanol-R= Methanol roots extract; QE= Quercetin equivalent.

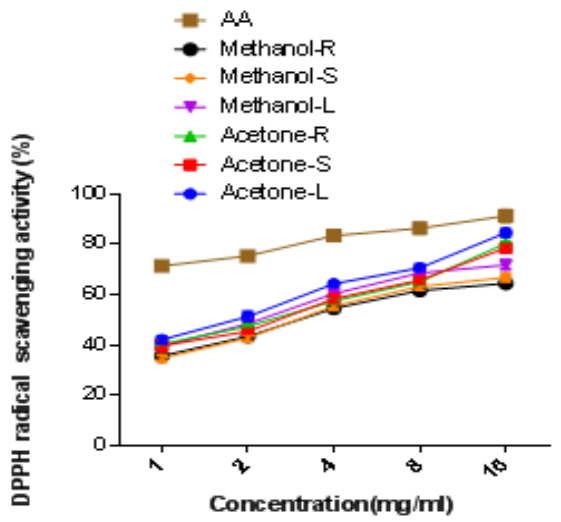

Fig. 3: DPPH radical scavenging activity of methanolic and acetone extracts of *V. amygdalina*

*Values are as mean ±SD of three determinations. **Acetone-L= Acetone leaves extract; Acetone-S=Acetone stem bark extract; Acetone-R-Acetone roots extract; Methanol-L= Methanol leaves extract; Methanol-S= Methanol stem bark extract; Methanol-R= Methanol roots extract; AA=Ascorbic acid.

The DPPH radical scavenging activity for extracts of *V. amygdalina* is shown in Fig. 3. The leaves extract obtained by acetone produced the highest DPPH radical scavenging activity at concentrations ranging from 42.01 L to 84.61 mg/mL. While the lowest yield was shown by Methanol-R extract with values ranging from 35.60 to 64.43 mg/mL. Comparatively, significant differences ($p< 0.05$) in the DPPH radical scavenging activity were observed in the following extracts/ standard at the lowest concentration (1.00 mg/mL): Acetone-L vs. Methanol-S; Acetone-L vs. Methanol-R; Acetone-L vs. AA; Acetone-S vs. Methanol-S; Acetone-S vs. Methanol-R; Acetone-S vs. AA; Acetone-R vs. Methanol-S; Acetone-R vs. Methanol-R; Acetone-R vs. AA; Methanol-L vs. Methanol-S; Methanol-L vs. Methanol-R; Methanol-L vs. AA; Methanol-S vs. AA; Methanol-R vs. AA. Similarly, significant differences ($p< 0.05$) were seen when all extracts/ standard were compared at the highest concentration (16 mg/ mL), except Acetone-S vs. Acetone-R and Methanol-S vs. Methanol-R. Also, the acetone-L extract gave high radical scavenging capacity comparable to the standard (at 16 mg/ mL). The observation in this study for the DPPH• free radical scavenging activity is similar to the report of (Thambiraj and Paulsamy, 2012; Vishnu *et al.*, 2013). Also a similar trend on DPPH radical scavenging activity had been reported for pineapple crude extract (Alothman *et al.*, 2009) and defatted wheat germ (Zhu *et al.*,2011). Higher flavonoids content was similarly seen in the leaves acetone extract and might have been responsible for the high scavenging activity, as flavonoids had been reported to be required for the scavenging activity of extracts (Senguttuvan *et al.*, 2014).

The result for antioxidant activity (%) in the β-carotene linoleate system is shown in Fig. 4. In this assay for β-carotene bleaching, linoleic acid generates hydroperoxides during incubation as free radicals. Antioxidants present in the extract thus minimize the oxidation of β-carotene by the hydroperoxides (Govindappa *et al.*, 2011). Thus, the antioxidant activity of extracts is a main determinant in the degradation of β-carotene linolate. In the present study, a major correlation was observed between the rate of β-carotene linolate degradation and phenolics and flavonoids content obtained, as the leaves acetone extract (with the greatest antioxidant capacity of 77.66%) also displayed the highest antioxidant activity, which may be as a result of these secondary metabolites. Among the extracts evaluated, leaf acetone and root acetone extracts had better activity (77.66% for leaf acetone and 72.66 for root acetone extracts). Also, the observed activity of the extracts was comparable to the activity of the standard used. The inhibitory capacity of the extracts/ standard was in the order: BHT > Acetone-L > Acetone-R > Acetone-S > Methanol-L > Methanol-R > Methanol-S.

The reducing power of the methanol and acetone extracts is shown in Fig. 5. The extracts demonstrated some degrees of electron-donating capacity according to concentrations. The best reducing power at the highest concentration (0.10 mg/mL) was found in the leaves acetone extract. Also, at the highest concentration, the standard and leaves acetone extract was statistically ($p < 0.05$) higher than all other extracts. Similarly, the value obtained for the Acetone-L extract was higher than that of all other extracts at the concentrations studied. While the lowest reducing power activity was shown by the Methanol-S extract.

It has been stated that the reducing power of extracts serves as a major indicator of its possible antioxidant activity (Oliveira *et al.*, 2008). The presence of reductones had been shown to produce antioxidant action by donating a hydrogen atom thereby breaking the free radical chain (Nair *et al.*, 2012). The reducing power in this study increased with corresponding increase in the extract concentrations, which may be a major indicator of the potential of the extracts' antioxidant activity. Thus, the results indicates that the leaves acetone extract possess high amount of reductones and so high antioxidant property (Senguttuvan *et al.*, 2014).

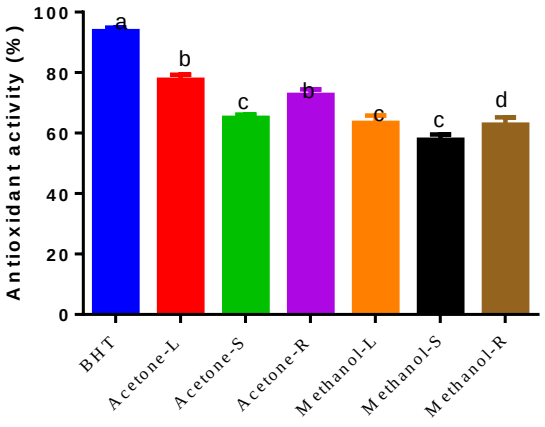

Fig. 4: Antioxidant activity (β-carotene-linoleate bleaching) of methanolic and acetone extracts of *V. amygdalina*

*Values are as mean ±SD of three determinations. Bars with different letters differ significantly ($p< 0.05$) **Acetone-L= Acetone leaves extract; Acetone-S=Acetone stem bark extract; Acetone-R-Acetone roots extract; Methanol-L= Methanol leaves extract; Methanol-S= Methanol stem bark extract; Methanol-R= Methanol roots extract. BHT= butylated hydroxyltoluene.

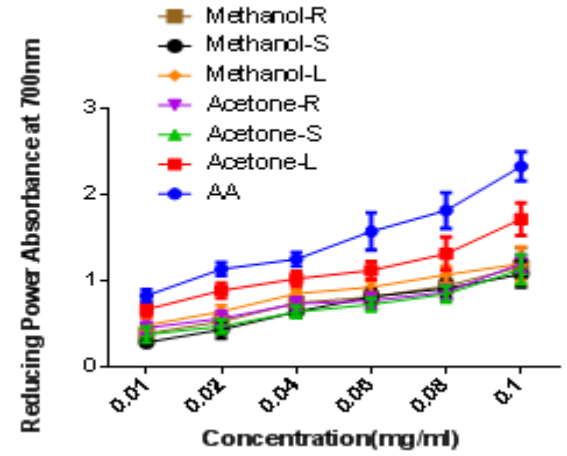

Fig. 5: Total reducing power of methanolic and acetone extracts of *V. amygdalina*

*Values are as mean ±SD of three determinations. **Acetone-L= Acetone leaves extract; Acetone-S=Acetone stem bark extract; Acetone-R-Acetone roots extract; Methanol-L= Methanol leaves extract; Methanol-S= Methanol stem bark extract; Methanol-R= Methanol roots extract; AA=Ascorbic acid.

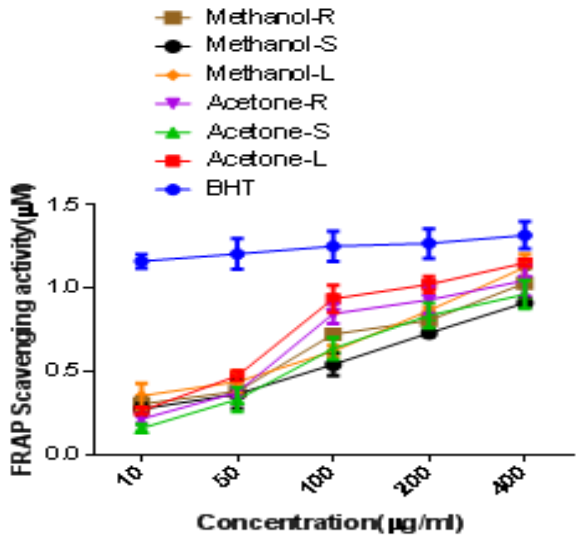

Fig. 6: Ferric reducing antioxidant power (FRAP) of methanolic and acetone extracts of *V. amygdalina*

*Values are as mean ±SD of three determinations. **Acetone-L= Acetone leaves extract; Acetone-S=Acetone stem bark extract; Acetone-R-Acetone roots extract; Methanol-L= Methanol leaves extract; Methanol-S= Methanol stem bark extract; Methanol-R= Methanol roots extract. BHT= butylated hydroxyltoluene

The FRAP of acetone and methanolic extracts of *V. amygdalina* is shown in Fig. 6. A significantly lower ($p < 0.05$) value of FRAP was noticed at 10 µg/mL for all extracts when compared with the standard used. However, there was a concentration dependent increase in activity of the extracts with the leaves acetone extract giving the highest activity at the highest concentration (400 µg/mL), among the extracts. Report suggests that plant phenolic compounds play significant role in determination of antioxidant properties and the general biological properties of plants (Pandey and Rizvi, 2009).

According to Kevers *et al*. (2007), a correlation exists between the antioxidant potential of plant and the phenolic content. Michiels *et al.* (2012) had similarly reported a correlation between the phenolic content and antioxidant capacity, which according to them varied from matrix to matrix. They reported positive and high connection between phenolic content and DPPH for apple and broccoli and for orange.

Comparison of the two extraction solvent on the one hand and the different parts (leaves, stems and roots) on the other hand revealed that the extract of both solvents produced similar concentration dependent antioxidant activities, however, the acetone leave extract seem to show significantly ($p < 0.05$) higher activity compared to all other extracts studied.

Several mechanisms like binding of transition-metal ion catalysts, radical scavenging, reductive capacity and preclusion of chain initiation, inhibition of continued hydrogen abstraction and decomposition of peroxides are reported to be responsible for the activity of antioxidants (Diplock, 1997; Yildirim *et al*., 2000).

Conclusion

This study revealed that the two solvents used for extraction have varied effects on the total phenol and antioxidant activities of the extracts, with the acetone extract of the leaves giving the highest total phenol content and antioxidant activity overall. Thus, on the basis of these results in terms of total phenol content, total flavonoid content, scavenging radicals, β-carotene bleaching, reducing power and ferric reducing antioxidant power, it is concluded that the extracts of *V. amygdalina* possessed antioxidant activity and that the leaves acetone extract could be a valuable therapeutic agent against oxidative stress.

Conflict of Interest

The author declares that there is none.

References

Aboa KA, Fred-Jaiyesimi AA & Jaiyesimi AEA 2008. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *J. Ethnopharmacol.*, 115: 67–7.

Adetutu A, Morgan WA & Corcoran O 2011. Ethnopharmacological survey and in vitro evaluation of wound healing plants used in South-western Nigeria. *J. Ethnopharmacol*, 137(1): 50–56.

Aiyegoro OA & Okoh AI 2010. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC *Complement Altern Med*, 10: 21.

Alothman M, Bhat R & Karim AA 2009. Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. *Food Chem.*, 115: 785-788.

Anokwuru CP, Esiaba I, Ajibaye O & Adesuyi AO 2011. Polyphenolic content and antioxidant activity of Hibiscus sabdariffa calyx. *Res. J. Med. Plant*, 5: 557-566.

Argheore EM, Makkar HPS & Becker K 1998. Feed value of some browse plants from the central zone of Delta State Nigeria. *Trop. Sci.*, 38: 97-104.

Benzie IFF & Strain JJ 1996. The ferric reducing ability of plasma as a measure of antioxidant power; the FRAP assay. *Anal. Biochem.*, 239: 70-76.

Brand-Williams W, Culvelier ME & Berset C 1995. Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft & Technologie*, 28: 25-30.

Diplock AT 1997. Will the good fairies please prove us that vitamin E lessens human degenerative disease? *Free Radic. Res.*, 27:511-532.

Farombi, EO & Owoeye O 2011. Antioxidative and chemopreventive properties of Vernonia amygdalina and Garcinia biflavonoid. *Int. J. Environ. Res*. Public Health, 8: 2533-2555.

Fecka I, Raj D & Krauze-Baranowska M 2007. Quantitative determination of four water-soluble compounds in herbal drug from Lamiaceae using different chromatographic techniques. *Chromatographia*, 66: 87-93.

Gonzalez-Montelongo R, Lobo MG & Gonzalez M 2010. Antioxidant activity in banana peel extracts: Testing extraction conditions and related bioactive compounds activity depends on the type and polarity of the extracting solvents the isolation procedures. *Food Chem*., 119, 1030-1039.

Govindappa M, Bharath N, Shruthi HB, Sadananda TS & Sharanappa P 2011. Antimicrobial, antioxidant and *in vitro* anti-inflammatory activity and phytochemical screening of Crotalaria pallida Aiton. *Afr. J. Pharm. Pharmacol.*, 5(21): 2359-2371.

Halliwell B & Gutteridge J 1999. Free Radicals in Biology and Medicine. 3rd ed. Oxford: Oxford University Press; p. 23-27.

Hayouni EA, Abedrabba M, Bouix M & Hamdi M 2007. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenica* L. fruit extracts. *Food Chem.*, 105: 1126-1134.

Ishida BK, Ma J & Bock C 2001. A simple rapid method for HPLC analysis of lycopene isomers. *Phytochem. Anal*., 12: 194-198.

Izevbigie EB, Bryant JL & Walker A 2004. A novel natural inhibitors of extracellular signal-regulated kinases and human breast cancer cell growth. *Experimental Bio. Med.*, 229: 163-169.

Kevers C, Falkowski M, Tabart J, Defraigne JO, Dommes J & Pincemail J 2007. Evolution of antioxidant capacity during storage of selected fruits and vegetables. *J. Agric. Food Chem.*, 55(21): 8596-8603.

Lafka TI, Sinanoglou V & Lazos ES 2007. On the extraction and antioxidant activity of phenolic compounds from winery wastes. *Food Chem.*, 104(3): 1206-1214.

Mbaebe BO, Edeoga HO & Afolayan AJ 2012. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of Schotia latifolia Jacq. *Asian Pac. J. Trop. Biomed.*, 2(2): 118-124.

Meenakshi S, Umayaparvathi S, Arumugam M & Balasubramanian T 2011. *In vitro* antioxidant properties of FTIR analysis of two sea weeds of Gulf of Mannar. *Asian Pac. J. Trop. Biomed.*, 1(Suppl 1): S66-S70.

Michiels JA, Kevers C, Pincemail J, Defraigne JO & Dommes J 2012. Extraction conditions can greatly influence antioxidant capacity assays in plant food matrices. *Food Chem.*, 130: 986-993.

Miliauskas G, Vensketonis PR & Van Beck TA 2004. Screening of radical scavenging of some medicinal and aromatic plant extracts. *Food Chem.*, 85: 231-237. http://dx.doi.org/10.1016/j.foodchem.2003.05.007

Nair VD, Paneerselvam R & Gopi R 2012. Studies on methanolic extract of Rawolfia species from Southern Western Ghats of India – *In vitro* antioxidant properties, characterization of nutrients and phytochemicals. *Ind. Crop Prod*., 39: 17-25.

Norhaizan ME, Ng SK, Norashareena MS & Abdah MA 2011. Antioxidant and Cytotoxicity Effect of Rice Bran Phytic Acid as an Anticancer Agent on Ovarian, Breast and Liver Cancer Cell Lines. *Mal. J. Nutr.*, 17(3): 367 – 375.

Oliveira I, Sousa A, Ferreira I, Bento A, Estevinho L & Pereira JA 2008. Total phenols, antioxidant potential and antimicrobial activity of walnut (*Juglans regia* L.) green husks. *Food Chem Toxicol*, 46: 2326-2331.

Oriakhi K, Oikeh EI, Ezeugwu N, Anoliefo O, Aguebor O & Omoregie ES 2014. Comparative antioxidant activities of extracts of Vernonia amygdalina and Ocimum gratissimum leaves. *J. Agric. Sci.*, 6: 13-20.

Oyaizu M 1986. Studies on product of browning reaction prepared from glucose amine. *Jpn. J. Nutr.*, 44: 307-315.

Pandey KB & Rizvi SI 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxid. *Med. Cell. Longevity*, 2: 270-278.

Sarikurkcu C, Arisoy K, Tepe B, Cakir A, Abali G & Mete E 2009. Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnus* Castus L. Fruits from Turkey. *Food Chem. Toxicol.*, 47(10): 2479-2483.

Sen S, Chakaraborty R, Sridhar C, Reddy Y & Biplab D 2010. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. *Int. J. Pharm. Sci. Rev. Res.*, 3(1): 91-100.

Senguttuvan J, Paulsamy S & Karthika K 2014. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, Hypochaeris radicata L. for in vitro antioxidant activities. *Asian Pac. J. Trop. Biomed.*, 4(Suppl 1): S359-S367.

Singleton VL, Orthofer R & Lamuela-Raventos RM 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods Enzymol*., 299: 152-178.

Sowndhararajan K & Kang SC 2013. Free radical scavenging activity from different extracts of leaves of *Bauhinia vahlii* Wight & Arn. *Saudi J. Biol. Sci.*, 20: 319-325.

Thambiraj J & Paulsamy S 2012. *In vitro* antioxidant potential of methanol extract of the medicinal plant, Acacia caesia (L.) Wild. *Asian Pac. J. Trop. Biomed.*, 2(Suppl 2): S732-S736.

Turkmen N, Sari F & Velioglu YS 2006. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and FolineCiocalteu methods. *Food Chem.*, 99: 835–841.

Vishnu R, Nisha R, Jamuna S & Paulsamy S 2013. Quantification of total phenolics and flavonoids and evaluation of *in vitro* antioxidant properties of methanolic leaf extract of *Tarenna asiatica* - An endemic medicinal plant species of Maruthamali hills, Western Ghats, Tami Nadu. *J. Res. Plant Sci.*, 2(2): 196-204.

Yildirim A, Mavi A, Oktay M, Kara AA, Algur OF & Bilaloglu V 2000. Comparison of antioxidant and antimicrobial activities of tilia (Tilia argentea Desf. Ex. D.C.) Sage (*Salvia triloba* L.) and black tea (*Camellia sinensis* L.) extracts. *J. Agric Food Chem.*, 48: 5030-5034.

Zhu KX, Lian CX & Guo XN 2011. Antioxidant activities and total phenolic contents of various extracts from defatted wheat germ. *Food Chem*, 126: 1122e6.