EVALUATION OF ANTIOXIDANT ACTIVITY OF FOUR FOLK ANTIDIABETIC MEDICINAL PLANTS OF INDIA

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Summary

Total antioxidant capacity of four folk antidiabetic medicinal plants of India was assessed for aqueous and methanolic extract. The total antioxidant activity of aqueous and methanolic extract was determined by ferric reducing power (FRAP) assay, measuring Fe^{3+}/ ferricyanide, using phosphomolybdenum complex and by DPPH radical scavenging activity. Quantitative estimation of total phenolic compound was achieved using Folin-Ciocalteu assay. In general, methanolic extracts were showing better antioxidant activity than aqueous extracts. Highest phenolic content (41.35mg GAE/g) was reported from methanolic extract of *Momordica charantia*, apart from this same extract was able to show highest FRAP value (86.19 mM Fe(II)/g dry weight), reducing power, total antioxidant activity (67.68 mg BHT/g dry weight) and DPPH radical scavenging activity (87%, IC_{50} 0.64mg/ml).

Key Words: Antioxidant, Antidiabetic, Reactive oxygen species

Introduction

Metabolism of oxygen is the constant source for spontaneous generation of free radicals and other reactive oxygen species (ROS) like hydroxyl radical ($^{\cdot}OH$), superoxide anion ($^{\cdot}O_2^-$) and hydrogen peroxide (H_2O_2) (1). The oxidative damages caused by ROS affect lipid, protein and nucleic acid in many ways (2,3,4) and eventually play an important role in the initiation and/or progression of various diseases such as diabetes (5), cataracts (6), muscular degeneration (7), impaired wound healing (8), gastrointestinal inflammatory diseases (9), atherosclerosis, inflammatory injury, aging (10), cancer (11), cardiovascular diseases (12), neurodegenerative diseases, including Parkinson's and Alzheimer's diseases (13,14). It has been reported that antioxidant compounds can offer a possible solution for curing some serious diseases like diabetes (15), cardiovascular diseases (16), and female reproductive diseases (17). The total antioxidant activity of a plant is the sum of individual activities of each of the antioxidant compounds present such as vitamin C, tocopherols,

carotenoids, and phenolic compounds (18,19). Moreover, these compounds render their effects via different mechanisms such as radical scavenging, metal chelation, inhibition of lipid peroxidation, quenching of singlet oxygen to act as antioxidants (20). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were the most widely used antioxidant compound which have been restricted from use because of their liver damage and carcinogenic potential (21,22,23,24). So in recent arena much attention has been given to search new natural antioxidants from medicinal plants because they can protect human body from various diseases by many ways. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs (25). In the present study attempts are made to determine the total phenolic contents and the antioxidant properties of aqueous and methanolic extracts of four folk antidiabetic medicinal plants (table-1) of India by various methods including FRAP assay, reducing power, DPPH radical assay and total antioxidant capacity.

Materials and Methods

Sample preparation

The samples were dried in hot air oven at 50 °C for 10 h. The dried material was ground to a fine powder and kept in an air-tight container at 4 °C until further use. For the aqueous extraction, 1.0 g of fine powder was extracted with 10 ml of distilled water at 90 °C for 30 mins in water bath. For methanolic extraction, 1.0 g of fine powder was extracted with 10 ml of 80% methanol at 40 °C for 24 h. The samples were cooled down to room temperature and centrifuged at 5000xg for 20 mins. The supernatant was collected and freeze dried. The dried sample of each extract was weighed to determine the yield of soluble constituents and stored at 4 °C until further use. The yield of aqueous extract was found to be 7.2% (w/w), while yield for methanolic extract was found to be 8.6% (w/w).

Determination of total phenolics

Total phenolics concentration was estimated by Folin-Ciocalteu method described by Singleton and Rossi (26). Two hundred microliters of 1:10 diluted samples was added to 1 ml of 1:10 diluted Folin-Ciocalteu reagent. After 4 mins, 800 µl of sodium carbonate (75 g/l) was added. After 2 h of incubation at room temperature, absorbance was measured at 765 nm. Gallic acid (0-500 mg/l) was used for calibration of standard curve. The result was expressed as mg gallic acid equivalents (GAE)/g dry weight of plant material. Triplicate measurements were taken and mean value was calculated.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed according to the procedure given by Benzie and Strain (27) with slight modifications. The FRAP reagent was prepared from acetate buffer (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl triazine) solution in 40 mM HCl and 20 mM iron (III) chloride solution in proportions of 10:1:1 (v/v) respectively. The FRAP reagent was prepared fresh and was warmed to 37 °C in a water bath prior to use. Fifty microliter of sample was added to 1.5 ml of FRAP reagent. After 4 mins, absorbance of reaction mixture was recorded at 593 nm. Standard curve was constructed using Iron (II) sulfate solution and the results were expressed as mM Fe(II)/g dry weight of plant material. All the measurements were taken in triplicate and the mean value was calculated.

Measurement of reducing power

The reducing power of plant extracts was determined according to the method given by Yen and Chen (28). Different concentrations of extracts (0.5-2.5mg/ml) were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). The tubes were capped and incubated at 50 °C for 20 mins. An equal volume of 1% trichloroacetic acid was added to the

mixture to stop the reaction. The mixture was centrifuged at 300xg for 10 mins. The supernatant was mixed with distilled water and 0.1% $FeCl_3$ in a ratio of 1:1:0.2 and absorbance was measured at 700 nm. The reducing power of tested extract increases with increasing absorbance value.

Evaluation of Total antioxidant capacity by phosphomolybdenum method

Total antioxidant activity of the plant extracts was evaluated by the standard method (29). 0.3 ml of the sample solution (three replicates) was mixed with 2.7 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molebdate). The tubes were capped and incubated at 95 °C for 90 mins. After the sample had cooled down to room temperature, the absorbance was measured at 695 nm against a blank. The antioxidant activity was expressed as mg BHT/g dry weight of plant material.

DPPH radical-scavenging activity

The hydrogen atom or electron-donation ability of the corresponding extracts was measured from the bleaching of a purple-coloured methanolic solution of DPPH. The antioxidant activity of the extracts was determined by standard method, based on the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical (30). An aliquot of the extract (0.1 ml, 2 mg/ml) was added to 3 ml of a 0.001 M DPPH in methanol. Absorbance at 517 nm was determined after 30 mins, and the percent inhibition of activity was calculated as [(Ao - Ae)/Ao] – 100.
(Ao = absorbance without extract, Ae = absorbance with extract).
The extract whose DPPH radical scavenging capacity was found to be highest was serially diluted to eight different concentrations to determine IC_{50}.

Table-1. List of folk antidiabetic plants and part used

Name of the Plant	Family	Part used	Reference
Eucalyptus globules	Myrtaceae	Leaves	(31)
Eugenia uniflora	Myrtaceae	Leaves	(32)
Momordica charantia	Cucurbitaceae	Seeds	(33)
Syzium cumini	Myrtaceae	Leaves	(34)

Results

Total phenolic contents of the various extracts

Total phenolic content in the aqueous and methanolic extracts was determined according to the Folin-Ciocalteu method and expressed as mg GAE/g dry weight of plant material. The total phenolics data for aqueous and methanolic extracts of four selected plants are shown in table-2. Methanol was found to be the most effective solvent in extraction of antioxidants from folk medicinal plants. This is similar to the previous reports that methanol is a widely used and effective solvent for extraction of antioxidants (35, 36). *Momordica charantia* was reported to have highest phenolic content in both aqueous extract (27.70 mg GAE/g) as well as in methanolic extract (41.35 mg GAE/g). Least phenolic content, 8.75 mg GAE/g for aqueous extract and 16.80 mg GAE/g for methanolic extract was reported from *Eugenia uniflora*.

Table-2. Total phenolic content of aqueous and methanolic extract from different plants.

Plant name	Phenolic content (mg GAE/g dry weight)	
	Aqueous extract	Methanolic extract
Eucalyptus globules	12.25±1.56	23.95±1.12
Eugenia uniflora	8.75±1.34	16.80±1.82
Momordica charantia	27.70±1.47	41.35±2.21
Syzium cumini	21.40±1.78	32.65±1.65

Many reports have revealed that the phenolic compounds such as flavonoids, phenolic acid and tannins in plants are related to their antioxidant activities. These antioxidant activities of phenolic compounds are because of their redox properties, which render them to act as reducing agents, hydrogen donor and singlet oxygen quenchers (37, 38, 39).

FRAP assay

The ability of the plants extracts to reduce ferric ions was determined using the FRAP assay. FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue coloured Fe^{II}- tripyridyltriazine compound from the colourless oxidized Fe^{III} form by the action of electron donating antioxidants (40). In FRAP assay the antioxidant activity of the extract under the test was calculated with reference to the reaction signal given by Fe^{2+} solution of known concentration, representing one electron exchange reaction. The FRAP values of aqueous and methanolic extract of various plants are given in table-3. *Momordica charantia* was reported to show highest ferric ion reducing activity among all the plants used. For aqueous extract FRAP values (mM Fe(II)/g dry weight) of *Momordica charantia* was reported to be 50.45 while it has increased to 86.19 in methanolic extract. Least FRAP value was shown by *Eugenia uniflora* which was 21.30 for aqueous extract and 42.15 for methanolic extract. The trend of FRAP value was reported to be *Momordica charantia* > *Syzium cumini* > *Eucalyptus globules* > *Eugenia uniflora*.

Table-3. FRAP value of aqueous and methanolic extracts from different plants.

Plant name	FRAP value (mM Fe(II)/g dry weight)	
	Aqueous extract	Methanolic extract
Eucalyptus globules	26.55±1.24	54.60±2.54
Eugenia uniflora	21.30±0.96	42.15±2.13
Momordica charantia	50.45±2.76	86.19±3.49
Syzium cumini	38.15±1.09	68.75±1.83

Since FRAP assay is reproducible and linearly related to the molar concentration of the antioxidants present it can be reported that folk antidiabetic medicinal plants used in this study may act as free radical scavenger, capable of transforming reactive free radical species into stable nonradical products. (27).

Reducing power of methanolic and aqueous extracts of various plant

In the reducing power assay, presence of reductants in the extracts would result in reduction of Fe^{3+}/ferricyanide to Fe^{2+} by donating an electron. The amount of Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm wavelength (41). Increase in absorbance at 700 nm reflects an increase in reductive ability. The reducing powers of aqueous and methanolic extracts are shown in table-4 and table-5 respectively. As a general trend, it was observed that as the concentration increased from 0.5 to 2.5 mg/ml, there was an increase in absorbance with both the solvent for all the plants studied.

Table-4. Reducing power of aqueous extract of various plants

Concentration (mg/ml)	Absorbance at 700nm				
	Momordica charantia	*Syzium cumini*	*Eucalyptus globules*	*Eugenia uniflora*	BHT
0.5	0.091±0.01	0.05±0.01	0.07±0.01	0.01±0.002	0.21±0.01
1.0	0.13±0.02	0.08±0.01	0.10±0.01	0.05±0.01	0.33±0.02
1.5	0.19±0.01	0.12±0.02	0.14±0.01	0.09±0.01	0.46±0.01
2.0	0.24±0.03	0.18±0.01	0.21±0.02	0.13±0.02	0.60±0.02
2.5	0.32±0.04	0.23±0.02	0.26±0.02	0.17±0.01	0.74±0.02

Table-5. Reducing power of methanolic extract of various plants

Concentration (mg/ml)	Absorbance at 700nm				
	Momordica charantia	*Syzium cumini*	*Eucalyptus globules*	*Eugenia uniflora*	BHT
0.5	0.11±0.01	0.09±0.01	0.11±0.01	0.03±0.01	0.21±0.02
1.0	0.16±0.02	0.12±0.01	0.14±0.01	0.08±0.01	0.33±0.01
1.5	0.22±0.01	0.16±0.02	0.17±0.02	0.12±0.01	0.46±0.02
2.0	0.27±0.01	0.24±0.03	0.28±0.02	0.16±0.03	0.60±0.03
2.5	0.38±0.03	0.29±0.02	0.32±0.04	0.21±0.02	0.74±0.02

The reducing powers of the samples were found to be in the following order BHT > *Momordica charantia* > *Eucalyptus globules* > *Syzium cumini* > *Eugenia uniflora* which remain same for both aqueous and methanolic extract. *Momordica charantia* showed the highest reducing power which was 0.32 for aqueous extract and 0.38 for methanolic extract, at 2.5 mg/ml concentration. Minimum reducing power was shown by *Eugenia uniflora*, which was 0.17 for aqueous extract and 0.21 for methanolic extract, at 2.5 mg/ml concentration. With these results it can be said that these plant extracts could serve as electron donors and terminate free radical chain reaction (28, 42, 43).

Total antioxidant capacity

Total antioxidant capacity of different plants is expressed as number of equivalents of mg BHT/g dry weight (table- 6). The phosphomolybdenum method is a quantitative assay to evaluate water-soluble and fat-soluble antioxidant capacity (29). The assay is based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. The extracts were found to have different levels of antioxidant activity. In this assay, methanolic extracts were found to have higher activity, aqueous extract again showed lower activity. In both methanolic as well as aqueous extract antioxidant activities of the plants were: *Momordica charantia* > *Syzium cumini* > *Eucalyptus globules* > *Eugenia uniflora*. The extracts demonstrated electron-donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products (44).

Table-6. Total antioxidant capacity of aqueous and methanolic extracts from different plants.

Plant name	Total antioxidant capacity (mg BHT/g dry weight)	
	Aqueous extract	Methanolic extract
Eucalyptus globules	23.32±0.96	41.90±1.78
Eugenia uniflora	14.65±0.57	22.75±1.24
Momordica charantia	42.45±1.13	67.68±3.11
Syzium cumini	35.79±2.45	52.81±1.89

DPPH radical-scavenging activity

The assay for scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging activity of various samples. Antioxidants, on interaction with DPPH transfer electrons or hydrogen atoms to DPPH, thus neutralizing free radical character (45). The colour of the reaction mixture changes from purple to yellow and its absorbance at wavelength 517 nm decreases. Table-7 shows the DPPH radical scavenging activities of four different medicinal plants. It was found that, the scavenging activities are in order of *Momordica charantia* > *Syzium cumini* > *Eucalyptus globules* > *Eugenia uniflora* for both aqueous and methanolic extracts. *Momordica charantia* exhibited highest antioxidant potential with 87.45% DPPH radical scavenging activity. IC_{50} was determined for methanolic extract of *Momordica charantia*. α-Toc had an IC_{50} value of 0.16 ± 0.01 mg/ml, whereas the IC_{50} for methanolic extract of *Momordica charantia* was found to be 0.64 ± 0.19 mg/ml.

Table-7. DPPH radical-scavenging capacity of various plants

Plant name	DPPH radical-scavenging capacity (%)	
	Aqueous extract	Methanolic extract
Eucalyptus globules	34.43 ± 0.49	56.67 ± 0.42
Eugenia uniflora	21.52 ± 0.32	28.76 ± 0.23
Momordica charantia	59.15 ± 0.95	87.45 ± 0.51
Syzium cumini	42.67 ± 0.63	68.84 ± 0.69

Discussion

In the present study phenolic content and antioxidant activity of aqueous and methanolic extracts of four folk antidiabetic plants was determined by FRAP assay, reducing power assay, total antioxidant assay using phosphomolybdenum and by DPPH radical scavenging activity. It was observed that *Momordica charantia* showed best results while *Eugenia uniflora* showed least values for the aforesaid antioxidant assays. Shifts in redox balances, auto-oxidation of glucose, decreased tissue concentrations of low molecular weight antioxidants such as glutathione (GSH), vitamin E and impaired activities of antioxidant defense enzymes such as superoxide dismutase (SOD) and catalase (46) could be the probable sources of oxidative stress in diabetes. Increased oxidative stress in diabetes is supposed to promote the development of neuropathy (47), nephropathy (48,49), myocardial injury (50) and retinopathy (51). Oxidative stress may decrease insulin sensitivity and injure the insulin-producing cells within the pancreas. For example, ROS can penetrate through cell membranes and cause damage to β-cells of pancreas (52). Since antioxidants have been observed to curtail ROS, we can say that they can also be useful against diabetes. Moreover, antioxidant treatment has been observed to exert beneficial effects in diabetes, with preservation of *in vivo* β-cell mass by reducing β-cell apoptosis (15). Polyphenolic compounds, like Apigenin isolated from leaves of *Myrcia multiflora*, could also play important role in diabetes. Apigenin was observed to have inhibitory effect on enzyme aldose reductase (53). This enzyme plays an important role in polyol pathways, by catalyzing the reduction of the glucose to sorbitol, which cannot diffuse out of cell membrane under normal conditions. Because of intracellular accumulation of sorbitol, chronic complication of diabetes such as neuropathy, retinopathy and cataracts can occur. These findings suggest that there could be a correlation between total phenolics concentration, antioxidant activity and antidiabetic potential of the plant extracts. Antioxidants may act at different levels, they may inhibit the formation of ROS, scavenge free radicals or increase the antioxidants defense enzyme capabilities. Since the extracts used in present study have high phenolic content and good antioxidant potential determined by various chemical assays, we can say that they can be potential antidiabetic agents as well as can protect the body against oxidative damage and their harmful consequences.

Conclusion

In conclusion, we might say that our results further support the view that the four chosen medicinal plants are promising sources of natural antioxidants. Total phenolic content and values for different antioxidant assays differ significantly among aqueous and methanolic extracts of selected medicinal plants. With the above results we can say that plants used in this study possess good antioxidant potential and it is possible that this high antioxidant potential could contribute in their folk antidiabetic and medicinal properties.

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References

1. Guetens G, Boeck GD, Highley M, Oosterom ATV, Bruijn EAD. Oxidative DNA damage: biological significance and methods of analysis. Crit Rev Clin Lab Sci 2001; 39(4–5): 331–457.
2. Kellog EW, Fridovich I. Superoxide, hydrogen peroxide, and singlet oxygen in lipid peroxidation by a xanthine oxidase system. J Biol Chem 1975; 250:8812–8817.
3. Lai CS, Piette LH. Hydroxyl radical production involved in lipid peroxidation of rat liver microsomes. Biochem Biophys Res Commun 1977; 78:51–59.
4. Wiseman H, Halliwell B. Damage to DNA by reactive oxygen and nitrogen species: Role of inflammatory disease and progression to cancer. J Biochem 1996; 313:17–29.
5. Johansen JS, Harris AK, Rychly DJ, Ergul A. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. Cardiovasc Diabetol 2005; 4(1):5-15.
6. Gerster H. Antioxidant vitamins in cataract prevention. Z Ernahrungswiss 1989; 28:56–75.
7. Vander-Hagen AM, Yolton DP, Kaminski MS, Yolton RL. Free radicals and antioxidant supplementation: a review of their roles in age-related macular degeneration. J Am Optom Assoc 1993; 64:871–878.
8. Wana A. Oxygen free radicals impair wound healing in ischemic rats. Ann Plast Surg 1997; 39(5):516–523.
9. Smirnov DA. Acute pancreatitis and biological antioxidants. Khirurgiia 1994; 3:30–32.
10. Dröge W, Schipper HM. Oxidative stress and aberrant signaling in aging and cognitive decline. Aging Cell 2007; 6(3):361–370.
11. Kinnula VL, Crapo JD. Superoxide dismutases in mallignant cells and human tumors. Free Radical Biol Med 2004; 36(6):718–744.
12. Minuz P, Fava C, Cominacini L. Oxidative stress, antioxidants, and vascular damage. Br J Clin Pharmacol 2006; 61(6):774–777.
13. Di-Matteo V, Esposito E. Biochemical and therapeutic effects of antioxidants in the treatment of Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis. Curr Drug Targets: CNS Neurol Disord 2003; 2:95–107.
14. Sas K, Robotka H, Toldi J, Ve´csei L. Mitochondria, metabolic disturbances, oxidative stress and the kynurenine system, with focus on neurodegenerative disorders. J Neurol Sci 2007; 257(1–2):221–239.
15. Kaneto H, Kajimoto Y, Miyagawa J, et al. Beneficial effects of antioxidants in diabetes possible protection of pancreatic β-Cells against glucose toxicity. Diabetes 1999; 48:2398–2406.
16. Obad A, Palada I, Valic Z, et al. The effect of acute oral antioxidants on diving-induced alterations in human cardiovascular function. J Physiol 2007; 578(3):859–870.
17. Agarwal A, Gupta S. Role of reactive oxygen species in female reproduction. Part 1. Oxidative stress: a general overview. Agro Food Ind Hi-Tech 2005; 16:21–25.
18. Javanmardi J, Stushnoff C, Locke E, Vivanco JM. Antioxidant activity and total phenolic content of Iranian Ocimum accessions. Food Chem 2003; 83:547–550.
19. Pizzale L, Bortolomeazzi R, Vichi S, Uberegger E, Conte LS. Antioxidant activity of sage (*Salvia officinalis* and *S. fruticosa*) and oregano (*Origanum onites* and *O. indercedens*) extracts related to their phenolic compound content. J Sci Food Agric 2002; 82:1645–1651.
20. Pryor WA. The antioxidant nutrients and disease prevention – what do we know and what do we need to find out? Am J Clin Nutr 1991; 53:391–391.
21. Buxiang S, Fukuhara M. Effects of co-administration of butylated hydroxytoluene, butylated hydroxyanisole and flavonoid on the activation of mutagens and drug-metabolizing enzymes in mice. Toxicology 1997; 122:61–72.
22. Hirose M, Takesada Y, Tanaka H, et al. Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4-methoxyphenol and catechol at low doses, either alone or in combination and

modulation of their effects in a rat medium-term multi-organ carcinogenesis model. Carcinogenesis 1998; 19:207–212.
23. Senevirathne M, Kim S, Siriwardhana N, et al. Antioxidant potential of *Ecklonia cava* on reactive oxygen species scavenging metal chelating, reducing power and lipid peroxidation inhibition. Food Sci Technol Int 2006; 12(1):27–38.
24. Suja KP, Jayalekshmy A, Arumughan C. Antioxidant activity of sesame cake extract. Food Chem 2005; 91:213–219.
25. Buenz EJ, Schnepple DJ, Bauer BA, et al. Techniques: bioprospecting historical herbal texts by hunting for new leads in old tomes. Trends Pharmacol Sci 2004; 25:494–498.
26. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphor-molybdic phosphotungstic acid reagent. Am J Enol Vitic 1965; 16(3):144–158.
27. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power – the FRAP assay. Anal Biochem 1996; 239:70–76.
28. Yen GC, Chen HY. Antioxidant activity of various tea extract in relation to their antimutagenicity. J Agric Food Chem 1995; 43(1):27–32.
29. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999; 269:337–341.
30. Braca A, Tommasi ND, Lorenzo B, et al. Antioxidant principles from *Bauhinia terapotensis*. J Nat Prod 2001; 64:892–895.
31. Gray AM, Flatt PR. Antihyperglycemic actions of *Eucalyptus globulus* (Eucalyptus) are associated with pancreatic and extra pancreatic effects in mice. J Nutr 1998; 128(12):2319–2323.
32. Arai I, Amagaya S, Komatsu Y, et al. Improving effects of the extracts from *Eugenia uniflora* on hyperglycemia and hypertriglyceridemia in mice. J Ethnopharmacol 1999; 68(1-3):307–314.
33. Ali L, Khan AK, Mamun MI, et al. Studies on hypoglycemic effects of fruit pulp. Seed and whole plant of *Momordica charantia* on normal and diabetic model rats. Planta Med 1993; 59(5):408–412.
34. Coimbra TC, Danni FF, Blotta RM, et al. Plants employed in the treatment of diabetes mellitus; results of an ethnopharmacological survey in Porto Algere, Brazil. Fitoterapia 1992; 63(4):320–322.
35. Hertog MGL, Hollman PCH, Van de Putte B. Content of potentially anticarcinogenic flavonoids of tea infusions, wines and fruit juices. J Agric Food Chem 1993; 41:1242–1246.
36. Yen G, Wu S, Duh P. Extraction and identification of antioxidant components from the leaves of mulberry (*Morus alba* L.). J Agric Food Chem 1996; 44:1687–1690.
37. Chang ST, Wu JH, Wang SY, et al. Antioxidant activity of extracts from *Acacia confuse* bark and heartwood. J Agric Food Chem 2001; 49:3420–3424.
38. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compound of 112 chinese medicinal plants accociated with anticancer. Life Sci 2004; 74:2157–2184.
39. Tang SY, Whiteman M, Peng ZF, et al. Characterisation of antioxidant and antiglycation properties and isolation of active ingredient from traditional Chinese medicine. Free Radical Biol Med 2004; 36:1575–1587.
40. Li YF, Guo CJ, Yang JJ, et al. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem 2006; 96:254–260.
41. Zou YP, Lu YH, Wei DZ. Antioxidant activity of flavonid rich extract of *Hypericum perforatum* L. *in vitro*. J Agric Food Chem 2004; 52:5032–5039.
42. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autooxidation of soybean oil in cyclodextrin emulsion. J Agric Food Chem 1992; 40:945–948.
43. Wang L, Yen JH, Ling HL, Wu MJ. Antioxidant effect of methanol extracts from lotus plumule and blossom (*Nelumbo nucifera Gertn*.). J Food Drug Anal 2003; 11:60-66.

44. Dorman HJD, Kosar M, Kahlos K, Holm Y, Hiltunen R. Antioxidant properties and composition of aqueous extracts from Mentha species, hybrids, varieties, and cultivars. J Agric Food Chem 2003; 51:4563–4569.
45. Naik GH, Priyadarsini KI, Satav JG, et al. Comparative antioxidant activity of individual herbal components used in Ayurvedic medicine. Phytochemistry 2003; 63:97–104.
46. Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental gaklactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. Diabetes 2001; 50(8):1938–1942.
47. Feldman EL. Oxidative stress and diabetic neuropathy: a new understanding of an old problem. J Clin Invest 2003; 111(4):431–433.
48. Ha H, Kim KH. Pathogenesis of diabetic nephropathy: the role of oxidative stress and protein kinase C. Diabetes Res Clin Pr 1999; 45(2-3):147–151.
49. Hinokio Y, Suzuki S, Hirai M, et al. Urinary excretion of 8-oxo-7, 8-dihydro-2´-deoxyguanosine as a predictor of the development of diabetic nephropathy. Diabetologia 2002; 45(6):877–882.
50. Cai L, Kang YJ. Oxidative stress and diabetic cardiomyopathy: a brief review. Cardiovasc Toxicol 2001; 1(3):181–193.
51. Kowluru RA. Effect of reinstitution of good glycemic control on retinal oxidative stress and nitrative stress in diabetic rats. Diabetes 2003; 52(3):818–823.
52. Maiese K, Morhan SD, Chong ZZ. Oxidative stress biology and cell injury during type 1 and type 2 diabetes mellitus. Curr Neurovasc Res 2007; 4(1):63–71.
53. Matsuda H, Morikawa T, Yoshikawa M. Antidiabetogenic constituents from several natural medicines. Pure Appl Chem 2002; 74:1301–1308.