Attanayake et al., Afr J Tradit Complement Altern Med. (2015) 12(4):28-33
http://dx.doi.org/10.4314/ajtcam.v12i4.5

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT POTENTIALS OF EXTRACTS OF TEN MEDICINAL PLANTS USED FOR THE TREATMENT OF DIABETES MELLITUS IN SRI LANKA

Anoja Priyadarshani Attanayake[1], Kamani Ayoma Perera Wijayawardana Jayatilaka[1], Chitra Pathirana[1], and Lakmini Kumari Boralugoda Mudduwa[2]

[1]Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka.
[2]Department of Pathology, Faculty of Medicine, University of Ruhuna, Sri Lanka.
Corresponding author: Anoja Priyadarshani Attanayake
E-mail address: anoja715@yahoo.com

Abstract

Background: Over the last decade, extensive research work has focused on the potential health benefits of antioxidants while many medicinal plant extracts have been evaluated for their antioxidant profile. Medicinal plants selected for this study are widely used in traditional medicine for the treatment of diabetes mellitus in Sri Lanka some of which are recommended as dietary supplements to the existing therapies. The present study aimed at determining the total polyphenol contents and total antioxidant activities of aqueous extracts of 10 selected Sri Lankan medicinal plants by three *in vitro* methods;
Materials and Methods: DPPH(2, 2-diphenyl-2-picrylhydrazyl), FRAP (ferric reducing power) and NO (nitric oxide) assays. The aqueous plant extracts were tested at the concentration of 0.05 g/mL. The total polyphenol content was determined according to the Folin-Ciocalteu method while the total antioxidant activity was evaluated by DPPH, FRAP and NO assays with L-ascorbic acid as reference compound.
Results: The total polyphenol content of the plant extracts varied from 0.41 to 13.00 mg GAE (gallic acid equivalents) per gram dry weight. The antioxidant activities ranged in IC_{50} of 36.89-101.27 µg/mL, IC_{50} of 139.56-419.93 µg/mL, 0.12-8.98 µM for DPPH, NO, FRAP assays, respectively. A significant positive correlation was observed between total polyphenol content and antioxidant activities ($P< 0.05$).
Conclusion: Phytochemical analysis revealed the presence of polyphenolic compounds, alkaloids and flavonoids in the plant materials which also possessed *in vitro* antioxidant potentials. Polyphenolic compounds contribute significantly to the total antioxidant capacities of medicinal plant extracts.

Key words: DPPH; FRAP; NO assay; total polyphenol content

Introduction

Over the years, research on antioxidants and medicinal plants has gained enormous popularity and proved as potential therapeutic avenue for preventing free radical-generated damage in the human body. Free radicals are well known for the pathogenesis of various degenerative diseases such as cardiovascular diseases, neurological disorder, diabetes mellitus, and also in aging (Shukla et al., 2009). Medicinal plants are being viewed as readily available, potent sources of natural antioxidants as they contain a mixture of different chemical compounds that may act individually or in synergy. The oxidative damage is a critical etiological factor implicated in several human diseases including diabetes mellitus (Pandey and Rizvi, 2009). The damage of lipids, proteins, and nucleic acids by free radicals with the simultaneous decline of antioxidant defense mechanisms leads to cell and tissue damage. It may be contributed to the secondary complications in diabetes (Bakirel et al., 2008). It has been postulated that most effects of oxidative stress in diabetes mellitus are diminished upon supplementation with certain plant antioxidants such as vitamins C, E and non-nutrient antioxidants as polyphenolic compounds (Sun et al., 2007). Thus, substantial effort is needed to ensure proper identification of safe natural antioxidants from plant species. Polyphenolic compounds virtually found in all families of plants are believed to possess preventive and curative effects on cellular oxidative damage. They are considered as affluent sources of free radical scavenging molecules which are rich in antioxidant activity (Rice-Evans et al., 1997). The evidence for their role in the inhibition of lipid peroxidation and scavenging reactive oxygen species such as hydroxyl, superoxide and peroxinitrite have been recognized in experimental diabetes (Kuttan and Sabu, 2009; Sharma et al., 2010).

As in many Asian countries in the world, Sri Lanka constitutes an apt example where medicinal plants are widely used in everyday life for culinary purposes and as medicinal remedies. The plants selected for this study are widely used in traditional medicine for the treatment of diabetes mellitus and some are recommended as dietary adjuncts to existing therapies (Ediriweera and Ratnasooriya, 2009). Till date, Sri Lankan medicinal plants included in the study have not been studied to assess their *in vitro* antioxidant potential and the relationship between their total polyphenol content and *in vitro* anti-oxidative activities.

Several reports indicate that the antioxidant potential of medicinal plants may be related to the concentration of the polyphenolic compounds (Anantharaman et al., 2010; Kiselova et al., 2006; Wong et al., 2006). The objectives of the study were to determine the total polyphenol content and *in vitro* antioxidant activities of aqueous extracts of ten selected Sri Lankan medicinal plants, in order to determine the relationship between their polyphenol content and antioxidant activities of plant extracts, and to examine the phytochemicals present in the plant extracts.

Materials and methods

Chemicals and reagents

L-Ascorbic acid, 2, 2′-diphenyl-2-picrylhydrazyl hydrate (DPPH), 95% ethanol (EtOH), Folin- Ciaocalteu reagent, ferric chloride ($FeCl_3$), gallic acid, acetic acid glacial (CH_3COOH), hydrogen chloride (HCl), N-(1-Naphthyl) ethylediaminedihydrochloride, orthophosphoric acid (H_3PO_4),

naphthylethylediaminedihydrochloride, sodium acetate trihydrate ($CH_3COONa·3H_2O$), sodium carbonate (Na_2CO_3), sodium chloride (NaCl), sodium nitroprusside (SNP), sulfanilamide and 2,4,6-tripyridyl-1,3,5-triazine (TPTZ) were purchased from Sigma-Aldrich Company (St. Louis, MO, USA). All chemicals and solvents were of analytical grade and used without any purification. A Sanyo Gallenkamp (model SP65) spectrophotometer was used for the measurements.

Plant material

A total of 10 plant samples representing ten Sri Lankan medicinal plant species from 10 families (*Spondias pinnata* Kurz. (Anacardiaceae), *Kokoona zeylanica* Thw. (Celastraceae), *Coccinia grandis* Kurz. (Cucurbitaceae), *Mommordica charantia* Linn. (Cucurbitaceae), *Sida alnifolia* Linn. (Malvaceae), *Syzygium caryophyllatum* (Linn.) Als. (Myrtaceae), *Nyctanthus arbo-tristis* Linn. (Oleacea), *Scoparia dulcis* Linn. (Scrophulariaceae), *Gmelina arborea* Linn. (Verbenaceae), and *Languas galanga* (Linn.) Stuntz. (Zingiberaceae) were collected during May-June 2009 from 3 different locations in southern region of Sri Lanka (Table 1). The Botanical identities of all plants were determined by the descriptions given by Jayaweera (1982), and confirmed by comparison with authentic samples at National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. Voucher specimens have been deposited at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka (A/Med/2009/1-10).

Preparation of extracts

Powdered plant material (5.0 g) was dissolved in 400.0 mL of distilled water and refluxed for 4 hr. The mixture was strained and the final volume was adjusted to 100.0 mL. The initial concentration of each plant extract was 0.05 g/mL. A series of extract dilutions were prepared (1-500 µg/mL) for DPPH and NO inhibition assays. All determinations were done in triplicates per plant species obtained from 3 different locations.
In the present study, we determined the total polyphenol content of selected medicinal plant extracts by classical Folin-Ciocalteu reagent method and total antioxidant activities by DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate) radical scavenging assay, FRAP (ferric reducing power)assay, and NO (nitric oxide) inhibition assay.

Total polyphenol content

Total polyphenol content was measured using Folin-Ciocalteu colorimetric method (Singleton et al., 1999). Plant extract (1.0mL) was mixed with 1.0 mL of 95% ethanol, 5.0 mL of distilled water and 0.5 mL of 50% Folin-Ciocalteu reagent. The mixture was allowed to react for 5 min and 1.0 mL of 5% Na_2CO_3 was added. Thereafter, it was thoroughly mixed and placed in dark at room temperature (25°C) for 1 hr and the absorbance was measured spectrophotometrically at 725 nm. Quantification was done with respect to the standard curve of gallic acid (0-50 µg/mL). The results were expressed in gallic acid equivalents mgGAE/g of the dry weight.

DPPH radical scavenging activity

The total antioxidant activity was measured by the DPPH radical scavenging assay method (Brand Williams et al., 1995). The radical scavenging activity of plant extract against stable DPPH radical (DPPH*) was determined. When DPPH* reacts with an antioxidant compound in the plant extract, the antioxidant donates hydrogen and reduced. The change in color from deep violet to light yellow was measured spectrophotometrically at 517nm. Briefly, 0.004% DPPH solution was prepared with EtOH and the absorbance of the DPPH solution alone was measured ($A_{control}$) at 517 nm. EtOH was used as the sample blank. 1.0 mL of each extract at different concentrations (1-500 µg/mL) was added to 3.0 mL of0.004% ethanol DPPH solution. The mixture was shaken vigorously, allowed to stand at 25°C in dark for 30 min. The decrease in absorbance of the resultant solution was measured spectrophotometrically at the same wave length (A_{sample}). L-Ascorbic acid was used as the reference compound. The antioxidant activity is expressed in terms of IC_{50} (concentration of the extract /reference compound required to inhibit DPPH radical formation by 50%).
% DPPH radical scavenging activity= $(A_{control}-A_{sample})/A_{control}$ x100% Where, $A_{control}$ represents the absorbance of the control without the plant extract/reference compound, A_{sample} represents the absorbance of the plant extract/reference compound.

Ferric reducing antioxidant potential (FRAP) assay

The FRAP assay was performed according to the method of Benzie and Strain (1999). The assay is based on the reducing power of a compound (antioxidant). A potential antioxidant reduces the ferric ion (Fe^{3+}) to ferrous (Fe^{2+}) ion; the latter forms a blue complex (Fe^{2+}/TPTZ), which increases the absorption at 593 nm. The FRAP working reagent was prepared by mixing acetate buffer (300 mM, pH 3.6), a solution of 10mM TPTZ in 40 mM HCl and 20 mM $FeCl_3$ at 10:1:1 (v/v/v). The FRAP reagent (3.0 mL) and sample solution (100 µL) were mixed and the absorbance of the resulting solution was measured (t=0) at 593 nm spectrophotometrically ($A_{sample\ t=o}$) against a sample blank (FRAP working reagent). Thereafter the sample was placed in a water bath (37°C) and absorption was measured at the same wave length after 4 min. ($A_{sample\ t=4}$). The ascorbic acid (100 µM) was used as the standard compound and preceded as in the same way. FRAP value of the plant extract (µM)=$(A_{sample\ t=0-4})/A_{standard\ t=0-4}$) x FRAP value of1000 µM ascorbic acid
Where, ($A_{sample\ t=0-4}$) represents the change in absorbance in a sample from 0 to 4 min, ($A_{standard\ t=0-4}$) is the change in absorbance in the standard from 0-4min. FRAP value of ascorbic acid is 2.

Nitric oxide (NO) radical scavenging assay

Nitric oxide generated from SNP in aqueous solution at physiological pH, interacts with the Griess reagent and the absorbance of the chromophore formed was measured spectrophotometrically (Marcocci et al., 1972). Scavengers of nitric oxide compete with oxygen leads to reduce

the production of nitric oxide. L-Ascorbic acid was used as the reference compound. 1.0 mL of SNP (5 mM) was mixed with 4.0 mL of the plant extract at different concentrations (1-500 µg/mL) and incubated the resultant solution at 29°C for 2 hr. Incubated solution (2.0 mL) was mixed with 1.2mL of Griess reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% N-(1-Naphthyl) ethylediamine dihydrochloride and measured the absorbance at 550 nm (A_{sample}) against a distilled water blank. The absorbance of the control without the plant extract/reference compound was also measured at the same wave length ($A_{control}$).The antioxidant activity is expressed in terms of IC_{50} (micro-molar concentration required to inhibit NO radical formation by 50%).

% NO radical scavenging activity= $(A_{control}-A_{sample})/A_{control}$ x 100%

Where, $A_{control}$ represents the absorbance of the control without the plant extract/reference compound, $A_{sample}$is the absorbance of the plant extract/reference compound.

Phytochemical screening

Preliminary qualitative phytochemical screening for the presence of alkaloids, cardenolide glycosides, phenols, flavonoids, phytosterols, saponins, tannins, reducing sugars, and proteins was carried out by the reported protocol (Trease and Evans, 1978).

Statistical analysis

The replicates of each sample were used for statistical analysis and the values were expressed as mean ± standard deviation. Pearson's correlation and regression analysis was carried out using Minitab software to establish the relationship between total polyphenol content and antioxidant activities of selected medicinal plant extracts. The level of significance was set at $P < 0.05$.

Results and discussion

Total polyphenol content

The total polyphenol content of the ten aqueous medicinal plant extracts was determined by regression equation of gallic acid calibration curve (y= -0.0203 + 0.0101x) and varied in a range of 0.41-13.00mgGAE/g of dry weight (Table 1). The highest and lowest polyphenol contents were in the extracts of *G. arborea* and *K. zeylanica*, respectively. Polyphenols possess ideal structural chemistry for free radical scavenging activity and are viewed as promising natural antioxidants. The antioxidant effect of polyphenolic compounds was mainly due to their redox properties and as a result of various possible mechanisms: free radical scavenging activity, metal chelating activity and/or singlet oxygen-quenching capacity (Corke et al., 2005).

Total antioxidant activities

The chemical approaches facilitate the study of the total antioxidant activity of medicinal plant extracts and the precise mechanisms of action of antioxidants. Thus far, numerous studies on antioxidant properties of many plant species have been conducted using different assay methods. The general recommendation is to employ at least three *in vitro* methods due to the presence of wide variety of oxidation systems (Kaul et al., 2010).

In the present study, antioxidant activity was evaluated by three spectrophotometric methods; DPPH, FRAP and NO assay. Water soluble antioxidant potentials are determined based on the traditional method in intake of plant extracts therapeutically. The IC_{50} values of extracts were calculated for DPPH and NO inhibition assays to compare the antioxidant activities at different concentrations as described by many authors (Adesegun et al., 2009; Marwah et al., 2007).The results of three *in vitro* assays (DPPH, FRAP, NO) for antioxidant properties of the 10 plant extracts are given in Table 1.

All extracts and the standard compound exhibited concentration-dependent radical scavenging activities in DPPH and NO inhibition assays. The radical scavenging activities were expressed in IC_{50} values in both assays to obtain a more precise single value over a range of concentration of plant extracts. The radical scavenging activity in DPPH assay was in the decreasing order of *G. arborea, S. pinnata, C. grandis, S. dulcis, N. arbo-tristis, S. caryophyllatum, S. alnifolia, M. charantia, L. galanga, K. zeylanica* followed by *G. arborea, C. grandis, S. pinnata, S. caryophyllatum, M. charantia, N. arbo-tristis, S. dulcis, S. alnifolia, L. galanga,* and *K. zeylanica*in NO assay.The high amount of polyphenol compounds leads to high radical scavenging activity (low IC_{50}) as shown in the extracts of *G. arborea, C. grandis* and *S. pinnata* in both assays. However the IC_{50} of standard compound (L-Ascorbic acid) as a powerful antioxidant was more pronounced in DPPH and NO radical scavenging assays and values are comparable with previous studies (Zhu et al., 2004).The DPPH assay is reported to be a direct and reliable method for the determination of radical scavenging activity where the structure of electron donor (e.g. plant extract) is not known. DPPH assay method can afford data on reduction potential of the sample and hence can be helpful in comparing the reduction potential of unknown compounds. A key mediator released by activated macrophages that has been implicated in toxicity is nitric oxide. It has been pointed out that modulating nitric oxide production can modify tissue injury (Laskin et al., 1990). Thus, development of specific nitric oxide scavengers is considered important due to lack of endogenous enzymes responsible for nitric oxide in activation (Alisi and Onyeze, 2008).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activities (Meir et al., 1995). As shown in Table 1, the reducing power of extracts were between 0.12-8.98µM at the concentration of 0.05g/mL of plant extract and followed the decreasing order of *G. arborea, C. grandis, S. pinnata, S. caryophyllatum,* and *N. arbo-tristis*. The extract of *K. zeylanica* had the lowest ferric reducing power among selected plant extracts. The antioxidant activity of polyphenols determined using different free radical methods showed similar results to those obtained using the FRAP assay suggesting that the reducing ability of polyphenols seemed to be an important factor dictating free radical scavenging capacity of these compounds. FRAP assay has many advantages over many radical scavenging assays such as excellent reproducibility, linearity over a wide range and high sensitivity. In contrast, the FRAP assay measures the reducing capability by increased sample

http://dx.doi.org/10.4314/ajtcam.v12i4.5

Table 1: Total polyphenol content and antioxidant activities of 10 selected Sri Lankan medicinal plant extracts

Plant name/ reference compound	Plant part tested	Total polyphenol content(mg GAE/g of dry weight)	Total antioxidant activities [a]		
			IC_{50} in DPPH assay µg/mL [b]	FRAP assay µM [c]	IC_{50} in NO assay µg/mL [d]
S. pinnata	bark	6.57±0.93	42.06±0.19	3.91±0.57	176.00 ± 4.43
K. zeylanica	bark	0.41±0	101.27±2.19	0.12±0.01	419.93±2.64
C. grandis	leaves	8.59±1.3	45.05±0.85	5.93±0.34	168.30±1.03
M. charantia	fruit	1.83±0.17	67.25±2.12	1.25±0.10	236.72 ± 4.52
S. alnifolia	leaves	4.56±1.01	63.14±0.98	1.69±0.32	262.18±1.02
S. caryophyllatum	bark	5.23±1.23	59.23±1.02	4.32±0.38	199.56±1.23
N. arbo-tristis	flowers	4.36±1.24	55.23±1.23	2.85±0.52	241.23±1.80
S. dulcis	aerial part	3.42±0.59	53.35±1.15	1.76±0.14	250.44±4.74
G. arborea	bark	13.00±1.10	36.89±1.23	8.98±0.09	139.56±4.20
L. galangal	root	1.95±0.33	77.39±2.50	1.24±0.05	327.88±2.98
L-ascorbic acid	NA	NA	4.52±0.11	NA	28.59±0.80

[a] All values were the mean of three measurements and expressed as mean ± SD.
[b,d] Radical scavenging activity by DPPH (2, 2-diphenyl-2-picrylhydrazyl hydrate)assay and NO(Nitric oxide) inhibition assay was expressed in IC_{50} (Concentration of the extract required to inhibit DPPH/NO radical formation by 50%) obtained by linear regression analysis.
[c] FRAP(ferric reducing power). NA; not applicabl

absorbance and the assay may not complete even several hours after the reaction starts, such that a single end point of the reaction cannot be determined (Prior et al., 2005).

Correlation between the total polyphenol content and antioxidant activity

The linear regression and correlation analysis was done to establish the correlation between total polyphenol content and antioxidant activities of medicinal plant extracts and to evaluate the suitability and reliability of the three *in vitro* assay methods. The correlation (*R*) is given in Table 2. A linear correlation between the total polyphenol content and antioxidant activity was established. This is consistent with previous findings for medicinal plant extracts (Sun et al., 2007). The total polyphenol content and antioxidant activity were positively correlated as*R*= 0.923, 0.978, 0.944 in DPPH, FRAP, and NO assays respectively ($P< 0.05$). This could be explained from the basic concept that polyphenolic antioxidants are reducing agents. All *R* values were positive at the $P<0.05$ significance level, indicating that the three antioxidant methods selected are suitable and reliable for assessing the total antioxidant potentials of plant extracts, although the differences in antioxidant capacities between assay methods were observed with samples.

Phytochemical screening

The phytochemical analysis conducted on all selected plant extracts revealed that the polyphenolic compounds, alkaloids and flavonoids are present in all plant species. Cardenolide glycosides were absent in the plant species of *K. zeylanica, C. grandis, S. alnifolia* and *S. dulcis*. In addition, saponins and sterols/tritepenoids were absent in the *S. alnifolia* and *L. galanga*. Anthracene glycosides and cyanogenic glycosides were not present in all of the selected plants.

R	DPPH	FRAP	NO
FRAP	0.884		
NO	0.947	0.948	
TPC	0.923	0.978	0.944

Table 2: The correlation between total polyphenol content and antioxidant activities of Sri Lankan medicinal plant extracts [a]

[a] The correlation (R) is given between total polyphenol content (TPC) and $1/IC_{50}$ in DPPH(2, 2-diphenyl-2-picrylhydrazyl hydrate), FRAP (ferric reducing power) value and IC_{50} in NO (nitric oxide) inhibition assay. (IC_{50} : Concentration of the extract required to inhibit DPPH/NO radical formation by 50%)
The correlation is statistically significant at $P < 0.05$.

Conclusion

The data presented in this study demonstrate that almost all the plant species investigated possessed antioxidant activities by the virtue of the variety of chemical constituents present. The positive correlations obtained in this study suggest that polyphenolic compounds contribute significantly to the total antioxidant capacities of medicinal plant extracts. Results revealed that, bark extracts of *G. arborea, S. pinnata* and leaf extract of *C. grandis* were excellent free-radical scavengers containing potent natural polyphenolic compounds. Therefore, these extracts are most likely to exert health benefits in free radical pathologies in diabetes mellitus.

Acknowledgements

The financial assistance given by University Grants Commission in Sri Lanka is greatly appreciated (UGC/ICD/CRF 2009/2/5). The authors wish to thank Dr D.A.B.N. Gunarathne, Faculty of Agriculture, University of Ruhuna, Sri Lanka for the guidance given in statistical data analysis and Mrs. B.M.S. Malkanthie of the Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Sri Lanka for laboratory assistance.

References

1. Adesegun, S.A., Fajana, A., Orabueze, C.I. and Coker, H.A.B. (2009). Evaluation of antioxidant properties of *Phauloppsis fascisepala* (Acanthaceae). J. Evid. Based Complementary Altern. Med. **6:** 227-231.
2. Alisi, C.S. and Onyeze, G.O.C. (2008). Nitric oxide scavenging ability of ethyl acetate fraction of methanolic leaf extracts of *Chromolaena odorata*. Afr. J. Biochem. Res. **2**: 145-150.
3. Anantharaman, P., Arumugam, R. and Kannan, R.R. (2010). *In vitro* antioxidant activities of ethanol extract from *Enhalus acoroides* (L.F.) Royle. Asian Pac. J. Trop. Med. **3**: 898-901.

4. Bakirel, T., Bakirel, U., Keles, O.U., Ulgen, S.G. and Yardibi, H. (2008). *In vivo* assessment of antidiabetic and antioxidative activities of Rosemary (*Rosmarinus officinalis*) in alloxan-diabetic rabbits. J. Ethnopharmacol. **116**: 64-67.
5. Benzie, I.F.F. and Strain, J.J. (1999). Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version of simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol. **299**: 15-27.
6. Brands Williams, W., Cuveilier, M.E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol. **28**: 25-30.
7. Corke, H., Shan, B. and Cai, Y.Z. (2005). Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. J. Agric. Food Chem. **53**: 7749-7759.
8. Ediriweera, E.R.H.S.S. and Rathnasooriya, W.D. (2009). A review of herbs used in diabetes mellitus by Sri Lankan Ayurvedic and traditional physicians. Ayu **30**: 373-391.
9. Jayaweera, D.M.A. (1982). Medicinal plants used in Ceylon. Sri Lanka: National Science foundation.
10. Kaul, V.K., Singh, B., Jaitak, V., Sharma, K., Kalia, K., Kumar, N. and Singh, H.P. (2010). Antioxidant activity of *Potentialla fulgens*: An alpine plant of Western Himalaya. J. Food Comp. Anal. **23**: 142-147.
11. Kiselova, Y., Ivanova, D., Chervenkov, T., Gerova, D., Galunska, B. and Yankova, T. (2006). Correlation between the *in vitro* antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. Phytother. Res. **20**: 961-965.
12. Kuttan, R. and Sabu, M.C. (2009). Antidiabetic activity and antioxidant activity of *Terminalia belerica* Roxb. Indian J. Exp. Biol. **47**: 270-275.
13. Laskin, J.D., Heck, D.E. and Laskin, D.L. (1990). Multifunctional role of nitric oxide in inflammation. Trends Endocrinol. Metab. **5**: 377-382.
14. Marcocci, I., Maguire, J.J., Droy-Lefix, M.T. and Packer I. (1972). The nitric oxide scavenging properties of *Ginko biloba* extract EGB 761. Biochem. Biophys. Res. Commun. **46**: 849-853.
15. Marwah, R.G., Fatope, M.O., Mahrooqi, R.A., Varma, G.B., Abadi, H.A. and Al-Burtamani, S.K.S. (2007) Antioxidant capacity of some edible and wound healing plants in Oman. Food Chem. **101**: 465-470.
16. Meir, S., Kanner, J., Akiri, B. and Hadas, S.P. (1995). Determination and involvement of aqueous reducing compounds in oxidative defence systems of various sensing leaves. J. Agric. Food Chem. **43**: 1813-1815.
17. Pandey, K.B. and Rizvi. S.I. (2009). Plant polyphenols as dietary antioxidants in human health and disease. Oxid. Med. Cell Longev. **2**: 270-278.
18. Prior, R.L., Wu, X.L. and Sachai, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in food and dietary supplements. J. Agric. Food Chem. **53**: 4290-4302.
19. Rice-Rice-Evans, C., Miller, N. and Paganga, G. (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci. **2**: 152-157.
20. Sharma, U., Sahu, R.K., Roy, A. and Golwala, D.K. (2010). *In vivo* antidiabetic and antioxidant potential of *Stephania hernandifolia* in streptozotocin-induced-diabetic rats. J Young Pharm, **2**: 255-260.
21. Shukla, S., Mehta, A., Bajpai, V.K. and Shukla, S. (2009). *In vitro* antioxidant activity and total polyphenol content of ethanolic leaf extract of *Stevia rebaudiana* Bert. Food Chem. Toxicol. **47**: 2338-2343.
22. Singleton, V.L., Ortofer, R. and Lamuda-Raventos, R.M. (1999). Analysis of total polyphenol and other oxidation substances and antioxidants by means of Folin-Ciocalteu regent. Methods Enzymol, **29**: 152-178.
23. Sun, M., Surveswaran, S., Cai, Y. and Corke, H. (2007). Systematic evaluation of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem. **102**: 938-953.
24. Trease, G.E. and Evans, W.C. Pharmacology. London: Bailliere Tindall Ltd, 60-7 Wong, C.C., Li, H.B., Cheng, K.W. and Chen, F. (2009) . A systematic survey of antioxidant activity of 30 Chinese medicinal plants using ferric reducing antioxidant power assay. Food Chem. **97**: 705-711.
25. Zhu, Y.Z., Huang, S.H., Tan, B.K.H., Sun, J., Whiteman, M. and Zhu, Y.C. (2004). Antioxidants in Chinese herbal medicine: A biochemical perspective. Nat. Prod. Rep. **21**: 478-489.