International Journal of Chemical Studies

P-ISSN2349–8528
E-ISSN 2321–4902
IJCS 2016; 4(3): 22-26

Received: 15-03-2016
Accepted: 16-04-2016

Pawar RK
Department of Chemistry Arts science commerceCollege, Surgana, [Nasik] Maharashtra, India

Bhagure GR
Department of Chemistry, Dnyanasadhana College [Thane] Maharashtra, India

Chavan RP
Department of Chemistry, Dnyanasadhana College [Thane] Maharashtra, India

Correspondence
Pawar RK
Department of Chemistry Arts Science Commerce College, Surgana, [Nasik] Maharashtra, India

Antioxidants and their role in nurture human life and industry: A review

Pawar RK, Bhagure GR, Chavan RP

Abstract
The present paper deals with the antioxidants and their role in human life. The comprehensive review has been taken in this paper which aims to state the types of different antioxidants present in plants and herbs, the constituents present in plant and their role as antioxidant is because of their phenolic and flavonoids contents. The paper deals with the antioxidant activity of a number of fruits, vegetables, leaf and grains of different plants. The selected fruits and vegetables such as mulberries, papaya, red grapes, mango, guava, tomato, red onion, red cauliflower, carrot, beetroot etc. are used in this study. The higher levels of radical scavenging capacity are observed in beetroot, red onion, red cauliflower, red grapes and mulberries as compared to other fruits and vegetables. The results can be compared with several other local fruits.

Keywords: Antioxidant, Phenolic, Flavonoid, Activities

Introduction
An antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants such as thiols or ascorbic acid (vitamin C) break down these chain reactions. To balance the oxidative state, plants and animals maintain complex systems of overlapping antioxidants, such as glutathione and enzymes (e.g., catalase and superoxide dismutase) produced internally or vitamin C, vitamin A and vitamin E obtained by ingestion.

Antioxidants are widely used as dietary supplements and have been investigated for the prevention of diseases such as cancer or coronary. Oxidative stress can be considered as either a cause or consequence of some diseases, stimulating drug development for potential antioxidant compounds for use as treatments. Antioxidants have been investigated for potential effects on neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.

Antioxidants are categorized into two groups such as enzymatic and non-enzymatic. Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase, endogenously produced in human systems and under normal conditions they act as defense agents against the free radicals and reactive oxygen species (ROS). However, antioxidant enzymes are weakened against radicals during the severe disease conditions [1]. Therefore, the external sources of dietary antioxidants are required to strengthen the human defense system. Non enzymatic antioxidants are polyphenols, carotenoids, vitamins and minerals found rich in fruits and vegetables [2].

Many research worker conducted research on sources of antioxidant in plants and their antioxidant activity was experimentally proved by different methods. Horubala *et al.* (1999); Borowska (2003) studied that antioxidants are the substances able to prevent or inhibit oxidation processes in human body as well as in food products. The natural antioxidants are a stable part of nutrition as they occur in almost all edible plant parts. Polyphenols are the most numerous group of antioxidant components, and they are present in fruits and vegetables, their products, leguminous plants, grains, teas, herbs, spices and wines [3, 4] Jacob and Burri, (1996); Ratnam *et al*., (2006); Stangeland *et al.* 2009 explained that free radicals are continuously generated in the human body as an effect of oxidative metabolism and thus results the incidence of severe illness such as coronary heart disease, cancer, neurodegenerative ailments, diabetes mellitus, autoimmune disease and aging [5-7]. Antioxidants are substance which can protect the human body from free radicals and ROS induced chronic diseases [8]. The dietary intake of fruits has a strong inverse correlation with the risk of developing coronary heart

Disease and cancer. In fruits, vitamins C, A and E, and Polyphenols are known to be responsible for such antioxidant activity, with polyphenols being the most active. A number of Studies have reported the content of some essential nutrients in wild edible fruits from western Africa [9, 10]. (Glew *et al*., 2005; Leung *et al*., 1968) including energy levels, ascorbic acid, vitamins, metals and trace minerals.

There are approximately 5000 known plant phenolic compounds and model studies have demonstrated that many of them have antioxidant activity [11]. The antioxidant activity of phenolic compounds is mainly because of their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators. Their antioxidant activity is generally based on the number and location of hydroxyl groups present as well as the presence of a 2-3 double bond and 4-oxofunction.

The flavonoids, a large family of low molecular weight polyphenolic compounds, include the flavones, flavonols, flavonones, isoflavones, flavan-3-ols and anthocyanins. [12] Although flavonoids are generally considered non-nutritive agents, interest in these substances has risen because of their possible effects on human health. In addition to their antioxidant activities, flavonoids inhibit enzymes such as prostaglandin synthase, lipoxygenase and cycloxygenase, closely related to tumorigenesis, and may induce detoxifying enzymes such as glutathione S- transferase. Many kinds of flavonoid have been reported in fruits and vegetables and their types and contents vary with cultivar and maturation [13].

Phenolic compounds are commonly found in vegetable based food and are important components of human diet. They have diverse properties such as antioxidant, anti-aging, anti-cancer, anti-inflammatory activity [14]. Phenolic compounds have been used as antioxidants and mainly inhibit free radicals and block their chain reactions [15].

Flavonoids are an important class of plant pigments, naturally found in fruit and vegetables. This class of naturally occurring polyphenolic compounds which cannot be synthesized by human possesses a series of biological properties acting on biological systems as antioxidants. Flavonoids act as antiviral, anti-inflammatory, and antitumoral agents, affecting capillary permeability and as exogenous antioxidants. Flavonoids capture and neutralize the oxidative agents and quench free radicals inhibiting several enzymes (ciclo-oxigenase, lipo-oxigenase, NADPH-oxidase, xantine-oxidase, fosfolipase) and stimulating enzymes with antioxidant activity (such as catalase and superoxide dismutase). Therefore flavonoids interfere directly in the formation and propagation of free radicals [15, 16] Flavonoids present in the diet are directly linked to the prevention of arthrosclerosis. Various studies show that the reduction of total blood cholesterol levels and the antioxidant effect lead to lower risks of arthrosclerosis, teratogenicity and coronary disease [17]. It has been reported that green vegetables are the source of polyphenolic compounds; so their anti-oxidant and antimutagenic capacity effects on apoptosis and antiproliferative mechanisms are related to the presence of these compounds in fruit and vegetables.

Prema Gurumurthy *et al*., 2013 studied antioxidant and free radical scavenging activity of triphala which is determined by using different in vitro models Triphala [‘three’ (tri) ‘fruits’ (phala)] is a traditional Ayurvedic herbal formulation consisting of the dried fruits of three medicinal plants, *Terminalia chebula*. Gaertn. *Terminalia bellirica* (Gaertn) Roxb. And *Phyllanthus emblica* [18]. This formulation, rich in antioxidants, is a frequently used ayurvedic medicine to treat many diseases such as anemia, jaundice, constipation, asthma, fever and chronic ulcers. It is an important medicine of the ‘rasayana’ group and is believed to promote health, immunity and longevity. It corrects constipation, cleanses and tonifies the gastrointestinal tract and also detoxifies the whole body, and improves digestion and assimilation. It exhibits anti-viral, anti-bacterial, antifungal and anti-allergic properties “Triphala” and its constituents act as cardio-tonic, control blood pressure, improve blood circulation and reduce cholesterol levels “Triphala” shows immunomodulatory properties and helps in improving the body’s defence system. In recent years, there are also several reports in the literature which suggest that “Triphala” possesses antimutagentic and radioprotecting activity [19]. Triphala has been reported to be a rich source of Vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, β-sitosterol and flavonoids [20].

The aim of the research paper is to study the variability of the composition in different plants, to know the antioxidant activity and also to know how different parts of plants exploited for some products formulation with high nutritional value and biomedical potentials. A comprehensive review has been taken in this paper which aims to state the types of different antioxidants present in plants and herbs, the constituents present in plant and their role as antioxidant is because of their phenolic and flavonoids contents. The paper deals with the antioxidant activity of a number of fruits, vegetables, leaf and grains of different plants.

The objective of this study was to analyze the antioxidant properties of certain foods, determining the total flavonoids as well as their antioxidant activity and fat concentration order to evaluate which types of food can help keep a healthy diet.

Objectives of the study

1. To analyze the total antioxidant activity and radical scavenging capacity of selected fruits and vegetables.
2. To know the overall, or ‘total’ antioxidant capacity of fruits and vegetables.
3. To find the antioxidant activity of food by using the DPPH, FRAP and ABTS methods.

Materials and Methods

Plant material Different plant fruits such as mulberries (*Morusnigra,* Moraceae), papaya (*Carica papaya*, Caricaceae), red grapes (*Vitus vinifera*, Vitaceae), mango (*Mangifera indica*, Anacardiaceae), guava (*Psidium guajava*, Myrtaceae), tomato (*Lycopersicone sculentum*, Solanaceae), red onion (*Allium cepa* variety cepa, Alliaceae), red cauliflower (*Brassica oleracea* variety botrytis, Cruciferae), carrot (*Daucus carota* subspecies sativus, Umbelliferae) and beetroot (Beta vulgaris variety conditiva, Chenopodiaceae) were acquired from a contact garden. Fruits and vegetables were freshly harvested in the morning and then, they were taken into the laboratory within a day at ambient temperature. The non-defected fruits and vegetables were selected and washed with distilled water and then proceed for the extraction process.

Plant material extraction

Plant fruits such as papaya, mango, red onion and beetroot have been homogenized without skin and remaining fruits and vegetables have been extracted with the skin. Plant extractions have been carried out based on the method [21]. of (Lim *et al*., 2007). Triplicate samples of 25 g of each samples edible portion have been used for the extraction in a mortar and pestle method at 4 °C with 50 ml of 80% methanol. Then, the homogenate will be transferred to 100 ml volumetric flask and made up the volume with 80% methanol. The mixture have been shaken in the orbital shaker for 20 min and then it have

been filtered by muslin cloth. The filtrate is used for the following experiments. All the experiments have been carried out in triplicate within 2 days after the extraction process.

Many research workers studied antioxidant activity by following different methods [22].

Antioxidant capacity assay	Principle of the method Spectrophotometry	End-product determination
DPPH	Antioxidant reaction with an organic radical	Colorimetry
ABTS	Antioxidant reaction with an organic cation radical	Colorimetry
FRAP	Antioxidant reaction with a Fe(III) complex	Colorimetry
PFRAP	Potassium ferricyanide reduction by antioxidants and subsequent reaction of potassium ferrocyanide with Fe^{3+}	Colorimetry
CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
ORAC	Antioxidant reaction with peroxyl radicals, induced by AAPH (2,2'-azobis-2-amidino-propane)	Loss of fluorescence of fluorescein
HORAC	Antioxidant capacity to quench OH radicals generated by a Co(II) based Fenton-like system	Loss of fluorescence of fluorescein
TRAP	Antioxidant capacity to scavenge luminol-derived radicals, generated from AAPH decomposition	Chemiluminescence quenching
Fluorimetry	Emission of light by a substance that has absorbed light or other electromagnetic radiation of a different wavelength	Recording of fluorescence excitation/emission spectra
	Electroanalytical Technique	
Cyclic voltammetry	The potential of a working electrode is linearly varied from an initial value to a final value and back, and the respective current intensity is recorded	Measurement of the intensity of the cathodic/ anodic peak
Amperometry	The potential of the working electrode is set at a fixed value with respect to a reference electrode	Measurement of the intensity of the current generated by the oxidation/reduction of an electro active analyte
Biamperometry	The reaction of the analyte (antioxidant) with the oxidized form of a reversible indicating redox couple	Measurement of the current flowing between two identical working electrodes, at a small potential difference and immersed in a solution containing the analysed sample and a reversible redox couple
	Chromatography	
Gas chromatography	Separation of the compounds in a mixture is based on the repartition between a liquid stationary phase and a gas mobile phase	Flame ionisation or thermal conductivity detection
High performance liquid chromatography	Separation of the compounds in a mixture is based on the repartition between a solid stationary phase and a liquid mobile phase with different polarities, at high flow rate and pressure of the mobile phase	UV-VIS (e.g. diode array) detection, fluorescence, mass spectrometry or electrochemical detection

Determination of total phenolics and total flavonoids

The total phenolics of each fruit extract are determined by the Folin-Ciocalteu method [23]. The diluted aqueous solution of each extract (0.5 mL) was mixed with Folin-Ciocalteu reagent (0.2 N, 2.5 mL). This mixture was allowed to stand at room temperature for 5 min and then sodium carbonate solution (75 g/L in water, 2 mL) was added. After 2 h of incubation, the absorbances were measured at 760 nm against a water blank. A standard calibration curve was plotted using gallic acid (0-200 mg/L). The results were expressed as mg of gallic acid equivalents (GAE)/100 g of fruit weight. The total flavonoids were estimated according to the Dowd method as adapted by Arvouet-Grand *et al.* A diluted methanolic solution (2 mL) of each fruit extract was mixed with a solution (2 mL) of aluminium trichloride ($AlCl_3$) in methanol (2 %). The absorbance was read at 415 nm after 10 min against a blank sample consisting of a methanol (2 mL) and plant extract (2 mL) without $AlCl_3$. Quercetin was used as reference compound to produce the standard curve, and the results were expressed as mg of quercetin equivalents (QE)/100 g of fruit weight.

Iron (III) to iron (II) reduction activity (FRAP)

Hinneburg *et al*., (2006) studied the total antioxidant capacity of each fruit extract using the iron (III) reduction method [24]. The diluted aqueous solution of each fruit extract (1 mL), at a concentration of 100 µg/mL, was mixed with phosphate buffer (0.2 M, pH 6.6, 2.5 mL) and 1% aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (2.5 mL). After 30 min incubation at 50°C, trichloroacetic acid (10 %, 2.5 mL) was added, and the mixture was centrifuged at 3000 rpm for 10 min. Then, the upper layer solution (2.5 mL) was mixed with water (2.5 mL) and an aqueous $FeCl_3$ (0.1 %) solution (0.5 mL). The absorbance was read at 700 nm and ascorbic acid was used to produce the calibration curve. The iron (III) reducing activity determination was expressed in mmol ascorbic acid equivalents/100 g of fruit weight.

DPPH radical method

Meda *et al*., (2005) described the method for the extract to scavenge the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical [25]. the antioxidant content will be determined using a standard curve of ascorbic acid (0 – 10 µg/mL). The results are expressed as mg of ascorbic acid equivalent antioxidant content (AEAC) per 100 g of fruit weight.

ABTS radical cation decolorization assay

Re *et al*. (1999) described the method for radical scavenging capacity of antioxidants for the ABTS (2,2'-azinobis-3-ethyl-benzothiazoline-6-sulphonate) radical cation [26]. $ABTS^{\cdot+}$ have been generated by mixing a 7 mM aqueous solution of ABTS

with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 12 h before use. The mixture will be diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using spectrophotometer. For each fruit, the diluted methanol solution of the extract (10 µL) is allowed to react with fresh $ABTS^{\cdot+}$ solution (990 µL), and then the absorbance will be measured 6 min after initial mixing. Ascorbic acid is used as a standard and the capacity of free radical scavenging is expressed as µmol ascorbic acid equivalents /100 g of fruit weight. Venkatachalam *et al*., 2014 studied the antioxidant activity of some fruits and vegetables as below [27].

Table 1: Total ascorbic acid, total phenolics and total flavonoids of selected fruits and vegetables.

Plant extract	Total ascorbic acid content (mg/100 g FW)*	Total phenolic contents (mg/100 g FW)*	Total flavonoid contents (mg/100 g FW)*	Ref. No.
Mulberries	32.19±0.5^{d}	42.12±1.1^{f}	7.57±0.7^{f}	27
Papaya	68.71±1.7^{i}	35.86±1.5^{d}	2.49±0.9^{a}	
Red grapes	43.97±1.1^{f}	36.08±0.8^{d}	12.69±1.1^{i}	
Red grapes	43.97±1.1^{f}	36.08±0.8^{d}	12.69±1.1^{i}	
Mango	39.84±1.2^{e}	33.44±1.7^{c}	4.64±0.5^{c}	
Guava	48.95±1.0^{g}	31.97±1.0^{b}	8.67±1.0^{g}	
Tomato	10.83±0.4^{a}	26.57±1.1^{a}	5.36±0.7^{d}	
Red onion	28.12±1.3^{c}	42.25±1.0^{f}	3.38±0.6^{b}	
Red cauliflower	22.12±0.5^{b}	44.23±2.2^{g}	6.69±0.5^{e}	
Carrot	54.50±0.2^{h}	39.76±1.3^{e}	5.78±0.9^{d}	
Beetroot	48.40±0.8^{g}	57.64±1.2^{h}	10.19±1.7^{h}	

Table 2: Total antioxidant and radical scavenging capacities of selected fruits and vegetables.

Plant exract	Total antioxidant capacity (mg/100 g FW)*	DPPH scavenging capacity (mg/100g FW)*	FRAP capacity (mg/100 g FW)*	Superoxide radical scavenging capacity (mg/100 g FW)*	Hydroxyl radical scavenging capacity (mg/100 g FW)*	Ref. No.
Mulberries	45.78±1.70 ^{d}	45.27±0.10^{f}	32.11±0.50^{c}	48.90±0.90^{h}	41.22±1.30^{e}	27
Papaya	40.05±2.50^{c}	38.24±0.90^{d}	39.72±1.00^{e}	33.71±1.20^{c}	36.50±0.50^{c}	
Red grapes	48.13±1.20^{e}	44.82±0.90^{f}	32.04±0.70^{e}	42.10±1.10^{f}	45.65±1.40^{f}	
Mango	31.21±1.70^{a}	28.99±1.20^{b}	33.42±0.80^{d}	30.78±1.00^{b}	28.17±1.00^{b}	
Guava	41.11±1.50^{c}	39.97±0.90^{d}	31.32±0.20^{c}	40.76±2.10^{e}	38.12±0.80^{d}	
Tomato	34.57±3.00^{b}	18.67±0.70^{a}	19.88±0.30^{a}	18.80±1.00^{a}	21.88±1.10^{a}	
Red onion	48.78±2.40^{f}	38.12±0.10^{d}	29.23±0.50^{b}	44.14±1.50^{g}	40.47±1.50^{e}	
Red cauliflower	41.29±1.80^{c}	40.53±0.50^{d}	43.81±0.60^{f}	39.37±1.00^{e}	38.87±1.80^{d}	
Carrot	47.65±1.20^{e}	34.44±0.10^{c}	33.29±0.90^{d}	37.77±0.90^{d}	35.41±1.20^{c}	
Beetroot	61.11±2.10^{g}	43.12±0.80^{e}	45.79±1.00^{g}	58.32±2.30^{i}	52.33±1.50^{g}	

The values are expressed in mean ± standard deviation. The superscript alphabets in the column show the significant difference ($P < 0.05$).

Conclusion

The selected fruits and vegetables studied by different research worker are highly beneficial to human nutrition and health which is revealed by their antioxidant activity and different scavenging capacity test. It has been concluded that many plants can be used as herbal medicine. The total phenol content and flavonoids are responsible for antioxidant activity and other medicinal properties. While using such a plants and their parts as a herbal medicine standardization of this should be carried out because plants are bio-accumulators. Plants are having ability to absorb heavy metals which are potentially health hazard. So the complete analysis of various parameters of plants and its critical interpretation must be carried out in concerned with health hazard.

The overall information of this present data will be very useful to nutritionists, dieticians, farmers and consumers.

References

1. Wootton-Beard PC, Ryan L. Improving public health? The role of antioxidant-rich fruit and vegetable beverages. Food Research International. 2011; 44(10):3135-3148.
2. Isabelle M, Lee BL, Lim MT, Koh WP, Huang D, Ong CN. Antioxidant activity and profiles of common vegetables in Singapore. Food Chemistry. 2010; 120(4):993-1003.
3. Borowska J. Owoce i war zywa jako źródło naturalnych przeci wutleniaczy [Fruits and vegetables as source of natural antioxidants]. Przem. Ferm. Owoc. Warz. 2003; 1:11-12.
4. Horubała A. Pojemność przeciwutleniająca i jejzmiany w procesach przetwarzania owoców i warzyw [Antioxidant capacity and their changes in fruit and vegetables processing]. Przem. Ferm. Owoc. Warz. 1999; 3:30-31.
5. Jacob RA, Burri BJ. Oxidative damage and defense. The American Journal of Clinical Nutrition. 1996; 63(6):985-990.
6. Ratnam DV, Ankola DD, Bhardwaj V, Sahana DK, Kumar MN, Role VR. of antioxidants in prophylaxis and therapy: A pharmaceutical perspective. Journal of Controlled Release. 2006; 113(3):189-207.
7. Stangeland T, Remberg SF, Lye KA. Total antioxidant activity in 35 Ugandan fruits and vegetables. Food Chemistry. 2009; 113(1):85-91.
8. Gülçin I, Berashvili D, Gepdiremen A. Antiradical and antioxidant activity of total anthocyanins from Perilla

pankinensisdecne. Journal of Ethno pharma cology. 2005; 101(1, 3):287-293.
9. Glew RS, Dorothy J, Chuang LT, Huang YS, Millson M, Glew RH. Nutrient content of four edible wild plants from West Africa. Plant Foods Hum. Nutr. 2005; 60:187-193.
10. Leung W. TW. Food composition table for use in Africa. FAO and US Department of Health, Education, and Welfare: Rome and Washington, 1968.
11. Robards K, Prenzler PD, Tucker G, Swatsitang P, Glover W. Phenolic compounds and their role in oxidative processes in fruits. Food Chem. 1999; 66:401-436.
12. Stewart AJ, Bozonnet S, Mullen W, Jenkins GI, Lean MEJ. A. Crozier. Occurrence of flavonols in tomatoes and tomato- based products. J Agric Food Chem. 2000; 48:2663-2669.
13. Hertog MGL, Hollman PCH, Venema DP. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J Agric. Food Chem. 1992; 40:1591-1598.
14. Han X, Shen T, Lou H. Dietary Polyphenols and Their Biological Significance. International Journal Molecular Science. 2007.
15. Segev A, Badani H, Kapulnik K, Shomer I, Oren-Shamir M, Galili S. Determination of Polyphenols, Flavonoids and Antioxidant Capacity in Colored Chick-Pea (Cicerarietinum L.). Journal Food Science. 2010; 75:S115- S119.
16. Fremont L, Gozzelino MT, Franchi MP, Linard A. Dietary Flavonoids Reduce Lipid Peroxidation in Rats Fed Polyunsaturated and Monounsaturated Fat Diets. Journal of Nutrition. 1998; 128:1495-1502.
17. Xu BJ, Yuan SH. Chang SKC. Comparative Analyses of Phenolic Composition, Antioxidant Capacity and Color of Cool Season Legumes and Other Selected Food Legumes. Journal of Food Science. 2007; 72:S167-S177.
18. Deepa Babu, Prema Gurumurthy, Sai Krishna Borra, Cherian KM, Antioxidant and free radical scavenging activity oftriphala determined by using different in vitro models. Journal of Medicinal Plant Research. 2013; 7(39):2898-2905.
19. Kaur S, Michael H, Arora S, Härkönen PL, Kumar S. The in vitro cytotoxic and apoptotic activity of Triphala--an Indian herbal drug. J Ethnopharmacol. 2005; 97(1):15-20.
20. Jagetia GC, Baliga MS, Malagi KJ, Sethukumar KM. The evaluation of the radioprotective effect of Triphala (an ayurvedic rejuvenating drug) in the mice exposed to gamma-radiation. Phytomed. 2002; 9(2):99-108.
21. Lim YY, Lim TT, Tee JJ. Antioxidant properties of several tropical fruits: A comparative study. Food Chemistry. 2007; 103(3):1003-1008.
22. Giardi MT, Rea G, Berra B. Bio Farms for Nutraceuticals: Functional Food and Safety Control by Biosensors. Landes Bioscience and Springer Science+Business Media, 2010.
23. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. Methods Enzymol. 1999; 299:152-178.
24. Hinneburg I, Dorman HJD, Hiltunen R. Antioxidant activities of extracts from selected culinary herbs and spices. Food Chem. 2006; 97:122-129.
25. Meda A, Lamien CE, Romito M, Millogo J, Nacoulma OG. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. Food Chem. 2005; 91:571-577.
26. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free Radical Biol. Med. 1999; 26:1231-1237.
27. Venkatachalam K, Rangasamy R, Krishnan V. Total antioxidant activity and radical scavenging capacity of selected fruits and vegetables from South India. International Food Research Journal. 2014; 21(3):1039-1043.