Effects of blanching on antioxidant property of Malabar Spinach (*Basella rubra*) ethanolic extract

J.O. Ogundele[1*]
A.A. Aliu[1]
K.C. Komolafe[2]
A.C. Akinmoladun[3]

[1]Department of Industrial Chemistry, Faculty of Science, Federal University Oye Ekiti, Ekiti State, Nigeria

[2]Department of Biochemistry, Faculty of Science, Federal University Oye Ekiti, Ekiti State, Nigeria

[3]Department of Biochemistry, School of Science, The Federal University of Technology Akure, Ondo State, Nigeria

(*) CORRESPONDING AUTHOR:
J.O. Ogundele
E-mail: joan.ogundele.joko@gmail.com
Gsm:+2348034752350

Malabar spinach (*B. rubra Linn*), a local vegetable among the Yoruba people in Nigeria has been reported to exhibit an immense potential in androgenic activity, antiulcer activity, antioxidant activity, antibacterial activity and anti-inflammatory activity. Blanching is a common cooking method used to prepare soup with this vegetable among the local people. This research work investigated the effects of blanching on the antioxidant property of the ethanolic extract of *B. rubra*. By assaying for 2, 2-diphenyl-1-pierylhydrazyl (DPPH) scavenging activity, metal chelating ability, reductive property, nitric oxide scavenging activities and lipid peroxidation inhibitory activities. In addition, total phenolic content, total flavonoid content and total antioxidant activity of the blanched and un-blanched *B. rubra* samples were determined. Blanching decreased the antioxidant activity of the vegetable at different concentrations except for the reductive property that slightly increased after blanching. At 80µg/ml, blanching Malabar leaves ethanolic extract scavenged DPPH at 22.88% while the un-blanched sample scavenged DPPH at 26.80%. In the metal chelating assay, the un-blanched Malabar spinach showed 93.79% of Fe^{2+} chelation at 100µg/ml while blanched Malabar spinach was 89.83%. The phenolic content of the plant is much more than its flavonoid. The phenolic content of the blanched and un-blanched Malabar spinach was 121.83 and 341.00 µg GAE/mg respectively; while the flavonoid content of the blanched and un-blanched Malabar spinach was 62.77 and 65.63 µg QE/mg respectively.

Keywords: Blanching, *B. rubra Linn*, Antioxidants, Flavonoid

Glossary of Acronyms
*BS (*Blanched sample), *DMSO* (Dimethyl sulphoxide), *DPPH* (2, 2-diphenyl-1-pierylhydrazyl), *EDTA* (Ethylenediaminetetra-acetic acid), *LPO* (Line 62 Lipid peroxidation), *RS* (Raw sample), *PEE* (Powdery ethanolic extracts), *SNP* (Sodium nitroprusside), *TBARS* (Thiobarbituric acid-reactive species), *TBA* (Thiobarbituric acid), *TCA* (Trichloroacetic acid).

1. INTRODUCTION

The Malabar Spinach (*B. rubra Linn*) is a popular tropical leafy-green vegetable, commonly grown as backyard herb in home gardens. Malabar spinach belongs to the *Basellaceae* family and has two chief cultivars, *Basella alba* that has green-stems and deep-green leaves, and *B. rubra* with purplish stems and dark green leaves with pink veins [1]. It is a fast-growing, soft-stemmed vine, reaching 10 meters (33 ft) in length. Its thick, semi-succulent, heart-shaped leaves have a mild flavour and mucilaginous texture. It is rich in vitamins A and C, iron and calcium. It has been shown to contain certain phenolic phytochemicals and has antioxidant properties [1]. It may be eaten raw in salads, boiled, steamed, stir-fried, or added to soups, stews, tofu dishes and curries.
Boiling helps to convert the raw food ingredient into consumable food or to transform food into other forms for consumption by humans or animals ei-

ther at home or by the food processing industry. It is a food cooking method consisting in just immersing them in water at 100°C and maintaining the water at that temperature till the food is tender. The effect of boiling on the anti-nutrient and antioxidant content depends on the sensitivity of the nutrient to the various conditions prevailing during the process, such as heat, oxygen, pH and light. The antioxidant retention may vary with a combination of conditions, such as the characteristics of the food being boiled, and the concentration of the nutrient in the food [2]. Nutrition wise, popular thinking is that fresh fruits and vegetables are better than cooked ones. However, carotenoids, the colourful pigments in a variety of red, yellow and orange vegetables are more available for absorption from cooked foods rather than raw ones. Cooked sweet yellow corn had 44% higher total antioxidant activity than the same corn before cooking [3]. Most vegetables are usually cooked by boiling in water or microwaving (unlike fruits that are usually consumed in their raw forms) before consumption. These cooking processes could bring about several changes in the physical characteristics and chemical composition of vegetables [3].

In recent years, antioxidants derived from natural sources, mainly plants, have been intensively used to prevent oxidative damage because of their advantages over synthetic ones. They are easily obtained, economical and have slight or negligible side effects [4]. Bioactive antioxidant agents present in herbs and spices may offer resistance against oxidative stress by scavenging free radicals and therefore inhibiting or preventing the deleterious consequences of oxidative stress [5].

Raw foods like meat, eggs, fruits and vegetables may harbour food poisoning bacteria that, if consumed, are likely to cause illness. The optimum temperature for the multiplication of most food poisoning bacteria is between 5-63°C, while, most bacteria are killed at temperatures over 70°C and most food bacteria can only multiply slowly or not at all below 5°C. Boiling heats foods to over 70°C so applying such a temperature for a carefully calculated time period will prevent many food borne illnesses that would otherwise occur when eating raw food [3]. Therefore, the aim of this research work was to determine the effect of blanching on the antioxidant property of Malabar spinach; the effects of blanching on the antioxidant properties of *B. rubra* (Malabar spinach).

2. MATERIALS AND METHODS

2.1. CHEMICALS

2, 2-diphenyl-1-pierylhydrazyl (DPPH), gallic acid and quercetin were obtained from sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade and obtained from standard suppliers.

2.2 PROCESSING OF SAMPLES INTO BLANCHED AND RAW FLOUR SAMPLES

Malabar spinach was purchased from Oja Oba Akure, Nigeria. The vegetable sample was processed into raw and blanched flour samples. A portion of the vegetable was weighed and rinsed in distilled water before processing. It was then cut, dried at room temperature (30°C-36°C) for some days and blended to obtain the raw sample (RS). Another portion was washed in distilled water, cut and blanched for two minutes in a stainless pot without extra water at low heat before drying at room temperature to obtain the blanched sample (BS).

After drying, they were blended in a blender and packaged in separate polythene bags, labelled, kept in airtight plastic containers and kept in a cool dry place.

2.3 PREPARATION OF ETHANOLIC EXTRACT

Samples (RS and BS) were separately weighed on a weight scale. The samples were soaked in 80% ethanol for 55 minutes and then filtered. The filtrates were kept at room temperature for 3 days for the solvent to vaporise at room temperature, leaving the powdery ethanolic extracts (PEE).

2.4 DETERMINATION OF ANTIOXIDANT COMPOSITION

2.4.1 Total phenol content

Total phenol content of PEE was determined as previously described [6]. Appropriate dilutions of the extracts were oxidised with 2.5 ml of 10% Folin–Ciocalteau's reagent (v/v) and neutralised by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45°C and the absorbance was measured at 765 nm on a colorimeter. The total phenol content was subsequently calculated using gallic acid as standard.

The phenolic content was calculated as gallic acid equivalents GAE/g of PEE based on a standard curve of gallic acid (5–500 mg/L, $Y = 0.0027x - 0.0055$, $R = 0.99$).

The total phenolic contents in all samples was calculated the using the formula:

$C = c\ V/m$

Where:

C = total phenolic content mg GAE/g dry PEE,

c = concentration of gallic acid obtained from calibration curve in mg/mL,

V = volume of PEE in ml,

m = mass of PEE in gram.

2.4.2 Total flavonoid content

The total flavonoid content of the PEE was determined using a slightly modified method [7]. Sample 0.5 ml was mixed with 0.5 ml methanol, 50 µl of 10%

$AlCl_3$, 50 µl of 1 mol L^{-1} potassium acetate and 1.4 ml water, and allowed an incubation at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm using a colorimeter. The total flavonoid was calculated using quercetin as standard.
The concentration of total flavonoid content in the test samples was calculated from the calibration plot ($Y = 0.0162x + 0.0044$; $R^2 = 0.999$) and expressed as mg quercetin equivalent (QE)/g of dried plant material.

2.5 EVALUATION OF ANTIOXIDANT ACTIVITIES

2.5.1 Ferric Reducing property

The ferric reducing property of the PEE was determined by assessing the ability of the extract to reduce the $FeCl_3$ solution as described by [8]. An aliquot of 2.5 ml was mixed with 2.5 ml of 200 mmolL^{-1} sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferric-cyanide. The mixture was incubated at 50°C for 20 min and then 2.5 ml of 10% trichloroacetic acid (TCA) was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance of the mixture was measured at 700 nm and the ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

2.5.2 Fe^{2+} chelation assay

The Fe^{2+} chelating ability of BS and RS PEE was determined using a modified method of Minotti and Augt (1987) [9]. Freshly prepared 500 µmol l^{-1} $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl of 0.1mol l^{-1}Tris-HCl (pH 7.4), 218 µl saline and the PEE ranging from (0 – 25 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.

2.5.3 DPPH Scavenging Activity

The method described by [10] was used to determine the DPPH Scavenging activity. Different volumes (2-20 µl) of PEE were made up to 40 µl with Dimethyl sulphoxide (DMSO) and 2.96 ml DPPH (0.1 mM) solution was added. The reaction mixture was incubated in dark condition at room temperature for 20 min. after 20 min the absorbance of the mixture was read at 517 nm. DPPH (3 ml) was used as a control. The radical scavenging activity of the plant extracts was calculated using the following formula:

$$\% \text{ DPPH radical scavenging activity} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

2.5.4. Total Antioxidant Capacity

Total antioxidant capacity of the RS PEE and BS PEE was determined by the phosphomolybdate method using ascorbic acid as a standard [11]. A stock solution of 1 mg/ml PEE was prepared and diluted to concentrations ranging from 0.05 to 1 mg/ml. An aliquot of 0.1 ml of sample solution was mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). Sample tubes were capped and incubated in a water bath at 95°C for 90 min. After cooling to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The blank contained 1 ml of the reagent solution along with an appropriate volume of the solvent and incubated under similar conditions.

2.5.5 Inhibition of lipid peroxidation

A modified thiobarbituric acid-reactive species (TBARS) assay [12] was used to measure the lipid peroxide formed. A laboratory rat brain homogenate was used as lipid-rich media [13]. The homogenate (500 µl of 10%, v/v in PBS (pH 7.4) and 100 µl of PEE samples were added to a test tube and made up to 1.0 ml with distilled water. Then, 50 µl of $FeSO_4$ (0.075 M) and 20 µl of L-ascorbic acid (0.1 M) were added and all were mixed and incubated for 1 h at 37°C to induce lipid peroxidation. Then, 0.2 ml of EDTA (0.1 M) and 1.5 ml of TBA reagent (3 g TBA, 120 g TCA and 10.4 ml 70% $HClO_4$ in 800 ml of distilled water) were added to the mixture and heated for 15 min at 100°C. After cooling, the samples were centrifuged for 10 min at 3,000 rpm and the absorbance of the supernatant was measured at 532 nm. Lipid peroxidation (LPO) inhibition was calculated using the equation:

$$\text{Inhibition of LPO (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \times 100$$

2.5.6 Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci et al., 1994 [14]. The reaction mixture (5.0 ml) containing SNP (5 mM) in phosphate-buffered saline (pH 7.3), with or without the PEE at different concentrations, was incubated at 25°C for 180 min in front of a visible polychromatic light source (25 W tungsten lamp). The Nitric oxide radical thus generated interacted with oxygen to produce the nitrite ion that was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylene- diaminedihydrochloride).

The absorbance of the chromophore (purple azo dye) formed during the diazotisation of the nitrite ions with sulphanilamide and subsequent coupling with naphthylethylene-diaminedihydrochloride was measured at 546 nm in a spectrophotometer. The nitrite generated in the presence or absence of the PEE was estimated using a standard curve based on sodium nitrite solutions of known concentrations.

2.6 STATISTICAL ANALYSIS OF RESULTS

Each experiment was carried out in replicates and the results presented as an average ± Standard deviation.

3. RESULTS AND DISCUSSION

3.1 TOTAL ANTIOXIDANT ACTIVITY, PHENOL AND FLAVONOIDS OF MALABAR SPINACH (*BASELLA RUBRA*).

The IC_{50} values in the above Table I show that the total antioxidant activity of the PEE is 22.33±1.53 for the blanched and 37.00±2.00 µg AAE/mg for the raw sample, which implies that the raw extract has a greater total antioxidant activity. It has been reported that the antioxidant activity of plant materials is well correlated with the content of their phenolic and flavonoid compounds [15]. Many plant constituents have been reported to have free radical scavenging activity [16]. Interestingly, flavonoids and other phenolic compounds of plant origin have been reported as scavengers of free radicals and inhibitors of lipid peroxidation [17]. Due to the potent free radical scavenging activity of the plant, it was subjected to some phytochemical analysis. As shown in Table I, phenolic compounds were a major class of bioactive components in this Malabar spinach plant extract. The medicinal properties of folk plants are mainly attributed to the presence of phenolic compounds mostly flavonoids, phenolic acids and antioxidant micronutrients [18]. Therefore, it is pertinent to explore these herbs and plants that can produce naturally occurring antioxidants. Plant phenols in fruits and vegetables have received considerable attention because of their potential antioxidant activities [19]. They are regarded to be the most important anti-oxidative components of plants while the correlations between the concentrations of plant phenolics and their total antioxidant capabilities have been reported [20].
Phenolic compounds may contribute directly to the antioxidative action. The total phenolic content of the blanched and raw sample is 121.83±9.46 and 341.00±15.21 µg gallic acid equivalents/mg dry weight PEE respectively, though the raw extract tends to have a greater phenolic content. The total flavonoid content of the blanched sample is 62.77±1.77 and that of the raw is 65.63±1.32 µg quercetin equivalents/mg dry weight PEE. The raw sample has more flavonoid content than the blanched sample.

3.2 REDUCTIVE PROPERTY OF RAW AND BLANCHED *B. RUBRA*

Table II shows the reductive property of the blanched and raw *B. rubra* PEE sample. The reducing capacity of the PEE and fractions were performed using Fe^{3+} to Fe^{2+} reduction assay. In this experiment, the yellow colour changes to pale green and blue depending on the concentration of antioxidants in the PEE samples and ascorbic acid is used as the standard reagent. The IC_{50} values on the Table shows that the reductive property of the PEE samples was effective with an increasing concentration from 20 µg/ml to 100 µg/ml but here, the blanched B. rubra PEE tend to have a slightly more reductive property than the raw PEE sample of Malabar spinach.

3.3 PERCENTAGE OF METAL CHELATING ACTIVITY OF RS AND BS *B. RUBRA*

Ferrozine can quantitatively form complexes with Fe^{2+}. However, in the presence of chelating agents, the complex formation is disrupted with the result that the red colour of the complex is decreased. Therefore, the colour reduction measurement, allows the estimation of the chelating activity of the coexisting chelator. The transition metal ion, Fe^{2+} possesses the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions, even starting with relatively non-reactive radicals [21]. The main strategy to avoid reactive oxidative species generation that is associated with redox active metal catalysis involving chelating of the metal ions.
Table III shows the metal chelating activity of the blanched and raw PEE Malabar spinach. In this Table, it is evident that the raw PEE sample can chelate free metallic ions than the blanched PEE sample and the metal chelating ability of the blanched and raw PEE Malabar spinach is effective with concentration from 20 µg/ml to 100 µg/ml.

Table I - Total Antioxidant activity, Total Phenol and Total Flavonoid of *B. rubra*

Extracts	Total antioxidant activity (µg AAE/mg)	Total phenol (µg GAE/mg)	Total flavonoid (µg QE/mg)
BLANCHED (BS)	22.33 ± 1.53	121.83 ± 9.46	62.77 ± 1.77
RAW (RS)	37.00 ± 2.00	341.00 ± 15.21	65.63 ± 1.32

Table II - Reductive property of raw and blanched *B. rubra*

CONC (µg/ml)	BLANCHED (BS)	RAW (RS)
20	0.829±0.006	0.781±0.009
40	0.845±0.003	0.815±0.015
60	0.858±0.006	0.834±0.004
80	0.875±0.007	0.875±0.003
100	0.905±0.013	0.897±0.014

3.4 DPPH SCAVENGING ACTIVITY OF RAW AND BLANCHED *B. RUBRA*

The DPPH radical scavenging activity of raw and blanched PEE Malabar spinach is shown in Table IV. DPPH scavenging activity measures the ability of an antioxidant compound to donate electrons or hydrogen, thereby converting the radical to a more stable species [22]. The advent of reactive oxygen species (ROS) and free radical generation from various cellular activities are well documented. In mitochondria, part of the electrons transported by the respiratory chain is delivered to oxygen in a process generating superoxide anion, O^{2-}, and subsequently H_2O_2 and reactive oxy and peroxyl radicals [23]. While this fraction may be as high as 2-3% in vitro, chronic production of reactive oxygen species (ROS) by mitochondria in vivo is probably much lower [23]. Also, under physiological conditions, oxidants and pro-oxidants such as superoxide (O^{2-}) and hydrogen peroxide (H_2O_2) producing ROS have been implicated in apoptosis and other related diseases such as hypertension, arteriosclerosis, cancer and diabetes [24, 25]. DPPH is a stable free radical and can be reduced in the presence of an antioxidant molecule; its usage has been widely applied for evaluating antioxidant activity in several studies [26]. The ability of a molecule to donate a hydrogen atom to a radical determines its antioxidant potentials. DPPH accepts a hydrogen atom from an antioxidant and becomes a stable diamagnetic molecule. The values in Table IV show that, at different concentrations, the raw PEE Malabar spinach extract scavenge free radicals more than the blanched PEE Malabar spinach except at 100 µg/ml (i.e. the IC_{50} value of the raw sample is 32.5±1.15 while that of blanched Malabar spinach is 33.66±1.18). The effectiveness of this radical scavenging ability of the plant increases with a concentration from 20 µg/ml to 100 µg/ml.

3.5 PERCENTAGE INHIBITION OF LIPID PEROXIDATION OF *B. RUBRA*

Table V shows the IC_{50} values of the percentage inhibition of lipid peroxidation. Lipid peroxidation is the degradation of lipids that occurs as a result of oxidative damage and is a useful marker for oxidative stress. Polyunsaturated lipids are susceptible to an oxidative attack, typically by reactive oxygen species, resulting in a well-defined chain reaction with the production of end products such as malondialdehyde (MDA).
In Table V, the IC_{50} values show that the percentage inhibition of lipid peroxidation of the raw PEE sample is higher than that of the blanched PEE sample and is effective with an increasing concentration from 20 µg/ml to 100 µg/ml.

3.6 PERCENTAGE INHIBITION OF NITRIC OXIDE *B. RUBRA*

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Nitric oxide is generated in biological tissues by specific nitric oxide synthesis (NOSs) that which metabolises arginine to citrulline with the formation of NO via a five-electron oxidative reaction. These compounds are responsible for altering the structural and functional behaviour of many cellular components. Incubation of solutions of sodium nitroprusside in PBS at 25°C for 2 hrs resulted in linear time dependent on nitrite production that is reduced by the tested PEE of the plants. Ascorbic acid is used as the standard reagent. In the above Table VI, it is evident that the percentage inhibition of nitric oxide of the raw PEE sample is more than the blanched PEE sample and the effectiveness of the nitric oxide scavenging activity increases with a concentration from 20 µg/ml to 100 µg/ml.

4. CONCLUSION

The conclusion of this research work is that the vegetable, *B. rubra* PEE exhibits various antioxidant properties that makes it a good vegetable that can scavenge free radicals liable of causing various oxi-

Table III - % Metal chelating activity of raw and blanched *B. rubra*

CONC (µg/ml)	BLANCHED (BS)	RAW (RS)	EDTA
20	74.01±0.98	81.36±1.69	81.92±0.98
40	77.97±1.69	84.75±1.19	85.88±0.98
60	83.62±0.98	89.27±0.98	87.57±1.95
80	87.01±1.96	90.96±0.97	92.09±0.98
100	89.83±1.69	93.79±0.98	97.17±0.98

Table IV - % DPPH Scavenging activity of blanched and raw Malabar Spinach (*Basella rubra*).

CONC (µg/ml)	BLANCHED (BS)	RAW (RS)
20	6.53±0.33	7.08±0.82
40	8.06±0.68	12.96±0.82
60	14.81±1.61	20.15±0.94
80	22.88±0.98	26.80±0.86
100	33.66±1.18	32.5±1.15

Table V - % Inhibition of Lipid Peroxidation of raw and blanched *B. rubra*

CONC (µg/ml)	BLANCHED (BS)	RAW (RS)
20	18.15±4.01	29.59±0.58
40	27.04±1.46	33.24±0.33
60	29.88±0.83	34.62±0.22
80	31.71±0.33	36.81±0.44
100	33.82±1.26	38.63±0.33

Table VI - % Inhibition of Nitric Oxide of blanched and raw *B. rubra*

CONC (µg/ml)	BLANCHED (BS)	RAW (RS)
20	10.27±3.72	28.85±8.28
40	28.67±1.62	35.58±0.81
60	31.50±1.06	38.05±1.11
80	39.65±1.86	41.59±0.92
100	45.13±1.71	43.19±0.92

dative stress (such as cancer, obesity, coronary heart disease, Alzheimer's disease, human ageing etc.) in the body.

PEE blanched *B. rubra*, have decreased DPPH scavenging activity, metal chelating activity, total flavonoid, total phenolic, total antioxidant activity, inhibition of lipid peroxidation, and inhibition of nitric oxide. However, after blanching, these values are still significantly high enough to meet the antioxidants needed in human nutrition.

This study has clearly shown that the effectiveness of bioactive compounds in *B. rubra* PEE were generally reduced by blanching.

REFERENCES

[1] M.J. Haskel, Daily consumption of Indian Spinach *(Baselle alba)* or Sweet potatoes has positive effect on total-body vitamin A store in Bangladeshi men. American Journal of Clinical Nutrition, *80*(3), 705-714 (2004).

[2] Z.U. Rehman, M. Islam, W.H. Shah, Effect of microwave and conventional cooking on insoluble dietary fibre compounds of vegetables. Food Chemistry, *80*, 237-240 (2003).

[3] F.J. Francis, Food colorants: anthocyanins. Critical. Review in Food Science and Nutrition, *28*(4) 273-314 (1989).

[4] E. Onay-ucar, A. Karagoz, N. Arda, Antioxidant activity of Viscium album spp. *Fitoterapia, 77*, 556-560 (2006).

[5] B. Halliwell, Antioxidants and Human Disease: A General Introduction. Nutrition Review, *52,* 253-265 (1997).

[6] L. Vernon, R. Singleton, R. M. Orthofer, Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Elsevier Methods in Enzymology *299*, 152-178 (1999).

[7] A. Meda, C.E. Lamien, M. Romito, J. Millogo, O.G. Nacoulma, Determination of the total phenolic, flavonoid and praline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chemistry, *91*, 571-577 (2005).

[8] M. Oyaizu, Studies on products of browning reaction: antioxidative activity of products of browning reaction prepared from glucosamine, Journal of Nutrition, *44,* 307-305 (1986).

[9] G. Minotti, S.D. Augt, An investigation into the mechanism of citrate- Fe^{2+} dependent lipid peroxidation. Free Radical Biology and Medicine, *3*, 379-387 (1987).

[10] C. Desmarchelier, E. Mongelli, J. Coussio, G. Ciccia, Evaluation of the in vitro antioxidant activity in extracts of Uncaria tomentosa (Willd) DC. Phytother Research, *11*, 254-256 (1997).

[11] N.G. Baydar, G. Ozkan, S. Yasar, Evaluation of the antiradical and antioxidant potential of grape extracts. Elsevier Food Control, *18,* 1131-6 (2007).

[12] F.M. Awah, P.N. Uzoegwu, J.O. Oyugi, J. Rutherford, P. Ifeonu, X. Yao, K.R. Fowke, M.O. Eze, Free radical scavenging activity and immunomodulatory effect of Stachytarpheta angustifolia leaf extract. Food Chemistry, *119*, 1409-1416 (2010).

[13] G. Ruberto, M.T. Baratta, S.G. Deans, H.J.D Dorman, Antioxidant and antimicrobial activity of foeniculum vulgare and crithmum maritimum essential oils. Plant medicine, *66*, 687-693 (2000)

[14] L. Marcocci, L. Packer, M.T. Droy-Lefaix, A. Sekaki, M. Gardès-Albert, Antioxidant action of Ginkgo biloba extract EGb 761. Methods Enzymol, *234,* 462-475 (1994).

[15] Y.S. Velioglu, G. Mazza, L. Gao, B.D. Oomah, Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. Journal of Agricultural Food Chemistry, *46* (10), 4113-4117 (1998).

[16] O.I. Arouma, Free Radicals, Oxidative Stress and antioxidants in Human Health and Disease. Journal of America Oil Chemical Society, *75*, 199-212 (1998).
[17] Y. Takanami, H. Iwane, Y. Kawai T. Shimoitsu, Vitamin E supplementation and endurance exercises? Are there benefits? Sports Medicine, *29*(2), 1381-1478 (2000).
[18] M.G. Repetto and S.F. Llesuy, Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazilian Journal of Medical and Biological Research, *35*, 523-34 (2002)
[19] I. Gulcin, M.E. Buyukokuroglu, M. Oktay, I.O. Kufrevioglu, The invitro antioxidant properties of Melatonin. Journal of Pineal Research, *33*(3), 167-171 (2002).
[20] S. McDonald, P.D. Prenzler, M. Antolovich, K. Robards, Phenolic content and Antioxidant activity of Olive extracts. Food Chemistry, *73*, 73-84 (2001).
[21] M.A. Aboul-Enein, F.K. Baz, G.S. El-Baroty, A.M. Youssef, H.H. Abd El-Baky, Antioxidant activity of algal extracts on lipid peroxidation. Journal of Medical Sciences, *3*, 87-98 (2003).
[22] T.L. Pownall, C.C. Udenigwe, R.E. Aluko, Amino acid composition and antioxidant properties of pea seed (Pisum sativum L.) enzymatic protein hydrolysate fractions. Journal of Agricultural and Food Chemistry, *58*, 4712-4718 (2010).
[23] M.P. Murphy, How mitochondria produce reactive oxygen species. Biochemical Journal, *417*, 1-13 (2009).
[24] R. Koren, I.E. Hadari-Naor, E. Zuck, C. Rotem, U.A. Liberman, A. Ravid, Mitochondrial Complex I Inhibitor Rotenone Induces Apoptosis. www.adultmito.net/wpcontent/upload.i-inflammatory 12/08/201 (2001).
[25] D. Doughan, E.K. Joan, The comprehensive Bio-Bibliography (review), Volume 64 Number 3. pp 514-516DOI 10.135/not 2008.0021 (2008).
[26] W. Brand-Williams. Use of a free radical method to evaluate antioxidant activity. Food Science Technology, (London) *28,* 25-30 (1995).

Received: January 18, 2019
Accepted: May 24, 2019