**International Research Journal of Natural and Applied Sciences**
ISSN: (2349-4077)
**Impact Factor**- 5.46, Volume 4, Issue 10, October 2017
**Website**- www.aarf.asia, **Email** : editor@aarf.asia , editoraarf@gmail.com

# PHOSPHOMOLYBDENUM ANTIOXIDATIVE POWER AND SCAVENGING ACTIVITY OF *MORINGA OLEIFERA* EXTRACT TOWARDS DPPH RADICAL.

**Okediran Ademola Philips[1] and Adeyemo Olaolu Elijah[2].**

[1, 2] Department of Science Laboratory Technology, Kwara State Polytechnic, Ilorin.

## ABSTRACT

*The differences in chemical composition of natural antioxidants provides challenges at separating, detecting, and quantifying individual non-enzymatic antioxidants in complex food/biological systems. Consequently, the antioxidant capacity is a better approach at assaying beneficial health effects that can result from the combined actions of individual antioxidant species. Antioxidant assay methods are based either on electron transfer (ET) or hydrogen atom transfer (HAT) mechanisms. The results obtained are different because of the mechanisms, redox potentials, and pH with reference to the solvent requirements of the various assay methods. Antioxidant activities of ethanolic extract of Moringa oleifera were evaluated and compared with that of caffeic acid using different in vitro methods of 1,1-diphenyl-2-picryl-hydrazyl free radical (DPPH) scavenging, and Phosphomolybdenum antioxidative power. At tested concentrations of 1mM and 2mM, caffeic acid showed an inhibition of 84.63% and 84.93% on the DPPH radical respectively, while Moringa oleifera extract gave 83.43% and 85.43% inhibition respectively. The extract showed significant antioxidant activities in the tested analytical methods. The study showed that extracts of the medicinal plant Moringa oleifera is a good scavenger of free radicals.*

**Keywords:** Antioxidant, Electron transfer, Hydrogen atom transfer, Non-enzymatic, Phosphomolybdenum.

## Introduction

A number of *in vitro* methods of assessing radical scavenging ability are available. Artificial species such as 2, 2´-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) [1], 1, 1-diphenyl-2-picrylhydrazyl (DPPH) [2], and *N*, *N*-dimethyl-*p*-phenylendiamine (DMPD) [3] have been used. The conditions of application may vary from one to another depending on pH, solvents, wavelength of measurement, which may lead to the achievement of different results [4]. These radicals are artificially generated and as such are not obtainable in vivo. Howbeit, they are useful in ranking antioxidant activity of food substances in which they occur. They may therefore be used in evaluating food antioxidant activity which is an indicator of the antioxidant potential prior to their consumption.

The importance of oxidation of foodstuffs in the human body cannot be underestimated as it is essential for the survival of cells [5]. A side effect of this oxidation is the production of free radicals and other reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet -}$), hydroxyl (•OH), peroxyl (ROO•), alkoxyl radicals (RO•), hydrogen peroxide ($H_2O_2$), singlet oxygen ($O_2{}^1\Delta g$) and reactive nitrogen species (RNS) mainly NO (nitric oxide), ONOO (peroxy nitrate), $NO_2$ (nitrogen dioxide) and $N_2O_3$ (dinitrogen trioxide). These may attack biological macromolecules, giving rise to protein, lipid, and DNA damage, cell aging, oxidative stress-originated diseases (e.g. cardiovascular and neurodegenerative diseases), and cancer [6, 7, 8, 9]. These free radicals affect the adjoining stable molecules by electron transfer. When this happens, the attacked molecule itself becomes another free radical setting up a chain of reactions, resulting in the destruction of the living cell [10]. Antioxidants scavenge or quench ROS and RNS products of respiration that can cause oxidative damage to cells [11, 12]. There is increasing evidence linking the involvement of such species with a variety of normal *in vivo* regulatory systems [13]. When ROS and RNS accumulates, they can disrupt the activities of protective enzymes such as superoxide dismutase, catalase and peroxidase and cause lethal cellular effects (e.g. senescence and apoptosis) by altering the normal structures of membrane lipids, cellular proteins, DNA and enzymes, thus down regulating cellular respiration [14]. Furthermore, ROS have been shown to influence cell signaling pathways [15, 16, 17]. Food items can also be oxidized resulting in chemical spoilage that leads to rancidity and /or deterioration of the

nutritional quality, color, flavor, texture and eventual safety of foods [18, 19, 20]. Evidence exists that half of the world's fruit and vegetable are lost due to postharvest deteriorations caused by oxidative reactions [21, 22, 23]. Defense mechanisms against the effects of excessive oxidations are augmented by the action of various natural antioxidants and thus the measurement of antioxidant activity is imperative [24, 25, 26]. Antioxidant activity relates to the kinetics of a reaction that takes place between an antioxidant and the prooxidant or the radical it reduces or scavenges, whereas antioxidant capacity determines the thermodynamic conversion efficiency of an oxidant probe upon reaction with an antioxidant [27, 28].

Any substance whose presence, at relatively low concentration, in comparison to those of the oxidizable substrate, reduces the oxidation of the substrate is referred to as an antioxidant [29, 30, 31]. They are classified as either primary/ chain-breaking antioxidants, or secondary/preventative antioxidants [32].

The methods of assessing antioxidant activity fall into two broad categories based on either activity in foods or bioactivity in humans [33]. There is the increasing need to assess the ability of the antioxidant(s) to provide protection for the food against spoilage that may result from oxidation and reduction reactions. The principles of other assay methods of determination of antioxidant activity involves measurement of activity in foods, particularly fruits, vegetables and beverages, bearing in mind the need to access the dietary burden as a result of *in vivo* activity of the active antioxidants present [34]. Oxidative stress in humans arises from an imbalance in the antioxidant status (ROS and RNS levels) in reference to defense and repair mechanisms [35, 36]. The body's endogenous defense machinery include enzymes such as superoxide dismutase, catalase and glutathione peroxidase, vitamin E, uric acid and serum albumins [37, 38]. In addition to these, food-derived antioxidants has been found to be important as it complements the body's endogenous defense machinery [39, 40]. The sum of endogenous and food-derived antioxidants represents the total antioxidant activity of the extracellular fluid [41]. Cooperation of all the different antioxidants provides greater protection against attack by reactive oxygen or nitrogen radicals, than any single compound alone. The overall antioxidant capacity gives a more relevant biological information compared to that obtained by the measurement of individual components, as it considers the cumulative effect of all antioxidants present in plasma and body fluids [42].

Antioxidants can deactivate radicals by two major mechanisms: Hydrogen Atom Transfer (HAT) and Single Electron Transfer (SET) [43, 44, 45]. Regardless of mechanism, the end result is the same, differing only in kinetics and potential for side reactions [46, 47]. Both reactions can occur in parallel, with the mechanism dominating a given system being determined by antioxidant structure and properties, solubility and partition coefficient, and solvent system [48, 49]. Ultimately, bond dissociation energy (BDE) and ionization potential (IP) are major factors that determine the mechanism and the efficacy of antioxidants [50, 51].

An organism's defense against the attack of ROS and RNS is provided by antioxidants and lack of equilibrium between free radicals and antioxidants leads to oxidative stress [52] which is a disturbance in the prooxidant-antioxidant balance in favor of the prooxidant that leads to potential damage. The term antioxidant is reserved for any compound, whose presence in low concentrations, has the ability to block or significantly delay the reaction of a substrate with molecular oxygen or ROS/RNS. ROS, RNS and RSS that result from the respirative cycle of oxidative phosphorylation may attack biological macromolecules like cellular DNA, giving rise to single- and double-strand breaks that may eventually cause cell ageing, cardiovascular diseases, mutagenic changes and cancerous tumor growth. When natural defenses of the organism (of enzymatic, non-enzymatic or dietary origin) are overwhelmed by an excessive generation of ROS/RNS, a situation of oxidative stress occurs, in which cellular and extracellular macromolecules (proteins, lipids and nucleic acids) can suffer oxidative damage, causing tissue injury. It has been established that the consumption of foods rich in natural antioxidants provides an efficient way of combating tissue injuries, undesired transformations and prevent health risks [53].

Some other compounds, known as retarder molecules, have the ability to reduce the rate of oxidation only when they are present in high concentrations and are often wrongly reported as antioxidants [54]. Consequently, in addressing the assessment of antioxidant effect, factors such the difference between antioxidant capacity and antioxidant activity are of primary importance. Considering antioxidant activity as the reaction between a single antioxidant species and the free radical, the antioxidant capacity is the reaction between an antioxidant solution, containing a mixture of antioxidant compounds and the radical [55]. While antioxidant activity is defined as the rate constant of the reaction between a unique antioxidant and a given free radical, antioxidant capacity is the number of moles of free radical scavenged by an antioxidant testing

solution that could lead to a different result for the same radical [56].The potential self-reactivity of radicals derived from parent antioxidants, versus the reaction products [57]. The specificity of the antioxidant compound towards the free radical as there are no universal antioxidants able to efficiently quench any type of reactive oxygen species [58].

Many studies have been conducted in the field of free radicals, oxidative stress and antioxidant activity of food. This gives antioxidants a prominent beneficial role, and regardless of the quantity ingested, the absorption is very limited, and in some cases prooxidants have been shown to be beneficial to human health [59,60]. The determination of antioxidant activity and specific antioxidant compounds can be achieved with the use of a large number of different assays, all of them with advantages and disadvantages [61] . Interest in antioxidants, particularly those intended to prevent the presumed deleterious effects of free radicals in the human body, as well as the deterioration of fats and other constituents of foodstuffs has increased in recent years [62, 63] .

**Methodology**

The sample of the plant is sourced in Ilorin, Kwara State, North Central Nigeria, where it grows as an ornamental plant. The chemical substances used in the experiments were all of analytical reagent grade. The visible spectra and absorption measurements were recorded in matched cuvettes using *Genesys 10S UV-VIS* spectrophotometer.

Fresh leaves of *Moringa Oleifera* were collected and dried under shade. 1g of the leaves was homogenized in 10ml of the solvent. The residue was weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain the desired concentration. Aqueous extracts were prepared fresh.

Stock solutions were prepared by dissolving a weighed amount in a total volume of 10ml ethanol to give 10mM concentration. Working solutions of 1mM and 2mM concentrations were prepared from the 10mM stock solutions for each assay [64]. All other reagents used were prepared by accurate dilutions from stock solutions. Reactions were carried out in triplicate.

**DPPH radical scavenging activity**

The free radical scavenging activity of the extract was measured by the 1, 1-diphenyl-2-picryl-hydrazil (DPPH•) wherein the bleaching rate of a stable free radical, DPPH• is monitored at a characteristic wavelength in the presence of the sample [65]. In its radical form, DPPH• absorbs at 517nm, but upon reduction by an antioxidant or a radical species its absorption decreases. Briefly, 0.1mM solution of DPPH• in ethanol was prepared and 1ml of this solution was added to 3ml of the extract solution in ethanol at different concentrations (1mM and 2mM). After 30minutes, the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity.

% inhibition calculated as: Scavenging effect (%) = [(OD of control-OD of sample) / (OD of control)] ×100.

The DPPH• radical displays an intense UV-VIS absorption spectrum. In this test, a solution of radical is decolorized after reduction with an antioxidant (AH) or a radical (R•) in accordance with the following scheme: DPPH• + AH → DPPH•-H + A•, DPPH• + R• → DPPH•-R.

**Phosphomolybdenum antioxidative power assay**

This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide[66]. The Phosphomolybdenum blue complex obtained is oxidized by the addition of nitrite and this causes a reduction in intensity of the blue color. Caffeic acid, 0.1ml (1mM - 2mM) was mixed with the Phosphomolybdenum reagent (1ml), (containing 0.6M $H_2SO_4$, 28mM $Na_2SO_4$ and 4mM ammonium molybdate). The mixture was incubated in a water bath for 90minutes at 95°C. Samples were cooled after the incubation and OD measured at 695nm against a blank in triplicate and percentage inhibition calculated according to:

Inhibition % = (1- OD of sample / OD of control) × 100

**Results**

The two *in vitro* methods presented in this study are based on the ability of antioxidants to reduce a radical cation / anion which can be monitored spectrophotometrically by determining decrease in absorbance. The ions monitored included 1, 1-diphenyl-2-picryl-hydrazil (DPPH) and Phosphomolybdenum.

**DPPH free radical scavenging activity**

Radical scavenging activities are very important due to the destructive role of free radicals in foods and in biological systems. The DPPH radical scavenging activity is generally quantified in terms of inhibition percentage of the pre-formed free radical by antioxidants. In the DPPH assay, the samples were able to reduce the stable DPPH radical to the yellow colored, diamagnetic diphenyl-picrylhydrazine, indicating their abilities to scavenge DPPH radical.

Figure 1.1 shows the percentage DPPH scavenging activity of 84.63% for caffeic acid and 83.43% for the extract at 1mM concentration. At 2mM concentration, the values obtained are 84.93% for Caffeic acid and 85.44% for the ethanolic extract of *Moringa oleifera*.

**Phosphomolybdenum Antioxidative Power (PAP) Assay**

PAP assay measures the reduction degree of Mo (VI) to Mo (V). It is a quantitative method to investigate the reduction reaction rate among antioxidant, oxidant and molybdenum ligand involving thermal generation of auto-oxidation during prolonged incubation period at higher temperature and acidic pH. It gives a direct estimation of reducing capacity of an antioxidant.

Being distinctive from FRAP and CUPRAC assays it remains intact irrespective of concentration of free metal ions and unlike CUPRAC and FRAP, it forms a green Phosphomolybdenum complex without induction of free metal ions in solution thus making it unique among *in vitro* antioxidant assays methods [67].

The result of this assay is presented in Fig.1.2. Caffeic and *Moringa oleifera* extract at 1mM concentration shows percentage inhibition of the phosphomolybdate ion of 67.81% and 71.90% respectively. At 2mM concentrations, the percentage values are 56.02% for Caffeic acid and 65.37% for the extract, an indication that *Moringa oleifera* extract is a better scavenger of the phosphomolybdate radical than caffeic acid in a concentration- dependent manner.

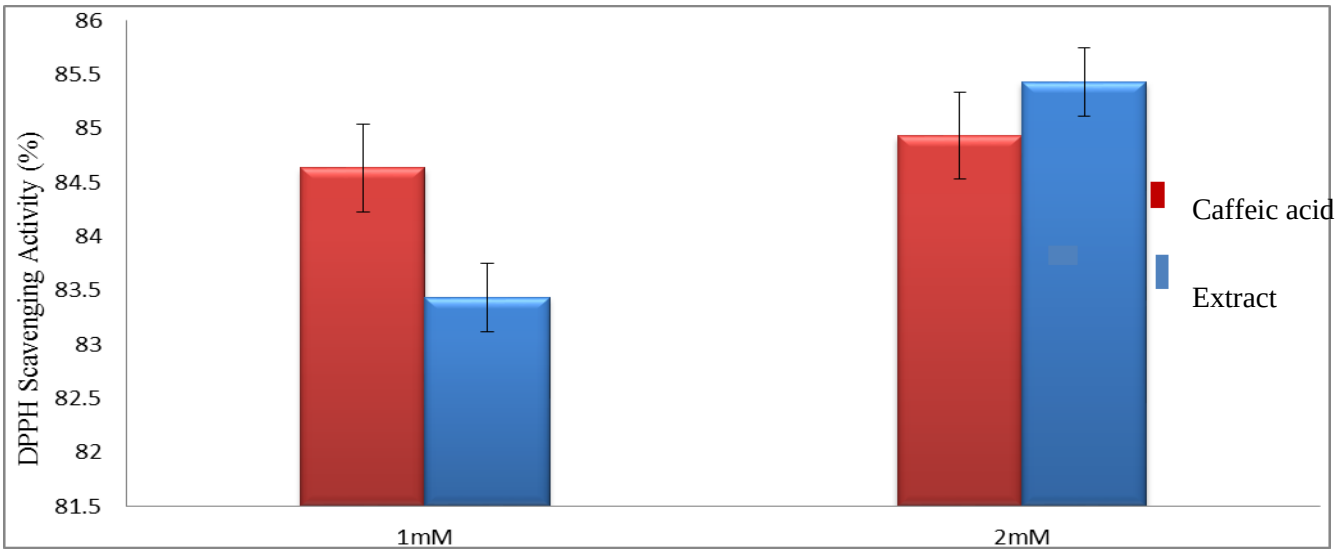


**Figure 1.1** DPPH free radical scavenging activity of caffeic acid and *Moringa oleifera* extract.

Solutions were prepared at 1mM and 2mM concentrations. DPPH (1ml, 0.1mM) solution was added to 3ml each of the aliquot of the solutions to make a total volume of 4ml reaction mixture. Each value is the average of three experiments with error bars indicating SEM.

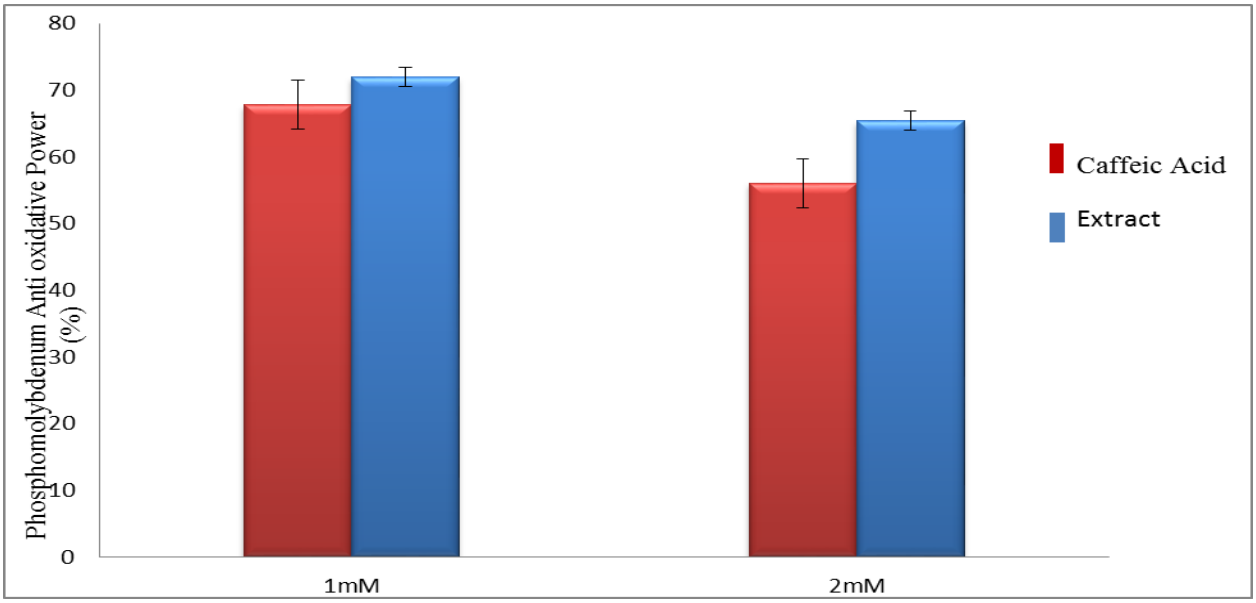


**Figure 1.2** Phosphomolybdenum antioxidative power of caffeic acid and *Moringa oleifera* extract.

Phosphomolybdenum solution, (1ml), was added to 0.1ml of Caffeic acid and the extract at molar concentrations of 1mM and 2mM. Each bar represents the mean ± SEM of experiments carried out in triplicates.

**Conclusions**

Nowadays, there seems to be an increased global interest in the identification and isolation of pharmacologically potent antioxidant compounds from natural sources, with low mammalian toxicity. The increase in the occurrence of different chemicals, pesticides, pollutants, coupled with smoking and alcohol intake and even some synthetic medicines has increased the chance of free radical-based diseases. Plants produce large amounts of secondary metabolites that may prevent oxidative stress, thus presenting a potential source of new compounds with antioxidant activity. Increasing knowledge of antioxidant phytochemicals and their inclusions can sufficiently support the human body's ability to combat diseases. In recent times, herbal medicines have become important in managing pathological conditions of diseases caused by free radicals. It is therefore imperative to explore and identify our traditional therapeutic knowledge of plant sources and interpret according to recent advancements at fighting oxidative stress, in order to accord it a deserving place.

The most effective way to eliminate free radicals that cause oxidative stress is with the help of antioxidants as they combat free radical-induced tissue damage by preventing the formation of radicals, scavenging them, thereby promoting their decomposition. Many synthetic drugs protect against oxidative damage, but they have adverse side effects. An alternative solution to the problem is the consumption of natural antioxidants from food supplements and traditional medicines. In almost all the traditional systems of medicine, medicinal plants play a major role and constitute their backbone. Prevention is a more effective strategy than treatment of chronic diseases. The incorporation of herbs into everyday meals may be beneficial as a diet in which culinary herbs are included generously provides a variety of active phytochemicals that could promote good health by protecting tissues against $O_2$- induced damage thereby prevent the onset of chronic diseases. Medicinal plants are the most important source of life saving drugs for the majority of the world's population.

The results obtained shows that the leaves of Moringa Oliefera possess considerable quantities of non-enzymatic antioxidants. Extracts of the leaves effectively scavenged or inhibited all the radicals tested.

It is evident from the study that extracts of the leaves of this plant exhibit effective antioxidant and radical scavenging activity *in -vitro,* implying they can be used in pharmacological and food industries due to its antioxidant properties.

**Acknowledgements**

The authors would like to express our sincere appreciation to *Tetfund* for grant-aiding this research work through Kwara State Polytechnic, Ilorin. Nigeria.

**References**

1. Chang, H.-C Chen, I.-C.; Yang, H.-W.; Chen, G.-L. Evaluation of total antioxidant activity of several popular vegetables and Chinese herbs: A fast approach with ABTS/H2O2/HRP system in microplates. *J. Food Drug Anal., 12,* 29-33. 2004.

2. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin *J. Sci. Technol. 26: 211-219.* 2003.

3. Fogliano V., V. Verde, G. Randazzo, A. Ritieni. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *J. Agric. Food Chem. 47,* 1035-1040 .1999.

4. P. Prieto, M. Pineda, M. Aguilar, Spectophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal. Biochem. 269,* 337–341. 1999.

5. Huang D., Ou B., Prior R.L. The chemistry behind antioxidant capacity assays. *J Agric Food Chem. 53,* 1841–56. 2005.

6. Ames, B.N., Shigenaga, M.K., Hagen, T.M. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. 90,* 7915–7922.1993.

7. Halliwell, B., Aeschbach, R., Lo Liger, J., Arouma, O.I. The characterization of antioxidants. *Food Chem. Toxicol. 33,* 601–617. 1995.

8. Ghiselli A., Serafini M., Natella F., Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. *Free Radic. Biol. Med. 29,* 1106–14. 2000.

9. Gulcin, I.; Bursal, E.; Sehitoglu, M.H.; Bilsel, M.; Goren, A.C. Polyphenol contents and antioxidant activity of lyophilized aqueous extract of *propolis* from Erzurum, Turkey. *Food Chem. Toxicol*. *48*, 2227-2238. 2010.

10. Frenkel E.N., German J.B. Antioxidants in foods and health: problems and fallacies in the field. *J. Sci. Food Agric.*86, 1999–2001. 2006.

11. Helmut Sies. Total Antioxidant Capacity: Appraisal of a Concept. *J. Nutr*. *137*, 1493–1495. .2007

12. Cao, G. H.; Alessio, H. M.; Cutler, R. G. Oxygen-radical absorbency capacity assay for antioxidants. *Free Radical Biol. Med. 14 (3),* 303-311. 1993.

13. Pulido, R.; Bravo, L.; Saura-Calixo, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *J. Agric. Food Chem*. *48*, 3396-3402. 2000.

14. Hopper, L., Cassidy, A. A review of the health care potentials of bioactive compounds. *J. Sci. Food Agric*. *86*, 1805–1813. 2006.

15. Vinson, J.A., Hao, Y., Su, X., Zubik, L. Phenol antioxidant quantity and quality in foods: vegetables. *J. Agric. Food Chem*. *46*, 3630–3634. 1998.

16. Kaneyuki, T., Noda, Y., Traber, M.G., Mori, A., Packer, L. Superoxide anion and hydroxylradical scavenging activities of vegetable extracts measured using electron spin resonance. *Biochem. Mol. Biol. Int*. *47*, 979–989. 1999.

17. Lee J., Durst, R.W., Wrolstad, R.E. Determination of total monomeric anthocyanin Pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: collaborative study. *J. AOAC Int*. *88*, 1269–1278. 2005.

18. Oyaizu, M. Studies on product of browning reaction prepared from glucoseamine. *Jpn*. *J. Nutr*. *44*, 307–315. 1986.

19. Dinis, T.C.P., Madeira, V.M.C., Almeida, L.M. Action of phenolic derivative (Acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Arch. Biochem. Biophys*.*315*, 161–169. 1994.

20. Fuleki, T., Francis, F.J. Determination of total anthocyanin and degradation index for Cranberry juice. *Food Sci*. *33*, 78–83. 1968.

21. Zhu, Q.V., Hackman, R.M., Jodilensunsa, X.X., Holt, R.R. Keen,C.L. Antioxidative activities of Oolong tea. *J. Agric. Food Chem*. *50*, 6229– 6934. 2002.

22. Brand Williams, W., Cuvelier, M.E., Berset, C. Use of free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol*. *28*, 25–30. 1995.

23. Badarinath A.V., K. Mallikarjuna , C.Madhu Sudhana Chetty, S. Ramkanth, T.V.S Rajan, K.Gnanaprakash. A Review on *In-vitro* Antioxidant Methods: Comparisons, Correlations and Considerations. *International Journal of Pharm. Tech Research 2*, 1276-1285. 2010.

24. Milan C, Hana C, Petko Denev, Maria Kratchanova, Anton Slavov, Antonín Lojek. Different methods for control and comparison of the antioxidant properties of vegetables. *Food Control 21*, 518–523. 2010.

25.Verica Dragovic -Uzelac ,Branka Levaj, Danijela Bursac ,Sandra Pedisic ,Ivana Radodci,

Ante Bisko. Total Phenolics and Antioxidant Capacity Assays of Selected Fruits. *Agriculturae Conspectus Scientificus 72 (4)*, 279-284. 2007.

26. Andreas R., Jeremy P. E. Spencer, Gunter K., Ulrich Hahn, Catherine A. Rice-Evans. Novel Biomarkers of the Metabolism of caffeic acid derivatives in vivo. *Free Radical Biology & Medicine 30 (11)*, pp. 1213–1222. 2001.

27. Max Romero , Benjamin Rojano , Jaime Mella-Raipán , Carlos David Pessoa-Mahana ,Eduardo Lissi, Camilo López-Alarcón. Antioxidant Capacity of Pure Compounds and Complex Mixtures Evaluated by the ORAC-Pyrogallol Red Assay in the Presence of Triton X-100 Micelles. *Molecules 15*, 6152-6167. 2010.

28. Alam Nur, Nusrat Jahan Bristi, Md. Rafiquzzaman. Review on *in vivo* and *in vitro*

methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal 21*, 143–152.

2013.

29. Apak, R., Kubilay Güçlü , Birsen Demirata , Mustafa Özyürek , Saliha Esin Çelik , Burcu Bektaşoğlu, K. Işıl Berker, Dilek Özyurt. Comparative Evaluation of Various Total

Antioxidant Capacity Assays Applied to Phenolic Compounds with the CUPRAC Assay. *Molecules 12*, 1496-1547. 2007.

30. Garry Clarke, Kang Nee Ting, Christophe Wiart, Jeffrey Fry. High Correlation of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging, Ferric Reducing Activity Potential and Total Phenolics Content Indicates Redundancy in Use of All Three Assays to Screen for Antioxidant Activity of Extracts of Plants from the Malaysian Rainforest. *Antioxidants 2*, 1-10. 2013.

31. Heng-Yuan Chang, Yu-Ling Ho, Ming-Jyh Sheu, Yaw-Huei Lin, Mu-Chuan Tseng,Sheng-Hua Wu Guan-Jhong Huang, Yuan-Shiun Chang. Antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts. *Botanical Studies* 48, 407-417. 2007.

32. Doughari,J.H. . Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents, Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, Dr. Venketeshwer Rao (Ed.), ISBN: 978-953-51-0296-0, InTech. 2012.

33. Rong Tsao. Chemistry and Biochemistry of Dietary Polyphenols. *Nutrients 2*, 1231- 1246. 2010.

34. Devanand L. Luthria, Sudarsan Mukhopadhyay, Donald T. Krizek. Content of total phenolics and phenolic acids in tomato (*Lycopersicon esculentum Mill*.) fruits as influenced by cultivar and solar UV radiation. *Journal of Food Composition and Analysis 19*, 771 – 777. 2006.

35. Terao, J., Hideyuki Karasawa, Hirofumi Arai, Akihiko Nagao, Tetsuya Suzuki & Kozo Takama. Peroxyl Radical Scavenging Activity of Caffeic Acid and Its Related Phenolic Compounds in Solution. *Bioscience, Biotechnology, and Biochemistry*, *57* (7), 1204-1205. 1993.

36. Jiang Hong Chen, Chi-Tang Ho. Antioxidant Activities of Caffeic Acid and Its Related Hydroxycinnamic Acid Compounds. *J. Agric Food Chem. 45*, 2374-2378. 1997.

37. Stalikas C.D. Extraction, separation, and detection methods for phenolic acids and flavonoids. *J. Sep. Sci. 30*, 3268 – 3295. 2007.

38. Gupta, D. Methods for Determination of Antioxidant Capacity: A Review. *Int J Pharm Sci Res*; *6 (2*), 546-66. 2015.

39. Mellina Damasceno, Rachid Santos, Aline de Paula Vitor, Jailton da Costa Carneiro, Domingos Sávio Campos Paciullo, Renato Camargo Matos, Maria Auxiliadora ,Costa Matos. Use of Ultrasound Bath in the Extraction and Quantification of Ester-Linked Phenolic Acids in Tropical Forages. *American Journal of Analytical Chemistry* 2, 344-351. 2011.

40. Moon, J. K.; Shibamoto, T. Antioxidant assays for plant and food components. *Journal of Agricultural and Food Chemistry 57 (5),* 1655-1666. 2009.

41. Khoddami, A., Meredith A. Wilkes, Thomas H. Roberts. Techniques for Analysis of Plant Phenolic Compounds. *Molecules 18*, 2328-2375. 2013.

42. MacDonald-Wicks, L. K.; Wood, L. G.; Garg, M. L. Methodology for the

determination of biological antioxidant capacity *in vitro*: A review. *J. Sci. Food Agric*. *86* 2046–2056. 2006.

43. Ronald L. Prior, Xianli Wu, and Karen Scaich. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *J. Agric. Food Chem*. *53*, 4290−4302. 2005.

44. Michael Antolovich, Paul D. Prenzler, Emilios Patsalides, Suzanne McDonald, Kevin Robards. Methods for testing antioxidant activity. *Analyst*, *127*, 183–198. 2002.

45. Apak, R., Shela Gorinstein, Volker Böhm, Karen M. Schaich, Mustafa Özyürek, Kubilay Güçlü. Methods of measurement and evaluation of natural antioxidant capacity/activity (IUPAC Technical Report). *Pure Appl. Chem*. *85, (5)* 957–998. 2013.

46. Jayanthi R., P. Subash. Antioxidant Effect of Caffeic Acid on Oxytetracycline Induced Lipid Peroxidation in Albino Rats. *Ind J Clin Biochem. 25 (4),* 371–375. 2010.

47. Charalampos Proestos, Konstantina Lytoudi, Olga Konstantina Mavromelanidou, Panagiotis Zoumpoulakis, Vassileia J. Sinanoglou. Antioxidant Capacity of Selected Plant Extracts and Their Essential Oils. *Antioxidants 2,* 11-22. 2013.

48. Tchinda, A.T., G.A. Agbor, D.E. Tsala, A.J.G. Yaya, E.N. Nga, E. Talla, J.N. Wauters, M. Frédérich. Antioxidant Activity of Flavonoids Isolated from the Fruits of *Xylopia parviflora* (A. Rich.) Benth. *International Journal of Pharmaceutical Sciences and Drug Research 6 (4)*, 323-328. 2014.

49. Simona Carmen Litescu, Sandra Eremia, Gabriel Lucian Radu. Methods for the Determination of Antioxidant Capacity in Food and Raw Materials Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors edited by Maria Teresa Giardi, Giuseppina Rea and Bruno Berra. 2010.

50. Rabeta, M.S., Nur Faraniza, R. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*. *International Food Research Journal 20 (4),* 1691-1696. 2013.

51. Jagtap, U.B., Panaskar, S.N., Bapat, V.A. Evaluation of Antioxidant Capacity and Phenol Content in Jackfruit (*Artocarpus heterophyllus* Lam.) Pulp. *Plant Food Hum. Nutr*. *65*, 99-104. 2010.

52. Sies H. Oxidative stress: from basic research to clinical application. *Am J Med.91* (3C), 31-38. 1991.

53. Halliwell B, Gutteridge J.M.C. Antioxidant defenses: endogenous and diet derived in Free Radicals in Biology and Medicine. 4th Ed. Oxford University Press: Clarendon. pp 79-81 2007.

54. Velioglu, Y.S.; Ekici, L.; Poyrazoglu, E.S. Phenolic Composition of European Cranberrybush (Viburnum opulus L.) Berries and Astringency Removal of Its Commercial Juice. *Int. J. Food Sci. Techol*. *41*, 1011–1015. 2006.

55. Prior, R.L., Cao G. *In vivo* total antioxidant capacity: comparison of different analytical methods. *Free Rad Biol and Med*. *27*, 1173-1181. 1999.

56. Shon, M.Y., Choi, S.D., Kahng, G.G., Nam, S.H.,Sung, N.J. Antimutagenic, Antioxidant and Free Radical Scavenging Activity of Ethyl Acetate Extracts From White, Yellow and Red Onions. *Food Chem. Tox*. 42, 659-666. 2004.

57. Pollyanna A. S. White, Rita C. M. Oliveira, Aldeidia P. Oliveira, Mairim R. Serafini ,Adriano A. S. Araújo, Daniel P. Gelain, Jose C. F. Moreira, Jackson R. G. S. Almeida, Jullyana S. S. Quintans, Lucindo J. Quintans-Junior, Marcio R. V. Santos. Antioxidant Activity and Mechanisms of Action of Natural Compounds Isolated from Lichens: A Systematic Review. *Molecules 19*, 14496-14527. 2014.

58. Simona Carmen Litescu, Sandra Eremia, Gabriel Lucian Radu. Methods for the Determination of Antioxidant Capacity in Food and Raw Materials Bio-Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors edited by Maria Teresa Giardi, Giuseppina Rea and Bruno Berra.2010.

59. Rice-Evans, C.A., Miller N.J., Paganga, G. Antioxidant properties of phenolic compounds. *Trends Plant Sci. 2*, 152–59. 1997.

60. Gupta, D. Methods for Determination of Antioxidant Capacity: A Review. *Int J Pharm Sci Res*; 6 (2), 546-66. 2015.

61. Mohammed Sulaiman, Hamzat Ibiyeye Tijani, Bashir Mohammed Abubakar, Saidu Haruna, Yusuf Hindatu, Jibrin Ndejiko Mohammed, Abdulrahman Idris. An Overview of Natural Plant Antioxidants: Analysis and Evaluation. *Advances in Biochemistry*. *1 (4)*, 64-72. 2013.

62. Pisoschi A.M., Negulescu G.P. Methods for Total Antioxidant Activity Determination: A Review. *Biochem & Anal Biochem 1*, 106. 2011.

63. Gupta, D. Methods for Determination of Antioxidant Capacity: A Review. *Int J Pharm Sci Res*; *6 (2)*, 546-66. 2015.

64. Adjimani, J.P., Prince A. Antioxidant and free radical scavenging activity of iron chelators. *Toxicology Reports 2*, 721–728. 2015.

65. Gulcin, I. Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology 217*, 213–220. 2006.

66. Prior, R.L., Cao G. *In vivo* total antioxidant capacity: comparison of different analytical methods. *Free Rad Biol and Med. 27*, 1173-1181. 1999.

67. Bruno Moukette Moukette, Constant Anatole Pieme, Jacques Romain Njimou, Cabral Prosper Nya Biapa, Bravi Marco, Jeanne Yonkeu Ngogang. *In vitro* antioxidant properties,

free radicals scavenging activities of extracts and polyphenol composition of a non-timber forest product used as spice: *Monodora myristica. Biological Research 48*, 15 1-17. 2015.