Phytochemical screening and *in vitro* antioxidant activity of *Psidium guajava*

Ravi Narayan Venkatachalam, Kanchanlata Singh, Thankamani Marar*

Department of Biotechnology & Bioinformatics, Padmashree Dr. D. Y. Patil University, Sector 15, CBD Belapur, Navi Mumbai, Maharashtra, India.

ABSTRACT

Free radicals or Reactive oxygen species (ROS), a class of highly reactive molecules derived from the metabolism of oxygen is believed to be involved in many health disorders. ROS including superoxide radicals, hydroxyl radical and hydrogen peroxide molecules are often generated as by products of biological reactions or from exogenous factors. Antioxidant-based drugs and formulations for the prevention and treatment of complex diseases have attracted a great deal of research interest in natural antioxidants. It is necessary to screen medicinal plants for their antioxidant potential. Determination of phytochemical constituents like phenols, flavonoids, tannins, chlorophyll and carotenoids and various tests like reducing power, ferric reducing antioxidant power, linoleic acid assay, DPPH, nitric oxide, superoxide and hydrogen peroxide radical scavenging assays for evaluation of *in vitro* antioxidant activity of methanolic and aqueous extracts of *Psidium guajava* (Guava) leaves were carried out. It was found that the methanolic extracts showed greater amount of phytochemicals and higher antioxidant activity than aqueous extracts. Present results indicate that guava is a good candidate for development of new nutraceuticals for treatment of diseases like diabetes. However, *in vivo* studies to determine the antioxidant potential of the plant should be conducted before they can be recommended as nutritional substitutes.

Keywords- flavanoids, phenols, DPPH, oxidative stress, reactive oxygen species, guava.

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Over 50% of all modern clinical drugs are of natural product origin and natural products play important roles in drug development in the pharmaceutical industry.[1] The isolation and identification of the active principles and elucidation of the mechanism of action of a drug is of paramount importance. Hence, works in both mixture of traditional medicine and single active compounds are very important.[2]

***Address for correspondence:**
Phone numbers: Department-+91 22-27563600
Mobile-+91 9892455625
Fax-+91 22 39286176
E-mail: dr.marar@yahoo.com

DOI: 10.5530/ax.2012.2.7

Oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems.[3] ROS (Reactive oxygen species) are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders[4] such as cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinsons disease, alcohol induced liver disease, ulcerative colitis, ageing, atherosclerosis[5-14]. Numerous studies have been carried out on the leaves of plants because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, phenols and flavonoids.[15] Flavonoids and phenolic compounds widely distributed in plants have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc.[16]

Psidium guajava (Common name: Guava) belongs to family of *Mystraecea* and is widely cultivated in India. Extracts from guava leaves are beneficial as therapeutic against cancer, bacterial infections, inflammation and pain. Essential oils

from guava leave display in vitro anti-cancer activity.[17] Guava is rich in tannins, phenols, triterpenes, flavonoids, essential oils, saponins, carotenoids, lectins, vitamins, fiber and fatty acids. The leaves of guava are rich in flavonoids and phenols. The flavonoids have demonstrated antibacterial activity. Guava leaves are also used in folk medicine as a remedy for diarrhea. Quercetin is thought to contribute to the anti-diarrhea effect of guava; it is able to relax the intestinal smooth muscle and inhibit bowel contractions. In addition, other flavonoids and triterpenes present in guava leaves show antispasmodic activity.[18]

The objective of the present study was to determine the phytochemical constituents and *in vitro* antioxidant activity of methanolic and aqueous extracts from leaves of guava through a number of testing methods. This study proposes to investigate the practical utility of consumption of guava leaves as an antidiabetic and antioxidant nutraceutical.

MATERIALS AND METHODS

Plant material and preparation of extracts

Fresh, young leaves of guava were collected from Navi Mumbai, authenticated, washed and allowed to dry at room temperature. The dried leaves were then ground to fine powder. 30 g of the dry powder was weighed and was used for extract preparation.

Extracts for the plant leaves were prepared using both methanol and distilled water. 30 g of the dry powder was ground to a paste in pestle and mortar using 150 ml of the respective solvents and was filtered twice through Whatman filter paper. The resulting filtrate was collected in a beaker and was subjected to evaporation in a Rotary Evaporator for 10 min at 100 °C (for aqueous extraction) and 60 °C (for methanolic extraction). The extract was suitably diluted for use. The yield of extract from methanol was 5.6 gm and from aqueous was 3.75 gm for 30 grams of dry guava leaves powder.

Estimation of Phytochemical constituents

1. Estimation of total phenol content (TPC)

The total phenol content was determined by Folin- Ciocalteu reagent method.[19] 0.5 ml of extract (1:5 dilution) and 0.1 ml of Folin-Ciocalteu reagent (0.5N) were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added, incubated for 30 min at room temperature and absorbance was measured at 760 nm. The total phenol content was expressed in terms of gallic acid equivalent (mg/g).[20]

2. Estimation of total flavonoids (TF)

The total flavonoid content was determined by Aluminum chloride method.[21] The reaction mixture (3.0 ml) that comprised of 1.0 ml of extract (1:10 dilution), 0.5 ml of aluminum chloride (1.2%) and 0.5 ml of potassium acetate (120 mM) was incubated at room temperature for 30 min and absorbance was measured at 415 nm. The total flavonoid content was expressed in terms of quercitin equivalent (mg/g).[22]

3. Estimation of sugars

Estimation of sugars in the extract was done by DNSA method.[22] 1 ml of the extract (1:20 dilution) was added to 1 ml DNSA, mixed and heated for 5 min. 2 ml of distilled water was added to the mixture and absorbance was measured at 525 nm. Total sugars were expressed in terms of maltose equivalent (mg/g).

4. Estimation of tannins[20]

The tannin content was determined by Folin-Ciocalteu reagent method. 1:10 diluted extract was added to Folin-Ciocalteu reagent (0.5N), mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added, incubated for 30 min at room temperature and absorbance measured at 760 nm. Tannins were expressed in terms of tannic acid equivalent (mg/g).

5. Estimation of chlorophyll and carotene[23]

1 g of leaf sample was weighed and was ground in pestle-mortar with 5 ml distilled water to a paste. The contents were transferred to a centrifuge tube and the total volume was made upto 10 ml with distilled water. 0.5 ml from the tube was transferred to a tube containing 4.5 ml of 80% acetone. The contents were centrifuged at 4000 rpm for 15 min. The absorbance of the supernatant was measured at the following wavelengths-645,663,490,638 nm and the content of chlorophyll was calculated.

Evaluation of antioxidant activity

1. α, α-diphenyl- β-picryl-hydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity was measured by using 2, 2-diphenyl-1-picryl-hydrazyl or 1, 1- diphenyl-2-picryl-hydrazyl by the method of McCune and Johns.[24] The reaction mixture consisted of 1.0 ml of DPPH in methanol (0.3 mM) and 1.0 ml of the extract. After incubation for 10 min in dark, the absorbance was measured at 517 nm.

DPPH scavenging activity was expressed in terms ascorbic acid equivalent (mg/g).[20]

2. Nitric oxide (NO) radical scavenging assay

3.0 ml of sodium nitroprusside in phosphate buffer (10 mM) was added to the extract and incubated at 25 °C for 60 min. 5.0 ml of Griess reagent (1% sulphanilamide, 0.1% naphthyethylene diamine dihydrochloride in 2% H_3PO_3) was added and absorbance of the chromophore formed was measured at 540 nm. NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).[20,25]

3. Ferric reducing antioxidant power (FRAP) assay

A suitable aliquot of diluted extract was added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM FeCl3. 6H2O solution) and the reaction mixture was incubated at 37 °C for 30 min. The increase in absorbance at 593 nm was measured. The antioxidant capacity based on the ability to reduce ferric ions of sample was expressed in terms of ascorbic acid equivalent (mg/g).[20]

4. Estimation of reducing power (RP)

The reducing power was determined by the method of Athukorala.[26] 1.0 ml extract was mixed with equal amounts of phosphate buffer (200 mM, pH 6.6) and potassium ferricyanide (30 mM) and incubated at 50 °C for 20 min. 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture, centrifuged for 10 min at 3000 rpm. The upper layer of solution was added to equal amount of distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance was measured at 700 nm. RP was expressed in terms of standard equivalent (mg/g).[20]

5. Superoxide anion (SO) radical scavenging assay

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski.[27] The superoxide anion radicals are generated in 3.0 ml of Tris-HCl buffer, containing 0.5 ml of NBT (0.3 mM), 0.5 ml NADH solution (0.936 mM), 1.0 ml diluted extract and 0.5 ml Tris-HCl buffer (16 mM, pH 8). The reaction was started by adding 0.5 ml PMS solution to the mixture, incubated at 25°C for 5 min and then the absorbance was measured at 560 nm. SO anion scavenging activity was expressed in terms of standard equivalent (mg/g).[20]

6. Hydrogen peroxide (H_2O_2) radical scavenging assay

The ability of plant extracts to scavenge hydrogen peroxide is determined according to the method of Ruch.[28] A solution of hydrogen peroxide (40 mM) is prepared in phosphate buffer (50 mM, pH 7.4) and 2 ml of the solution is added to 1 ml extract (1:20 dilution). The absorbance at 230 nm is determined after 10 mins. H_2O_2 radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

7. Total antioxidant activity

The antioxidant activity was determined by the conjugated diene method.[29] The method allows dynamic quantification of conjugated diene as a result of initiation of PUFA (Polyunsaturated Fatty acid) oxidation by measuring UV absorbance at 234 nm. The principle of this assay is that during linoleic acid oxidation, the double bonds are converted into conjugated double bonds which are characterized by strong UV absorption at 234 nm. 0.1 ml extract was mixed with 2.0 ml of linoleic acid emulsion (10 mM) in sodium phosphate buffer (pH 6.6) and kept in dark at 37 °C to accelerate oxidation. After incubation for 15 h, 0.1 ml from each tube was mixed with 7.0 ml methanol in deionized water (80%) and the absorbance of the mixture was measured at 234 nm. Total antioxidant activity was expressed in terms of ascorbic acid equivalent (mg/g).

RESULTS AND DISCUSSION

There are many reports of herbal extracts being used in ayurvedic literature which are directly or indirectly used for the preparation of many modern drugs. Gauva plants have been used in African and Mexican medicine for treatment of diabetes, diarrhea and bacterial infection. Table 1 and 2 represent the phytochemical constituents present in guava leaves. It was observed that methanolic extraction had a higher content of the phytochemicals than the aqueous extract. Guava is rich in phytochemicals like tannins, phenols, triterpenes, flavonoids, saponins, lectins etc. The leaves of guava are rich in flavonoids and phenols which contribute to its antioxidant potential.[30] The sugar content of the leaves was also high since guava is a fruit bearing plant.

Table 1: Phytochemical constituents in *Psidium guajava* leaf extracts

Tests	Standard Equivalent in Methanolic Extract (mg/g)	Standard Equivanent in Aqueous Extract (mg/g)
Total Phenol Content	8.00 ± 0.1	7.38 ± 0.09
Total Flavanoids	5.03 ± 0.149	4.525 ± 0.050
Sugar Content	24.00 ± 0.86	21.33 ± 0.76
Tannin Content	3.81 ± 0.029	3.46 ± .06

(The results obtained were expressed as Mean ± S.D. of triplicates).

Table 2: Total chlorophyll and carotene content in *Psidium guajava* leaves

Plant	Total Chlorophyll (g/l)	Chlorophyll a (g/l)	Chlorophyll b (g/l)	Carotene (g/l)
Psidium guajava	0.011 ± 0.0002	0.004 ± 0.001	0.006 ± 0.001	0.276 ± 0.038

(The results obtained were expressed as Mean ± S.D. of triplicates).

Table 3: Antioxidant activity of *Psidium guajava* leaf extracts

Tests	Standard equivalent in methanolic extract (mg/g)	Standard equivalent in aqueous extract (mg/g)
DPPH Scavenging assay	42.33 ± 0.76	39.33 ± 0.29
NO radical scavenging assay	47.5 ± 1.00	32.00 ± 1.00
FRAP assay	68.66 ± 3.75	43.50 ± 0.26
Reducing power assay	9.24 ± 0.39	8.3 ± 0.30
SO radical scavenging assay	285.00 ± 0.83	270.83 ± 3.82
H_2O_2 radical scavenging assay	14.66 ± 0.35	13.7 ± 0.30
Linoleic acid assay	60.06 ± 0.76	57.5 ± 0.86

(The results obtained were expressed as Mean ± S.D. of triplicates).

The *in vitro* methods for evaluation of antioxidant activity have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. Table 3 shows the reducing power and radical scavenging activity of guava leaves which are directly correlated to antioxidant activity.

DPPH is a stable free radical and is widely used to assess the radical scavenging activity of antioxidant compounds. The DPPH scavenging activity in 1 gram of guava leaves was found to be 42 mg ascorbic acid equivalent in methanolic extract and 39 mg ascorbic acid equivalent in aqueous extract indicating only a slight difference between both extracts of guava leaves. (Table 3) DPPH scavenging activity of the extracts can be correlated to the presence of flavonoids.[31-33]

Antioxidants act by scavenging the NO radicals.[20] The NO scavenging activity in guava leaves was found to be higher in methanolic extracts than in aqueous extract (Table 3). Nitric oxide radical scavenging activity is correlated to the presence of phenolic compounds.[34]

FRAP assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), forming an intense blue Fe^{2+}-TPTZ.[22] The FRAP in guava leaves was estimated to be 68.66 mg equivalent ascorbic acid per gram leaves and equivalent in methonolic extract and 43.50 mg/gram equivalent in aqueous extract. This shows a significant difference between both extracts of guava leaves (Table 3). The FRAP activity is correlated to catechin, ferulic acid and total phenols which are present in guava.[32,35]

Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity. The methanolic extract exhibited a higher reducing power than the aqueous extract. The reducing power is mainly correlated to the presence of reductones like ascorbic acid and guava is reported to be rich in ascorbic acid.[36]

Superoxide anion is a weak oxidant; still it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress.[20] The SO scavenging activity was found to be very high in both the extracts. SO scavenging activity is associated to total flavanoids which is higher in the methanolic extract.[37,20]

Hydrogen peroxide occurs naturally at low concentration levels in the air, water, human body, plants, microorganisms, food and beverages. The H_2O_2 scavenging activity in guava leaves was estimated to be higher in the methanolic extract. This can be correlated to the presence of total phenols in the extract.[38]

The oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of hydrogen atoms from diallylic methylene groups of linoleic acid. The free radicals will further oxidize the highly unsaturated beta carotene. Consequently, the orange coloured chromophore of beta carotene is degraded.[20] The antioxidant activity in 1 gram guava leaves was estimated to be 60.06 ± 0.76 mg ascorbic acid equivalent in methanolic extract and 57.5 ± 0.86 mg ascorbic acid equivalent in aqueous extract. This shows a significant difference between both extracts indicating high antioxidant activity of guava leaves (Table III). Total antioxidant activity is associated to total phenol content.[39]

The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, like iron and copper, and inhibition of enzymes responsible for free-radical generation. Depending on their structure, flavonoids are able to scavenge practically all known ROS.[20]

Antioxidative properties of polyphenols arise from the ability of the polyphenol derived radical to stabilize and

delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction). Plants having more phenol content show good antioxidant activity indicating a direct correlation between TPC and antioxidant.[20]

Tannin is actually a bitter plant polyphenolic compound and an astringent that binds to and precipitates proteins and various other organic compounds including amino acids and alkaloids. This tannin-protein complex can provide persistent antioxidant activity.[22] The tannin content in guava leaves was estimated to be 114.5 ± 0.87 mg tannic acid equivalent in methanolic extract and 104 ± 1.73 mg tannic acid equivalents in aqueous extract.

Chlorophyll has been suggested as an effective antioxidant since it scavenges free radicals such as 1, 1-diphenyl-2-picrylhydrazyl.[40] Carotenes have the ability to detoxify various forms of activated oxygen and triplet chlorophyll that are produced as a result of excitation of the photosynthetic complexes by light. In terms of its antioxidant properties carotenoids can protect the photosystems in one of four ways – by reacting with lipid peroxidation products to terminate chain reactions or by scavenging singlet oxygen and dissipating the energy as heat or by reacting with triplet or excited chlorophyll molecules to prevent formation of singlet oxygen or by the dissipation of excess excitation energy through the xanthophyll cycle.[3] The total chlorophyll content in guava leaves was found to be 0.011 g/l and the carotene content was 0.276 g/l.

In the current investigation, both extracts from guava leaves gave good results indicating that it possesses significant amount of phytochemicals and *in vitro* antioxidant activity. The methanolic extracts showed higher content of phytochemical constituents and higher *in vitro* antioxidant activity than aqueous extracts. Phenolic compounds and other phytochemicals appear to be responsible for the *in vitro* antioxidant activity of the extracts and may contribute to the therapeutic activity observed. On the basis of the results obtained, guava leaf extracts are rich sources of natural antioxidants and could be developed into functional food or drug against diseases and for a variety of beneficial chemo-preventive effects. Our previous report have also revealed anti-diabetic potential of guava.[41] Further studies are in progress in our laboratory to evaluate the *in vivo* antioxidant potential of guava leaves in various animal models. Phytochemical studies required to establish the types of compounds responsible for the bioactivity are also currently being pursued.

REFERENCES

1. Preethi R, Devanathan V, Loganathan M. Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants Against Food Borne Pathogens. Adv. in Biological Res 2010; 4:122-125.
2. Nagavani V, Rao D, Madhavi Y. Free radical scavenging activity and qualitative analysis of polyphenols by RP-HPLC in the flowers of Couroupita guianensis abul. Electronic Journal of Environmental.Agricultural and Food Chemistry 2010; 9:1471-1484.
3. Slater A, Scott W. Plant Biotechnology: The genetic manipulation of plants. Oxford University Press 2008, pp. 229.
4. Rackova L, Kostalova D, Oblozinsky M. Free radical scavenging activity and lipoxygenase inhibition of Mahonia aquifolium extract and isoquinoline alkaloids. *Journal of* Inflammation 2007; 4:1-7.
5. Kinnula V, Crapo J. Superoxide Dismutases in the Lung and Human Lung Diseases. American Journal of Respiratory and Critical Care Medicine 2003; 167:1600-1619.
6. Singh U, Jiala I, Devraj S. Vitamin E, Oxidative Stress, and Inflammation. Annual Review of Nutrition 2005; 25:151-174.
7. Sas K, Robotka H, Toldi, J. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. Journal of the Neurological Sciences 2007; 257:221-239.
8. Smith M, Perry G, Raina A. Oxidative stress in Alzheimer's disease. Biochimica et Biophysica Acta 2000; 1502:139-144.
9. Guidi I, Baron P, Lonati S. Oxidative imbalance in patients with mild cognitive impairment and Alzheimer's disease. Neurobiology of Aging 2006; 27:262-269.
10. Bolton J, Trevor M, Trush M. Role of Quinones in Toxicology. Chemical Research in Toxicology 2000; 13:135-160.
11. Arteel G. Oxidants and antioxidants in alcohol-induced liver disease. Gastroenterology 2003; 124:778-790.
12. Ramakrishna B, Jayakumar S, Varghese R. Circulating antioxidants in ulcerative colitis and their relationship to disease severity and activity. Journal of Gastroenterology and Hepatology 2008; 12:490-494.
13. Hyun D, Jo D, Emerson S. Calorie restriction up-regulates the plasma membrane redox system in brain cells and suppresses oxidative stress during aging. The Proceedings of the National Academy of Sciences 2006; 103:19908-12.
14. Upston J, Kritharides L, Stocker R. The role of vitamin E in atherosclerosis. Progress in Lipid Research 2003; 42:405-422.
15. Miladi S, Damak M. *In vitro* antioxidant activities of *Aloe vera* leaf skin extracts. Journal de la Société Chimique de Tunisie 2008; 10:101-109.
16. Deshraj C, Balakrishnan B, Jayakar B. *In vitro* antioxidant activities of roots of Thespesia lampas dalz and Gibs. Pak. J. Pharm. Sci. 2009; 22:368-372.
17. Gutierrez R, Mitchell S, Solis R. *Psidium guajava*: a review of its traditional uses, phytochemistry and pharmacology. J. Ethnopharmacol 2008; 117:1-27
18. Oh W, Lee C, Lee M. Antidiabetic effects of extracts from *Psidium guajava*. *J.* Ethnopharmacol. 2005; 96:411-415.
19. McDonald S, Prenzler P, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chemistry 2001; 73:73-84.
20. Chandha S, Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. African Journal of Microbiology Research 2009; 3:981-996.
21. Chang C, Wen H, Yang M. Estimation of Total Flavonoid Content in Propolis by Two Complementary Colorimetric Methods. Journal of Food and Drug Analysis 2002; 10:178-182.
22. Mohun AF, Cook IJY. An improved dinitrosalicylic acid method for determining blood and cerebrospinal fluid sugar levels. J. clin. Path 1962; 15:169-180.
23. Jayaraman, J. Estimation of Chlorophyll and Carotenoids. In Laboratory Manual in Biochemistry. New Age International 2011; pp: 141-142.
24. McCune L, Johns T. Antioxidant activity in medicinal plants associated with the symptoms of diabetes mellitus used by the Indigenous Peoples of the North American boreal forest. Journal of Ethno pharmacology 2002; 82:197-2005.
25. Green L, Wagner D, Young V. Nitrate biosynthesis in man. The Proceedings of the National Academy of Sciences 1981; 78:7764-7768.

26. Athukorala Y, Jeon Y, Kim K. Antiproliferative and antioxidant properties of an enzymatic hydrolysate from brown alga, Ecklonia cava. Food and Chemical Toxicology 2006; 44:1065-1074.
27. Robak J, Gryglewski J. Flavanoids are scavengers of superoxide anions. Biochem. Pharmacol 1998; 37:837-841.
28. Ruch J, Klaunig J. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989; 10:1003-1008.
29. Lingnert H. Measurement of Antioxidative effect in model system. Journal of Food Processing and Preservation 1979; 3:87-103.
30. Chen H, Yen G. Antioxidant activity and free radical-scavenging capacity of extracts from guava (*Psidium guajava*) leaves. Journal of Agricultural and Food Chemistry 2006; 2:686-694.
31. Ypuwei Z, Jinlian Z, Yonghong P. A comparative study on the free radical scavenging activities of some fresh flowers in southern China. LWT-Food Science & Technology 2008; 41:1586-1591.
32. Thaipong K, Byrne D, Crosby K. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 2006; 19:669-675.
33. Khalaf N, Ashok K, Shakya A. Antioxidant Activity of Some Common Plants. Turk J Bio. 2008; 32:51-55.
34. Sonawane I, Dhasade V, Nirmal S. Antioxidant effect of Tephrosia Purpurea L. roots. International Journal of Pharmaceutical Sciences and Research 2010; 1:57-60.
35. Adebayo J, Ayoade A, Adesokan, A. Effect of ethanolic extract of Bougainvillea spectabilis leaves on haematological and serum lipid variables in rats. Biokemistri 2005; 17:45-50.
36. Li H, Li Y, Wang X. Comparative Study of Antioxidant Activity of Grape (*Vitis vinifera*) Seed Powder Assessed by Different Methods. Journal of Food and Drug Analysis 2008; 16:1-7.
37. Chen H, Yen G. Antioxidant activity and free radical-scavenging capacity of extracts from guava (Psidium guajava L.) leaves. Journal of Agricultural and Food Chemistry 2006; 2:686-694.
38. Elmastas M, Ibao K, Gulcin I. Radical Scavenging Activity and Antioxidant Capacity of Bay Leaf Extracts. Journal of the Iranian Chemical Society 2006; 3:258-266.
39. Anwar F, Qayyum H, Iqbal S. Antioxidant activity of 100% and 80% methanol extracts from barley seeds (Hordeum vulgare L.): stabilization of sunflower oil. Grasas Y Aceites 2010; 61:237-243.
40. Ferruzzi M, Courtney P, Bohm V.Antioxidant and Antimutagenic Activity of Dietary Chlorophyll Derivatives Determined by Radical Scavenging and Bacterial Reverse Mutagenesis Assays. Journal of Food Science 2002*;* 67:2589-2595.
41. Singh A, Marar T. Inhibitory effect of extracts of syzygium cumini and psidium guava on glycosidases. J.Cell and Tissue Res 2011; 11:2535-2539.