ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparative Analysis of Phytochemical Profile and Antioxidant Activity of Some Indian Culinary Herbs

Deepa Garg *, Aditya Muley, Nishtha Khare, Thankamani Marar

Department of Biotechnology & Bioinformatics, Padmashree Dr. D. Y. Patil University, Sector 15, CBD Belapur, Navi Mumbai, Maharashtra, India-400614.

ABSTRACT

In the current study, we carried out a comparative analysis of the antioxidant activities of methanolic and aqueous extracts of the selected leaves of herbs commonly used in Indian cuisine. Total content of phenols, carotenes, tannins and flavonoids was quantitatively estimated from leaves of lemon grass, mint, coriander and curry leaves. Antioxidant activity of the two extracts using free radical scavenging assays like DPPH, FRAP, SO, NO and H_2O_2 was determined. It was observed that presence of greater amount of phenolic compounds leads to a more powerful radical scavenging effect as was shown by methanolic extract of the leaves when compared to the aqueous extracts. Among the herbs investigated by us lemon grass exhibited the maximum content of phenols and hence greatest antioxidant profile. Mint showed significant concentration of phenols and thus good activity against deleterious oxidants. The results show that use of the natural antioxidants occurring in herbs used in the Indian diet, or their extracts, is a viable option for the food industry as long as the organoleptic characteristics of the food product are not affected.

Keywords: mint, curry leaves, antioxidants, lemon grass, coriander, culinary herbs.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Free radicals and reactive oxygen species (ROS) have been associated with the etiology and/or progression of a number of diseases and in aging. Oxygen-free radicals (OFR), or more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) are products of normal cellular metabolism. ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems [1]. So a search for better and effective antioxidants has always been a priority. Phenolic acids and their derivatives are widely distributed in plants [2]. The role of phenolic acid and flavonoids as natural antioxidants and free radical scavenger has been of interest due to their pharmacological behavior [3].

Indian cooking basically contains a handful of herbs which help to enhance the flavor of the dish. Lemon grass (*Cymbopogon citrtus*), curry leaves (*Murraya koenigii*), mint (*Mentha arvensis*) and coriander(*Coriandrum sativam*) leaves are common ingredients in Indian cooking added in the end to garnish the dishes. Since these leaves do not undergo extensive cooking process the natural antioxidants are preserved [4, 5].

Mint (*Mentha arvensis*) is a genus of flowering plants in the family Lamiaceae. It is well known for its properties related to indigestion, stomach cramps, menstrual cramps, flatulence, upset stomach, nausea, vomiting, and colic in children [6]. It is also a source of effective antioxidants. Workers have separated and identified phenolic acids, flavonoids, terpenoids and other volatile compounds from different extracts of mint [7].

Curry leaves (*Murraya koenigii*) is a tropical to sub-tropical tree in the family Rutaceae, which is native to India. The leaves are used in Ayurvedic medicine.Their properties include anti-diabetic, antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and anti-hypercholesterolemic [8, 9].

Lemon Grass (*Cymbopogon citratus*) is a tropical grass in the family Poaceae. It is used extensively in Ayurvedic medicine. Lemongrass oil is used as a pesticide and preservative and has anti-fungal properties [10]. It helps to relieve congestion, coughing, bladder disorders, headaches, fever, stomach aches, digestive problems, diarrhea, gas, bowel spasms, vomiting, flu symptoms, as a mild sedative, and to promote perspiration and as a possible cholesterol lowering agent[11,12].

Coriander (*Coriandrum sativum*) is an annual herb in the family Apiaceae. Coriander has been used as a folk medicine for the relief of anxiety and insomnia in Iran. Experiments in mice support its use as an anxiolytic [13]. Seeds are used as a drug for indigestion, against worms, rheumatism and pain in the joints. Recent studies have also demonstrated hypoglycaemic action and effects on carbohydrate metabolism [14, 15].

The objective of this research was to evaluate and compare the phytochemical and antioxidant activities of the methanolic and aqueous extracts of above mentioned herbs.

MATERIALS AND METHODS

Preparation of Extracts

Fresh, young leaves of mint, curry leaves, coriander and lemon grass were collected, authenticated, washed and soaked in water for 3 hours. The leaves were then allowed to dry at room temperature under a clean cloth for 24 hours. 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 (methanolic extract) and distilled water (aqueous extract) as solvents. Extracts were prepared using the soxhlet apparatus. The extracts were diluted appropriately before use.

Estimation of Phytochemical constituents

1. Estimation of total phenol content (TPC)

The total phenol content was determined by Folin-Ciocalteu reagent method [16,17]. The diluted extract 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 and further incubated for 30 min at room temperature and absorbance measured at 760 nm. The total phenol content was expressed in terms of Gallic acid equivalent (mg/g).

2. Estimation of total flavonoids (TF)

The total flavonoid content was determined by aluminum chloride method [18]. The reaction mixture comprising of extract, 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).

3. Estimation of sugars

Estimation of sugars in the extract was done by DNSA method [19]. 1 ml of the diluted extract was added to 1 ml DNSA. The contents were mixed and allowed to boil for 5 min. 2 ml of distilled water was added to the mixture and absorbance was measured at 525 nm. Sugar content was expressed in terms of maltose equivalent (mg/g).

4. Estimation of tannins

The tannin content was determined by Folin-Ciocalteu reagent method. Appropriately diluted extract and 0.1 ml of Folin-Ciocalteu reagent were mixed and incubated at room temperature for 15 min. 2.5 ml saturated sodium carbonate was added and further incubated for 30 min at room temperature and absorbance measured at 760 nm. The tannin content was expressed in terms of tannic acid equivalent (mg/g) [17].

5. Estimation of chlorophyll and carotene

1g 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 up to 10ml with distilled water. 0.5 ml from the tube was transferred to a tube containing 4.5ml 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 [20].

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 [21]. The reaction mixture (2.0 ml) consisted of 1ml of DPPH in methanol (0.3 mM) and 1ml of the extract (1:100 dilution). After incubation for 10 min in dark, the absorbance was measured at 517 nm. The standard used was ascorbic acid (0.05 - 0.4 mg/ml) [17]. DPPH scavenging activity was expressed in terms ascorbic acid equivalent (mg/g).

2. Nitric oxide (NO) radical scavenging assay

3.0 ml of sodium nitroprusside in phosphate buffer (10 mM) was added to 2.0 ml of extract (1:200 dilutions). The resulting solution was then incubated at 25°C for 60 min. To 5.0 ml of the incubated sample, 5 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. Ascorbic acid was used as standard (0.02 - 0.1 mg/ml). NO radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g) [17, 22].

3. Ferric reducing antioxidant power (FRAP) assay

0.2 ml of the extract (1:20 dilution) was added to 3.8 ml of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10mM TPTZ solution and 1 part of 20 mM $FeCl_3$ $6H_2O$ solution) and the reaction mixture was incubated at 37°C for 30 min and the

increase in absorbance at 593 nm was measured. Ascorbic acid was used as standard (0.02 - 0.1 mg/ml) [23]. The antioxidant capacity based on the ability to reduce ferric ions of sample was expressed in terms of ascorbic acid equivalent (mg/g).

4. Estimation of reducing power (RP)

The reducing power was determined by the method of Athukorala [24]. 1.0 ml extract was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of 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 (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (6 mM) and absorbance was measured at 700 nm. Ascorbic acid was used as standard. RP was expressed in terms of standard equivalent (mg/g) [25].

5. Superoxide anion (SO) radical scavenging assay

The superoxide anion scavenging activity was measured as described by Robak and Gryglewski [26]. 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 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. Ascorbic acid was used as standard (0.1 - 0.5 mg/ml) [17]. SO anion scavenging activity was expressed in terms of standard equivalent (mg/g).

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 [27]. 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. Ascorbic acid was used as standard [17]. H_2O_2 radical scavenging activity was expressed in terms of ascorbic acid equivalent (mg/g).

Statistical analysis: Mean ± SD for samples in triplicate was used for comparisons.

RESULTS AND DISCUSSION

Spices and herbs are an excellent source of phenolic compounds (flavonoids, phenolic acid and alcohols, stilbenes, tocopherols, tocotrienols), ascorbic acid and carotenoids which have been reported to show good antioxidant activity [28].

Table 1 represents the phytochemical constituents in *Mentha arvensis*, *Murraya koenigii*, *Cymbopogon citratus*, and *Coriandrum sativam* leaf extracts. The highest total

ISSN: 0975-8585

phenolic content was found in the methanolic extracts of lemon grass followed by mint and curry leaves.

TABLE 1: Phytochemical constituents in *Mentha arvensis* , *Murraya koenigii*, Cymbopogan citratus, and *Coriandrum sativam* leaf extracts

TESTS	Standard equivalent in methanolic extract (mg/g)				Standard equivalent in aqueous extract (mg/g)			
	Mentha arvensis	*Murraya koenigii*	*Cymbopogon citratus*	*Coriandrum sativam*	*Mentha arvensis*	*Murraya koenigii*	*Cymbopogon citratus*	*Coriandrum sativam*
TOTAL PHENOL CONTENT	5.13± 0.25	3.0± 0.26	5.73± 0.15	0.6 ±0.1	3.13± 0.25	3.34 ± 0.05	3.14± 0.31	1.67± 0.05
TOTAL FLAVONOIDS	1.57±0.13	2.57±0.07	5.14± 0.12	0.94 ±0.05	1.94±0.33	3.73 ±0.11	3.55± 0.28	1.0±0.05
SUGAR CONTENT	2.76±0.26	11.4±0.29	4.63± 0.12	6.0± 0.86	5.94± 0.09	8.17 ±0.61	11.07± 0.24	5.16 ±0.57
TANNIN CONTENT	2.25±0.26	1.3 ±0.05	2.67± 0.76	0.74 ±0.05	1.44±0.13	1.46 ±0.03	1.45± 0.14	0.27 ±0.05

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

Flavonoid content was again highest in methanolic lemon grass extracts while curry leaves showed minimum flavonoid content. Reducing sugar content was highest in curry leaves (methanolic) extract, followed by lemon grass (aqueous) extract.

Tannin is actually an astringent, bitter plant polyphenolic compound 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. The tannin content was highest in lemon grass extracts [29].

TABLE 2: **Total chlorophyll and carotene content in *Murraya koenigii* , *Mentha arvensis*, *Coriandrum sativam* and Cymbopogon citrates leaf extracts.**

PLANT	Total Chlorophyll (g/l)	Chlorophyll a (g/l)	Chlorophyll b (g/l)	Carotene (g/l)
Mentha arvensis	0.0096	0.0064	0.0052	0.094
Murraya koenigii	0.0264	0.00065	0.00381	0.0445
Cymbopogon citratus	0.0051	0.0031	0.0039	0.055
Coriandrum sativam	0.0112	0.00329	0.00147	0.071

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

Chlorophyll content was found to be higher in curry leaves hence the darker shade of green, then in coriander whereas carotene content was more in mint followed by coriander (Table 2). Chlorophyll has been suggested as an effective antioxidant since it scavenges free radicals such as 1, 1-diphenyl-2-picrylhydrazyl [30]. 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 [31].

TABLE 3: Antioxidant activity of *Murraya koenigii* , *Mentha arvensis*, *Coriandrum sativam* and *Cymbopogon citratus* leaf extracts.

TESTS	STANDARD EQUIVALENT IN METHANOLIC EXTRACT (mg/g)				STANDARD EQUIVALENT IN AQUEOUS EXTRACT(mg/g)			
	Mentha arvensis	*Murraya koenigii*	*Cymbopogon citratus*	*Coriandrum sativam*	*Mentha arvensis*	*Murraya koenigii*	*Cymbopogon citratus*	*Coriandrum sativam*
DPPH SCAVENGING ASSAY	16.4 ±0.37	6.9 ±0.05	17.25 ±0.45	6.9 ±0.05	1.42 ±0.69	7.86 ±0.05	8.05 ±0.38	7.0 ±0.05
NO RADICAL SCAVENGING ASSAY	45.1 ±1.26	5.4 ±0.1	51.4 ±1.26	5.73 ±0.05	37.3 ±6.67	5.43 ±0.16	39.6 ±2.75	2.0 ±0.07
FRAP ASSAY	13.0 ±0.17	1.49 ±0.56	14.5 ±0.61	10.73 ±0.24	20.02 ±0.31	20.86 ± 0.12	26.2 ±0.35	24.26 ±0.02
REDUCING POWER ASSAY	0.56 ±0.15	5.64 0.24	5.96 ±0.47	0.84 ±0.15	2.94 ±0.95	4.46 ±0.23	3.0 ±0.31	0.64 ±0.09
SO RADICAL SCAVENGING ASSAY	234 ±12.8	41.8 0.29	416 ±12.8	40.33 ±0.28	281 ±20.8	42.3 ±0.29	339 ±12.8	40.83 ±0.88
H_2O_2 RADICAL SCAVENGING ASSAY	2.85 ±0.45	10.04 0.91	33.5 ±0.35	2.93 ±0.36	1.3 ±0.21	2.26 ±0.28	2.44 ±0.35	1.5 ±0.25

(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 *Mentha arvensis*, *Murraya koenigii*, *Cymbopogon citratus*, and *Coriandrum sativam* leaf extracts which are directly correlated to their antioxidant activity.

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule and is widely used to assess the radical scavenging activity of antioxidant compounds [32, 33]. The results revealed that methanolic extract of lemon grass is a more effective scavenger than the aqueous extract and it is comparable to that of mint. DPPH scavenging activity can be correlated to the presence of tannins, flavonoids and various phenolic compounds which are highest in lemon grass as seen in Table 1. β-Sitosterol, Myrcene and Selenium in lemon grass are responsible for its antioxidative behavior [34].

Under physiological conditions, nitric oxide is continuously released by vascular endothelial cells as consequence of the shear stress generated by the blood flow, antioxidants act by scavenging the NO radicals [17]. Our experimental results indicated that lemon grass and mint proved to be good NO-suppressors, whereas coriander and curry leaves were found poor NO-suppressors. Nitric oxide radical scavenging activity is correlated to the presence of phenolic compounds.

FRAP activity was extensively displayed by the aqueous extracts. Compound of medium polarity are most potent, even if their total antioxidant contribution in the plant is small. Lemon grass and coriander leaves showed good FRAP activity. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species [35].

The reducing power is mainly correlated to the presence of reductones. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [36]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [37]. Curry leaves show significant reducing power which correlates well with the reducing sugar content which is high in both extracts of curry leaves. Lemon grass also has higher concentration of sugars and consequently increased RP compared to the other extracts.

SO scavenging activity is correlated to the total flavonoids. Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress [38]. The SO scavenging activity in lemon grass extracts was estimated to be 416 ± 12.8 mg/g in methanolic extract and 339 ± 12.0 mg/g ascorbic acid equivalent in aqueous extract. Mint leaves exhibited a SO scavenging activity which was 44 percent of that of lemon grass. This can be explained in terms of a higher flavanoid content of lemon grass [17, 39].

Hydrogen Peroxide radical scavenging activity is correlated to the presence of phenolic compounds. Hydrogen donation is the main mechanism of phenolics as antioxidants. The lower strength of the O–H bond present in phenolics corresponds to a higher scavenging activity. Elevated hydrogen peroxide radical scavenging activity found in curry leaves.

A high correlation between antioxidant capacities and their total phenolic contents indicated that phenolic compounds were a major contributor of antioxidant activity of these plants and this study provided evidence on the potential health benefits of these plants.

CONCLUSION

Polyphenols are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Therefore, the phenolic content of plants may

contribute directly to their antioxidant action. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors, 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) .

In our study, a significant linear correlation was found between the concentration of phenolic compounds and the antioxidant activity of extracts from different herbs. Among the four herbs investigated we found that lemon grass exhibited maximum antioxidant activity. Mint was also rich in antioxidant activity, curry leaves and coriander leaves were promising. The results reveal that use of the natural antioxidants occurring in herbs used in the Indian diet, or their extracts, is a viable alternative for the food industry, as long as the organoleptic characteristics of the food product are not affected. Further generous use of these herbs in Indian cuisine should be encouraged for a healthy life.

REFERENCES

[1] Valko M, Leibfritz D, Moncola J, Cronin TDM, Mazur M. Telser J Int J Biochem Cell Bio 2007; 39: 44–84.
[2] Hakkinen S, Heinonen M, Karenlampi S, Mykkanen M, Ruskanen M. Torronen R Food Res Int 1999; 32(5): 345–353.
[3] Chen JH, Ho JCT. Agric Food Chem 1997; 45: 2374–2378.
[4] Ambasta SP. The useful plants of India. New Delhi: Council of Scientific and Industrial Research1986; 365-366.
[5] Medicinal Plant of India, Mentha Linn. (Lamiaceae; Labiatae) New Delhi. Indian Council of Medical Research 1987; II: 230–239.
[6] Benavente -García O, Castillo J, Marin FR, Ortuñ A, José A, Del Río. Agric Food Chem 1997; 45 (12):4505–4515.
[7] Chopra RN, Nayar S L, Chopra IC, In: Glossary of Indian medicinal plants. New Delhi: Council of Scientific and Industrial Research, 1956; 165-166.
[8] Xie JT, Chang WT, Wang CZ, Mehendale SR, Li J, Ambihaipahar R. Am J Chin Med 2006; 34: 279–284.
[9] Dasgupta T, Raoa A R, Yadavaa P K. 2003; 23: 1427–1446.
[10] Kasali AA, Oyedeji AO, Ashilokun AO. Flavour Fragrance J 2001; 16(5): 377–378.
[11] Borrelli F, Izzo AA. Phytother Res 2000; 14(8): 581–591.
[12] Runnie I, Salleh M N, Mohamed S, Head R, Abeywardena M. J Ethnopharmacol 2005; 96(3): 365–370.
[13] Masoumeh, E.; Mohammad, K.; Maryam, F.A. Journal of ethanopharmacology 2005; 96(3): 365–370.
[14] Craig WJ. Am J Clin Nutr 1999; 70; 491–499.
[15] Chithra V, Leelamma. J Ethnopharmacol 2000; 71: 457–463.
[16] McDonald S, Prenzler P, Robards K. 2001; 73: 73-84.
[17] Chandha S, Dave R. Afr J Microb Res 2009; 3 (13): 981-996.
[18] Chang C, Wen H, Yang M. J Food Drug Anal 2002; 10 (3): 178-182.

[19] Mohun AF, Cook IY. J Clin Path 1962; 15: 169- 180.
[20] Jayaraman J. Laboratory Manual in Biochemistry. New Age International, 2011, pp141-142.
[21] McCune L, Johns T. J Ethnopharmacol 2002; 82 (2); 197-200.
[22] Green L, Wagner D, Young V.1981; 78 (12): 7764-7768.
[23] Athukorala Y, Jeon Y, Kim K. Food Chem Toxicol 2006; 44 (7): 1065-1074.
[24] Robak J, Gryglewski. J Biochem Pharmacol 1998; 37 (5): 837-841.
[25] Ruch R, Cheng S, Klaunig. J Carcinogenesis 1989; 10 (6): 1003-1008.
[26] Riedl KM, Carando S, Alessio HM, McCarthy M , Hagerman AE. ACS Symp Ser 2002; 807: 188-200.
[27] Ferruzzi M, Courtney P, Bohm V. J Food Sci 2002; 67 (7): 2589-2595.
[28] Slater A, Scott W. Plant Biotechnology: The genetic manipulation of plants. Oxford University Press , 2008; 229.
[29] Ypuwei Z, Jinlian Z, Yonghong P. LWT- Food Sci Tech 2008; 41: 1586-1591.
[30] Khalaf N, Ashok K, Shakya A. Turk J Bio 2008; 32: 51-55.
[31] Miguel MG. Molecules 2010; 15: 9252-9287.
[32] Oktay M, Gulcin I, Kufrevioglu OI. Lebensm Wiss Techol 2003; 36**:** 263–271.
[33] Gulcin I, Oktay M, Kirecci E, Kufrevioglu I. Food Chem 2003; 83: 371-382.
[34] Chen H, Yen G. J Agric Food Chem 2006; 2 (47): 686-694.
[35] Meyer AS, Isaksen A. Trends Food Sci Tech 1995; 6: 300–30.
[36] Mohun AF, Cook IY. J Clin Path 1962; 1: 169-180.
[37] Anwar F, Qayyum HMA, Hussain AI, Iqbal S. Grasas Y Aceites 2010; 61 (3): 237-243.
[38] Thaipong K, Byrne D, Crosby K. J Food Compos Anal 2006; 19: 669-675.
[39] Vinayagam A, Sudha P N. IJPSR 2011; 2 (6): 1548-1553.