

Evaluation of antioxidant potentials and total phenolic contents of selected Indian herbs powder extracts

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Abstract

The aim of the study was to compare 19 commonly used antidiabetic plants for their antioxidant potential by seven assays and identify the plants that are good sources of natural antioxidants. The antioxidant potential was evaluated by estimating reducing power (using potassium ferricyanide), lipid peroxidation inhibitory activity (thiobarbituric acid assay) and scavenging activities of DPPH[•], ABTS^{•+}, NO[•] and H₂O₂^{•-} radicals; whereas total phenolic contents were also estimated. *T. chebula* extract demonstrated high phenolic content with significant antioxidant activity. Total phenolic content were positively correlated with reducing power and ABTS scavenging activity. However, three plant extracts (*C. mukul*, *P. crispum* and *W. somnifera*) having less phenolic contents exhibited excellent antioxidant potential and some plants (*T. arjuna*, *S. reticulata* and *G. glabra*) with high phenolics where devoid of comparable antioxidant property. Results indicate that the plants extracts offer promising sources of natural antioxidants and in addition to phenolics, there could be other non phenolic antioxidants which contribute to antioxidant potential.

Keywords

Indian herbs powder extracts
antioxidant potentials
total phenolic contents
reducing power
lipid peroxidation
radical scavenging activities

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Introduction

The prevalence of diabetes mellitus is rapidly rising all over the world (Huizinga and Rothman, 2006) creating a significant clinical and economic burden on the society (Wild *et al.*, 2004). Though development of modern medicine resulted in the advent of modern pharmacotherapeutics including insulin, biguanides, sulfonylureas and thiazolidinediones, there is still a need to look for new drugs as no drug (except strict glycemic control with insulin) has been shown to modify the course of diabetic complications (Grover *et al.*, 2002). The onset and subsequent complications of diabetes are due to induction of multiple cellular pathways by oxidative stress which ultimately leading to the pathogenesis (Maiese *et al.*, 2007). Oxidative stress is an insult caused by excessive generation of reactive oxygen species including superoxide free radicals (O²⁻), hydrogen peroxide (H₂O₂), singlet oxygen and other chemical entities such as nitric oxide (NO) and peroxynitrite (Pitocco *et al.*, 2010). Therapeutic approach for the control of oxidative stress mainly involves - (i) prevention of free radicals generation and (ii) enhancing antioxidant defense (Pitocco *et al.*, 2010). It is hypothesized that development of type 2

diabetes may be reduced by the intake of antioxidants in the diet (Montonen *et al.*, 2004). Antioxidant refers to a compound that can delay or inhibit the oxidation of biomolecules by inhibiting the initiation or propagation of oxidative chain reactions and which can thus prevent or repair damage done to the body's cells by reactive oxygen species (Tachakittirungrod *et al.*, 2007). However large scale clinical trials with classic antioxidants failed to demonstrate any benefit for diabetic patients (Johansen *et al.*, 2005). Therefore, it is of great importance to find new sources of safe and inexpensive antioxidants as a complementary therapy for diabetes and subsequently a combination of diverse antioxidants to quench various reactive oxygen species altogether will be more effective. In recent decades dietary intake of plant based antioxidants has been increased due to its abundant source and least side effects (Sing *et al.*, 2012). Plants contains a wide variety of free radical scavenging molecules, such as phenolic compounds, non phenolic compounds like nitrogen compounds, vitamins, and some other endogenous metabolites, which are rich in antioxidant activity (Nurul and Asmah, 2012).

India has an ancient history of using various potent antidiabetic plants and plant components for

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treating diabetes (Grover *et al.*, 2002; Modak *et al.*, 2007). Such plants and their products have been widely prescribed for diabetic management all around the world with less known mechanistic basis of their functioning. Many Indian plants have previously been investigated for their beneficial use in different types of diabetes; however majority of the rich diversity of Indian medicinal plants is yet to be scientifically evaluated for their biological/antioxidant properties. Few plants are well studied for their antioxidant potential in vitro and in vivo with different animal models including human beings; however their complete profiling for antioxidant capacity is still not known. Therefore continuing research is necessary to elucidate the pharmacological activities of plants being used to treat diabetes. In the present study 19 traditionally used antidiabetic medicinal plants have been carefully selected out of which few plants are well studied and many are less explored for their identical comparison through seven in vitro estimations. The selections of 19 plants were based on their traditional usage and also taking into consideration previous studies that have demonstrated their antidiabetic properties (Table 1). The aim of present study was to comparatively investigate the antioxidant potential of these plant extracts through the estimation of total phenolic contents, reducing power, lipid peroxidation inhibitory activity and scavenging activities of DPPH[•], ABTS[•], NO[•] and H₂O₂[•] radicals. There are no reports about systematic evaluations of these activities for selected antidiabetic plants. This study will determine the plants that are good sources of natural antioxidants, which will be useful for isolating newer antioxidants in future and provide a direct validation of the antidiabetic properties of these ayurvedic plants.

Materials and Methods

Chemicals

2,2'-diphenyl-1-picrylhydrazyl (DPPH[•]), 2,2'-azinobis (3 ethyl-benzothiazoline 6 sulphonic acid (ABTS[•]), thiobarbituric acid (TBA), gallic acid, butylated hydroxytoluene were obtained from Sigma Chemical Company (St. Louis, MO, USA). Folin-Ciocalteu reagent, ascorbic acid, methanol, potassium persulfate, Griess reagent A, Griess reagent B, Curcumin, sodium nitroprusside, butylated hydroxytoluene, potassium ferricyanide were purchased from SRL (India).

Plant materials and their extraction

The botanical names, English names and part used for analysis of 19 dietary plants are listed in Table 1. The powders of specified plant and parts

were obtained from the local herbal and Ayurvedic medicine store (*Ambadas Vanaushadhalaya*, Pune, India) where the authenticated plant parts were collected, dehydrated (in a chamber below 40°C for 48 h), powdered with a mechanical grinder and stored in an air-tight container.

The methanolic plant extracts were prepared by adding 1 g of dry powders of selected materials in 100 mL methanol, further stirring at 150 rpm (steelmet incubator shaker, India) at ambient temperature for 3 h. Insoluble residues from the solutions were removed by centrifugation at 8,000 g for 10 min (SuperspinR-V/FM, Plasto craft, India) and the clear supernatants were used for analysis. The extracts were stored at 4°C in plastic vials, till further use. All the estimations were performed in triplicates.

Estimation of total phenolic content

Total phenolic content was determined using the Folin-Ciocalteu reagent (Lim and Quah, 2007). Briefly 300 µL of plant extracts were thoroughly mixed with 1.5 mL of freshly diluted Folin-Ciocalteu reagent (1 N), to which 1.2 mL of sodium carbonate solution (7.5%) was added and the mixture was incubated for 30 min in the dark. The absorbance was measured at 765 nm using Spectrophotometer (UV-10 Spectrophotometer, Thermo Scientific, U. S.). Gallic acid (0.002-0.02 mg/mL) was used as a reference standard. The concentration of phenolic content was expressed as mg of gallic acid equivalents (GAE) per g dry weight.

Estimation of reducing power (RP)

The RP was estimated by the method of Oyaizu (1986). The mixture containing 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1% w/v) was added to 1 mL of the plant extract and incubated at 50°C for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10% w/v) was added and the solutions were centrifuged at 700 g for 10 min. The upper layer (2.5 mL) was collected and mixed with 2.5 mL distilled water and 0.5 mL of FeCl₃ (0.1%, w/v). The absorbance was measured at 700 nm against a blank (UV-10 Spectrophotometer, Thermo Scientific, U. S.). Higher absorbance of the reaction mixture indicated increased RP.

Lipid peroxidation (LPO) inhibition assay

TBA reactive species were used as a measure of the LPO inhibition (Ohkawa *et al.*, 1979). Plant extracts (0.1 mL) was mixed with 0.5 mL egg yolk homogenate (10%) and volume made up to 1 mL with distilled water. LPO was induced by adding 0.05 mL of ferrous sulphate (0.07 M) and incubated for 30 min. After incubation, 1.5 mL of acetic acid (20%) and

Table 1. Scientific name of plants with family, English name, part used for analysis and reported antidiabetic effect

Scientific Name	Family	English Name	Part used	Reported antidiabetic effect
<i>Aegle marmelos</i>	Rutaceae	Bael, Bilva	Leaf	Streptozotocin-induced diabetic rats (Narendhirakannan and Subramanian, 2010)
<i>Aloe barbadensis</i>	Aloaceae	Aloe vera	Latex	Type 2 diabetic mice (Tanaka et al., 2006)
<i>Andrographis paniculata</i>	Acanthaceae	Indian echinacea	Leaf	Alloxan-induced diabetic rats (Reyes et al., 2005)
<i>Bacopa monnieri</i>	Scrophulariaceae	Bacopa	Leaf	Diabetic rats (Ghosh et al., 2008)
<i>Boerhavia diffusa</i>	Nyctaginaceae	Spreading hogweed	Leaf	Alloxan induced diabetic rats (Pari and Amamath, 2004)
<i>Caesalpinia bonducella</i>	Cesalpiniaceae	Bonducella nut	Fruit	Alloxan induced diabetic rats (Kamur et al., 2006)
<i>Commiphora mukul</i>	Burseraceae	Salaitree	Leaf	High fat diet induced diabetic rats (Sharma et al., 2009)
<i>Glycyrrhiza glabra</i>	Fabaceae	Licorice, Liquorice	Root	Streptozotocin-induced diabetes in rats (Sen and Roy, 2011)
<i>Gymnema sylvestre</i>	Asclepiadaceae	Gymnema	Leaf	Alloxan-induced diabetic rats (Ahmed et al., 2010)
<i>Hemidesmus indicus</i>	Asclepiadaceae	Indian sarsaparilla	Root	Streptozotocin-induced diabetic rats (Gayathri and Karmahin, 2010)
<i>Ocimum basilicum</i>	Lamiaceae	Common basil	Seed	In vitro study (El-Beshbishy and Bahashwan, 2012)
<i>Ocimum sanctum</i>	Lamiaceae	Holy basil	Leaf	Alloxan diabetic albino rats (Kar et al., 2003)
<i>Ocimum sanctum</i>	Lamiaceae	Holy basil	Seed	Cholesterol fed rabbits (Gupta et al., 2006)
<i>Petroselinum crispum</i>	Apiaceae	Parsley	Leaf	Streptozotocin-induced diabetic rats (Ozesoy-Sacan et al., 2006)
<i>Salacia reticulata</i>	Celastraceae	Salacia	Root	Diabetic human and rodents (Li et al., 2008)
<i>Silybum marianum</i>	Asteraceae	Milk thistle	Seed	Type II diabetic human patients (Husaini et al., 2006)
<i>Sweria chirayita</i>	Gentianaceae	Indian gentian	Leaf	Streptozotocin-induced diabetic rats (Saxena et al., 1996)
<i>Terminalia arjuna</i>	Combretaceae	Arjuna	Bark	Diabetic human patients (Dwivedi and Aggarwal, 2009)
<i>Terminalia chebula</i>	Combretaceae	Myrobalan	Fruit	Streptozotocin-induced diabetic rats (Murali et al., 2007)
<i>Wihania somnifera</i>	Solanaceae	Winter cherry	Root	Alloxan diabetic albino rats (Udayakumar et al., 2009)

1.5 mL of TBA (0.8% w/v in 1.1% sodium dodecyl sulfate) were added, vortexed and the reaction mixture was heated at 95°C for 60 min. After cooling, 5 ml of n-butan-ol was added to each tube and centrifuged at 700 g for 10 min. The absorbance of the upper layer was measured at 532 nm. Inhibition of LPO (%) was calculated as $[(1 - A_1)/A_0] \times 100\%$. Where, A0 and A1 were the absorbance of the reaction control and with extracts respectively.

DPPH• radical scavenging activity

The DPPH• radical scavenging activity was estimated by measuring the decrease in the absorbance of methanolic solution of DPPH• (Brand-Williams *et al.*, 1995). In brief, to 5 mL DPPH• solution (3.3 mg of DPPH in 100 mL methanol), 1 mL of each plant extracts were added, incubated for 30 min in the dark and the absorbance (A_1) was read at 517 nm. The absorbance (A_0) of a reaction control (methanol instead of plant extract) was also recorded at the same wavelength. Ascorbic acid (5-50 µg/mL) was used as a standard. Scavenging ability (%) was calculated by using the formula: DPPH• radical scavenging activity (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of reaction control and A_1 was the absorbance of extracts or standards.

ABTS• radical scavenging activity

The ABTS• cation radical scavenging activity of the extracts was determined according to the modified method of Re *et al.* (1999). A stock solution of ABTS was produced by mixing 7 mM aqueous solution of ABTS• with potassium persulfate (2.45 mM) in the dark at ambient temperature for 12–16 h before use.

The radical cation solution was further diluted until the initial absorbance value of 0.7 ± 0.005 at 734 nm was reached. For assaying test samples, 0.98 mL of ABTS solution was mixed with 0.02 mL of the plant extracts. The decrease in absorbance was recorded at 0 min. and after 6 min. Scavenging ability relative to the reaction control (without plant extract as 100%) was calculated by using the formula: ABTS• radical scavenging activity (%) = $[(\text{Initial reading} - \text{final reading})/\text{Initial reading}] \times 100$, where initial reading is absorbance at 0 min. and final reading is absorbance at 6 min.

NO• scavenging activity

Scavenging activity of NO• radicals was determined as per Garratt (1964). The mixture of 2 mL of sodium nitroprusside (10 mM), 0.5 mL of phosphate buffer saline (pH 7.4) and 0.5 mL of plant extract was incubated at 25°C for 150 min. From this reaction mixture, 0.5 mL taken and 1 mL of Griess reagent A (1% sulfanilamide in 5% phosphoric acid) was added and allowed to stand for 5 min. for diazotization. Subsequently 1 mL of Griess reagent B (Naphthylethylenediamine dihydrochloride 0.1% w/v) was added in the above mixture and incubated at 25°C for 60 min. The absorbance was recorded at 540 nm. Curcumin (50-500 µg/mL) was used as a reference standard. Scavenging ability (%) was calculated by using the formula- NO• radical Scavenging (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 was the absorbance of control and A_1 was the absorbance in the presence of extracts.

H2O2• scavenging activity

Scavenging activity of H₂O₂• was determined as per Ruch *et al.* (1989). Plant extracts (4 mL) were mixed with 0.6 mL of 4 mM H₂O₂ solution prepared in phosphate buffer (0.1 M, pH 7.0) and incubated for 10 min at 37 °C. Butylated hydroxytoluene (0.02-0.08 mg/mL) was used as a reference standard. The absorbance of the solution was taken at 230 nm. Scavenging ability (%) was calculated by using the formula-

$$\text{H}_2\text{O}_2\text{• radical scavenging activity (\%)} = \frac{[(A_0 - A_1)/A_0] \times 100}{}$$

where, A_0 was the absorbance of control and A_1 was the absorbance of extracts or standards.

Statistical analysis

Pearson correlation matrix was applied to the analytical data to find the relationships between the different analytical methods, which were expressed as the correlation coefficient 'R'. The associations were

determined at first between total phenolic content of the extracts with the antioxidant parameters and then within various antioxidant assays. The significance level (p) of correlation coefficients was related to the critical values for the Pearson product moment correlation coefficient

Results

Total phenolic content

Total phenolic content of the plants was measured using the Folin-Ciocalteu method and the results are presented in Table 2. There was a wide range of phenol concentrations in the medicinal plants as the values varied from 2.34-152.32mg GAE/g. *T. arjuna* extract showed highest total phenolic content (152.32mg GAE/g) followed by *T. chebula* (144 mg GAE/g) and *S. reticulata* (103.42 mg GAE/g). In *O. sanctum*, results indicated that total phenolic content was four folds higher in leaves (46.43 mg GAE/g) than seeds (10.33 mg GAE/g). Whereas, lowest content of phenolics were observed in *A. barbadensis* (2.34 mg GAE/g) extract.

RP estimation

As can be seen from Table 2, the plant extracts had shown reducing activity in terms of Absorbance at 700 nm in the range from 0.13 - 8.01. Thus Fe^{3+} was transformed to Fe^{2+} in the presence of plant extracts. Among plants RP of *C. mukul* was particularly high, indicating that *C. mukul* extracts has strong capacity of donating electrons, though it has low polyphenols content. Moderately high RP value is shown by *T. chebula* (4.29) and *T. arjuna* (4.26), which exhibited very high phenolic levels. While lowest reducing capacity was demonstrated by *A. barbadensis* which also had least phenolic compounds.

LPO inhibition activity

Table 2 shows anti-LPO activities of extracts which ranged from 21.72 to 98.34 %. High LPO inhibitory activity was found in *A. paniculata* extract (98.34%) followed by *B. monnieri* (96.98%), *H. indicus* (95.48%) and *O. sanctum*- leaf (93.18%), whereas lowest inhibition was noted in *G. glabra* extract (21.72%). In *O. sanctum* distinct variation in LPO inhibition was seen in the different parts as leaves extract showed higher inhibition (93.18%) than seeds extracts (56.03%).

DPPH• radical scavenging ability

Table 3 illustrates a variation in DPPH• scavenging activity of the plants which ranged from 26.26 to 96.01%. In several plants potent (> 90%) activity was

Table 2. Total phenolic contents of selected traditional medicinal plants along with their RP and LPO inhibition activity

Plants	Total Phenolic content (mg GAE /gm sample)	RP †(A ‡700nm)	LPO¶ (% inhibition)
<i>Aegle marmelos</i>	63.01 ± 0.21	2.67 ± 0.01	46.54 ± 8.02
<i>Aloe barbadensis</i>	2.34 ± 0.37	0.13 ± 0.01	65.64 ± 10.1
<i>Andrographis paniculata</i>	22.39 ± 2.04	0.44 ± 0.01	98.34 ± 2.14
<i>Bacopa monnieri</i>	12.94 ± 1.2	0.56 ± 0.01	96.98 ± 0.52
<i>Boerhavia diffusa</i>	6.36 ± 0.24	0.48 ± 0.02	71.40 ± 4.49
<i>Caesalpinia bonducella</i>	37.09 ± 0.37	1.00 ± 0.01	42.53 ± 3.17
<i>Commiphora mukul</i>	83.61 ± 1.82	8.01 ± 0.12	84.77 ± 3.69
<i>Glycyrrhiza glabra</i>	71.29 ± 4.67	0.92 ± 0.01	21.72 ± 12.6
<i>Gymnema sylvestre</i>	35.92 ± 0.8	1.3 ± 0.03	68.79 ± 3.73
<i>Hemidesmus indicus</i>	92.66 ± 3.2	1.12 ± 0.06	95.48 ± 1.20
<i>Ocimum basilium</i>	15.33 ± 1.82	0.41 ± 0.04	55.90 ± 9.23
<i>Ocimum sanctum</i> (leaf)	46.43 ± 2.08	1.25 ± 0.03	93.17 ± 0.81
<i>Ocimum sanctum</i> (seed)	10.33 ± 0.23	0.35 ± 0.02	56.03 ± 4.96
<i>Petroselinum crispum</i>	25.62 ± 0.15	0.61 ± 0.04	60.48 ± 9.88
<i>Salacia reticulata</i>	103.42 ± 0.49	0.87 ± 0.02	83.86 ± 2.14
<i>Silybum maritanum</i>	18.33 ± 0.16	1.87 ± 0.01	61 ± 3.35
<i>Swertia chirayita</i>	38.40 ± 0.41	0.58 ± 0.03	62.60 ± 17.1
<i>Terminalia arjuna</i>	152.32 ± 2.66	4.26 ± 0.04	54.75 ± 6.09
<i>Terminalia chebula</i>	144.00 ± 1.42	4.29 ± 0.16	69.68 ± 4.46
<i>Withania somnifera</i>	6.16 ± 0.63	0.78 ± 0.02	72.55 ± 1.83

Values are mean ± S. D., n=3.
 * GAE= Gallic Acid Equivalent
 † RP= Reducing Potential
 ‡ A= Absorbance
 ¶ LPO= Lipid peroxidation

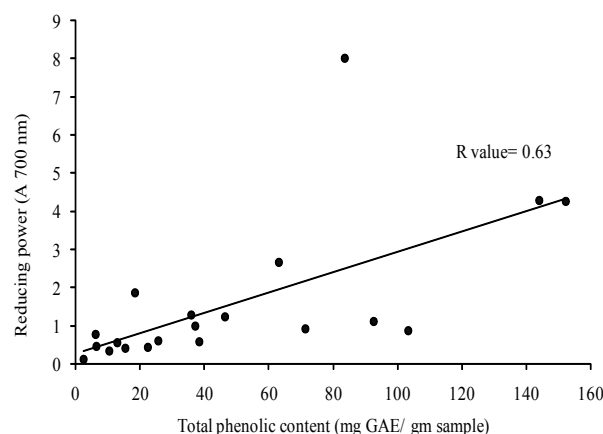


Figure 1. Correlation between the total polyphenols and the RP value of plants

noted indicating they have effective hydrogen donors or electron acceptor thus scavenging the free DPPH• radical. *C. bonducella* exhibited the highest (96.01%) activity, closely pursued by *W. somnifera* (95.31%) and *A. marmelos* (95.18 %). These plants had low phenolic content, whereas those plants with high quantity of phenolics like *T. arjuna*, *S. reticulata*, *H. indicus* showed comparatively weaker scavenging activity (< 90%) except *T. Chebula* which had equally robust properties. Lowest scavenging activity was

Table 3. Scavenging activities of plants for DPPH, ABTS, NO and H₂O₂ radicals

Plants	DPPH* (% scavenging activity)	ABTS† (% scavenging activity)	NO ‡ (% scavenging activity)	H ₂ O ₂ § (% scavenging activity)
<i>Aegle marmelos</i>	95.18 ± 3.33	98.91 ± 0.46	32.80 ± 1.73	16.52 ± 3.3
<i>Aloe barbadensis</i>	26.26 ± 4.92	8.33 ± 1.08	ND §	10 ± 2.26
<i>Andrographis paniculata</i>	58.41 ± 1.39	57.27 ± 4.88	ND §	33.22 ± 4.02
<i>Bacopa monnieri</i>	89.14 ± 0.43	29.41 ± 0.41	30.63 ± 8.54	17.73 ± 3.29
<i>Boerhavia diffusa</i>	40.67 ± 1.07	23.08 ± 0.79	13.52 ± 2.2	26.86 ± 0.58
<i>Caesalpinia bonducella</i>	96.01 ± 0.34	72.66 ± 12.3	20.12 ± 2.27	25.40 ± 3.59
<i>Commiphora mukul</i>	92.06 ± 0.28	97.07 ± 0.46	32.90 ± 1.15	33.22 ± 27.2
<i>Glycyrrhiza glabra</i>	87.64 ± 0.7	87.86 ± 2.99	51.44 ± 0.22	14.51 ± 4.86
<i>Gymnema sylvestre</i>	91.78 ± 0.4	96.78 ± 0.5	31.02 ± 1.9	5.32 ± 3.47
<i>Hemidesmus indicus</i>	87.62 ± 0.18	99.43 ± 0.14	16.78 ± 2.94	17.52 ± 2.32
<i>Ocimum basilicum</i>	93.93 ± 0.39	20.61 ± 1.72	29.35 ± 2.85	9.50 ± 3.89
<i>Ocimum sanctum (leaf)</i>	84.47 ± 0.89	95.85 ± 0.38	25.05 ± 0.43	15.33 ± 2.89
<i>Ocimum sanctum (seed)</i>	78.45 ± 2.73	33.75 ± 3.69	5.073 ± 2.25	8.87 ± 5.14
<i>Petroselinum crispum</i>	93.33 ± 0.84	74.40 ± 2.84	25.68 ± 0.65	60.27 ± 16.8
<i>Salacia reticulata</i>	84.27 ± 0.54	99.95 ± 0.08	25.15 ± 0.54	4.35 ± 2.84
<i>Silybum marianum</i>	92.45 ± 1.91	53.44 ± 0.71	34.69 ± 1.19	20.75 ± 1.08
<i>Swertia chirayita</i>	87.58 ± 0.37	71.67 ± 1.72	25.05 ± 0.48	45.51 ± 1.24
<i>Terminalia arjuna</i>	79.01 ± 0.12	98.91 ± 0.33	33.61 ± 0.82	ND §
<i>Terminalia chebula</i>	92.42 ± 0.96	100 ± 0.21	34.66 ± 2.31	27.70 ± 15.2
<i>Withania somnifera</i>	95.31 ± 0.7	29.83 ± 5.2	13.20 ± 0.83	84.49 ± 7.72

Values are mean ± S. D., n=3.
 * DPPH= 2,2'-diphenyl-1-picrylhydrazyl
 † ABTS= 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
 ‡ NO= Nitric oxide
 § H₂O₂ = Hydrogen peroxide
 ¶ ND=Not detectable.

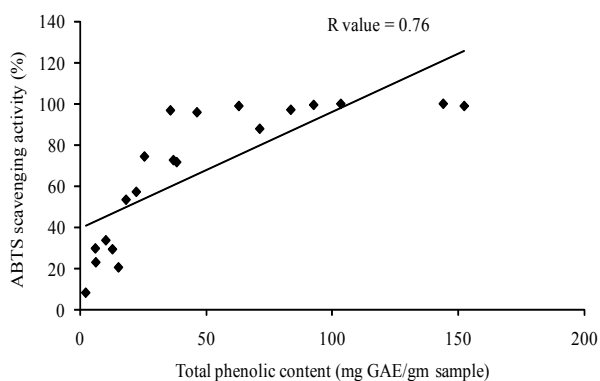


Figure 2. Correlation between total polyphenols and ABTS scavenging activity of plants

demonstrated by *A. barbadensis* which had least phenolic compounds.

ABTS• radical scavenging ability

As presented in Table 3, ABTS• radical scavenging ability, ranged from 8.33 to 100%. *T. chebula* extract showed the highest scavenging capacity (100%) followed by *S. reticulata* (99.95%), *H. indicus* (99.43%), *A. marmelos* (98.91%) and *T. arjun* (98.91%), whereas *A. barbadensis* exhibited

lowest activity (8.33 %).

NO• scavenging activity

The analyzed plant extracts showed the scavenging of NO• radicals in the range from negligible to 51.44% (Table 3). Maximum activity was found in the extracts of *G. glabra* (51.44%) and many plant extracts showed scavenging from 30.6 to 34.6%. *A. barbadensis* and *A. paniculata* extracts were totally devoid of this activity.

H₂O₂• scavenging activity

The ability of extracts to scavenge H₂O₂• is shown in Table 3 where a wide variation in scavenging activity was observed from negligible to 84.49%. *W. somnifera* exhibited maximum scavenging activity (84.49%), followed by *P. crispum* (60.27%). Surprisingly, higher H₂O₂• scavenging activity was noticed in *W. somnifera*, *P. crispum*, *S. chirayita* extracts, which have very less phenols contents, while in plants with high phenolic levels such as *T. chebula* and *S. reticulata*, less scavenging activity was exhibited. It is interesting to note the totally absent H₂O₂• scavenging activity in *T. arjuna* extract which exhibited highest phenolic content.

Correlation between antioxidant characteristics

A correlation analysis was used to determine the relationship between various assay parameters. The correlation coefficients of total phenolic content with other parameters were as follows: total phenolic content vs. RP (R = 0.63, p<0.001, Fig. 1); total phenolic content vs. LPO (R = -0.02, not significant); total phenolic content vs. DPPH• free scavenging activity (R = 0.28, not significant); total phenolic content vs. ABTS• scavenging activity (R = 0.78, p<0.001, Fig. 2); total phenolic content vs. NO• scavenging activity (R= 0.48, p<0.05); total phenolic content vs. H₂O₂• scavenging activity (R value = -0.3, not significant).

Additionally within antioxidant assays, it was found that ABTS• scavenging activity was marginally correlated with RP (R= 0.52, p<0.05) and DPPH• free scavenging activity (R = 0.5, p<0.01) whereas between DPPH• scavenging activity and LPO inhibition negative correlation (R= - 0.16, not significant) was noticed.

Discussion

The antioxidant potential of the plant extracts are influenced by various factors and largely depends on both the composition of the extract and the analytical test system. Thus, it is necessary to perform more than one type of antioxidant capacity measurements

to take into account the various mechanisms of antioxidant action (Frankel and Meyer, 2000) which will give more reliable results. Hence the assessment of antioxidant capacities of 19 selected antidiabetic medicinal plants were done by seven assays which are based on different approaches through estimation of i) total phenolic content, ii) RP, iii) LPO and scavenging ability of- iv) DPPH•, v) ABTS•, vi) NO• and vii) H₂O₂• radicals. The systematic evaluation of antioxidant content of the many currently used plants is not yet available, which is desirable to validate traditional knowledge based on scientific evidence. The results indicated that the same plants extracts had different antioxidant capacities in relation to the various method applied.

Highest phenolic content was observed in *T. arjuna* which contains 'arjunolic acid' a triterpenoid saponin which is a potent antioxidant, free radical scavenger and reported to have a protective role against type I, type II diabetes even in diabetic renal dysfunctions (Hemalatha et al., 2010). From *T. chebula* extracts, Manosroi et al. (2010) have identified six phenolic compounds as gallic acid, punicalagin, isoterchebulin, 1,3,6-tri-O-galloyl-β-D-glucopyranose, chebulagic acid and chebulinic acid which have antioxidant activities of different magnitudes of potency (Manosroi et al., 2010). Hazra et al. (2010) reported the total phenolic content from *T. chebula* was about 127.60 mg GAE/100 mg extract, which is found to be 144 mg GAE/1 g in the current study. In *O. sanctum*, higher phenolic content in leaves than seeds in this study was comparable to that reported by Hakkim et al. (2006), even though our values were higher than their data. Our results of *A. barbadensis* where lowest phenolic content was found are in accordance with the literature as Zheng et al. (2003) also observed lowest phenolic concentration (0.23 mg of GAE/g of fresh weight) among the various 39 plants used for their study. However its polyphenol-rich extract was reported to be effective for the control of insulin resistance in mice (Pérez et al., 2007). The slight disparities in values may be attributed to their geo-climatic conditions, state of maturity and method of sample preparation.

Polyphenols possess ideal structural chemistry for free radical-scavenging activities which have shown to be more effective antioxidants in vitro than vitamins E and C on a molar basis (Rice-Evans et al., 1997). The chemical activities of polyphenols in terms of their reducing properties as hydrogen or electron-donating agents predict their potential for action as free-radical scavengers (antioxidants). The activity of an antioxidant is determined by: (i) Its reactivity as a hydrogen or electron-donating agent

(which relates to its reduction potential i.e. RP), (ii) The fate of the resulting antioxidant-derived radical, which is governed by its ability to stabilize and delocalize the unpaired electron, (iii) Its reactivity with other antioxidants and (iv) The transition metal-chelating potential.

In RP assay, Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action (Nabavi et al., 2009) as the presence of antioxidants in the samples would result in the reducing Fe³⁺ to Fe²⁺ by donating an electron. Amount of Fe²⁺ complex can then be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Higher absorbance at 700 nm indicates greater reductive ability (Chung et al., 2002). Among plants, maximum RP value was observed in *C. mukul* which is reported to contain various steroids including the two isomers E- and Z-guggulsterone (cis- and trans-4, 17(20)-pregnadiene-3, 16-dione) (Deng, 2007). The RP exhibited by *T. chebula* (at 1.0 g/ mL) was 4.26 much lower than reported values as 0.25 at 1.0 mg/ mL (Hazra et al., 2010).

For LPO assay, egg yolk lipids undergo non-enzymatic peroxidation upon incubation in the presence of FeSO₄, resulting in formation of malondialdehyde that form pink chromogen with TBA, absorbing at 532 nm (Kosugi et al., 1987). In this assay, iron induces LPO through Fenton reaction (H₂O₂ + Fe²⁺ = Fe³⁺ + OH• + OH•) which leads to the formation of lipid hydroperoxides. Oxidative and reductive decomposition of hydroperoxides into peroxy and alkoxy radicals that can perpetuate the chain reaction further amplifies the peroxidation process (Halliwell, 1991). The presence of chain-breaking phenolic antioxidants provides a means of intercepting this peroxidation process (Rice-Evans et al., 1997). Maximum LPO inhibition was displayed by *A. paniculata* though it contained fewer amounts of phenolic compounds (22.39 mg GAE/g), which is in consensus with the similar reported value of about 80% LPO inhibition (Pérez et al., 2007). This plant contains two bioactive compounds, andrographolide and 14-deoxy-11, 12-didehydroandrographolide demonstrated to exhibit LPO inhibition and free radical scavenging activities by Akowuah et al. (2006). The negative correlation between LPO and total phenolic content may be explained by the fact that although hydrogen abstraction is the most important mechanism other factors e.g., oil-water partition behavior, electron-transfer process, stability of phenoxyl radicals and absolute chemical hardness also regulate the anti-LPO potentials of phenols to a significant degree (Cheng et al., 2003). The current

results also suggest the presence of non-phenolic components in extracts, which may be inhibiting the LPO reactions predominantly.

DPPH• is relatively stable nitrogen centered free radical and gives absorbance at 515 nm (visible deep purple color) that decolorizes easily by accepting an electron or hydrogen radical donated by an antioxidant compound to become a stable diamagnetic molecule. This assay is a sensitive way to survey the antioxidant activity (Singh *et al.*, 2012) which is strongly dependent on solvent type, pH and temperature of the system (Settharaksa *et al.*, 2012). Maximum DPPH• scavenging activity was noticed in *C. bonducella* which was analogous to the reported study by Shukla *et al.* (2010). In *O. sanctum*, higher DPPH• scavenging activity in leaves than seeds in this study was in coherence with previous study by Hakkim *et al.* (2006), where they reported IC₅₀ value 0.46 mg/mL for leaves extracts and 0.83 mg/mL for extracts of inflorescence.

The ABTS• radical scavenging assay is one of the popular indirect methods of determining the antioxidative capacity of compounds (Roginsky and Lissi, 2005). ABTS• is a blue chromophore produced by the reaction between ABTS and potassium persulfate. In the absence of antioxidants, the ABTS• radical is rather stable, but it reacts energetically with a hydrogen atom donor and is converted into a noncolored form of ABTS• and thus addition of the plant extracts to the pre-formed radical cation reduced it. The highest ABTS• radical scavenging activity of *T. chebula* indicated that all ABTS• radicals in the assay system were scavenged by the plant extract; this is supported by parallel investigation by Manosroi *et al.* (2010). From literature survey on *S. reticulata*, it appears that present study for the first time reporting its *in vitro* antioxidant activity values, although many researchers summarily proved its antioxidant potential in rodent models (Vasi and Austin, 2009).

It is well known that NO• has an important role in various inflammatory processes. Sustained levels of NO• production are directly toxic to tissues and contribute to the vascular collapse associated with septic shock, whereas chronic expression of NO• radical is associated with inflammatory conditions including juvenile diabetes (Taylor *et al.*, 1997). The toxicity of NO• increases greatly when it reacts with superoxide radical, forming the highly reactive peroxy nitrite anion (ONOO⁻) (Miller *et al.*, 1993). The NO• generated from sodium nitroprusside reacts with oxygen to form nitrite and the antioxidant inhibits nitrite formation by directly competing with oxygen. For the tested plants extracts, almost the same order for NO• scavenging activity was reported

by Jagetia and Baliga (2004). *G. gabra* root extract which is known to contain number of biologically active components such as glycyrrhizin, flavonoids, glycyrrhetic acid (Obolentseva *et al.*, 1999) and recently it is discovered that in THP-1 cells, it inhibits the NO• synthase activity therefore improve antioxidant condition and thus prevents inflammation (Franceschelli *et al.*, 2011). The NO scavenging activity exhibited by *T. chebula* (at 1.0 g/ mL) was 34% much lower than reported values as 59% at 1.0 mg/ mL (Hazra *et al.* 2010).

In vivo by many oxidizing enzymes such as superoxide dismutase H₂O₂• is formed, which crosses membranes and oxidizes a number of compounds. It inactivates a few enzymes directly by oxidation of essential thiol (-SH) groups. Intracellularly it also reacts with Fe²⁺ and possibly Cu²⁺ ions to form hydroxyl radicals which further amplify its toxic effects (Valko *et al.*, 2007). The scavenging activity exhibited by *O. sanctum* plant part extracts (at 1.0 g/ mL) on H₂O₂• was 15% (leaves) and 8.8% (seed) was much lower than reported values as 68% (leaves) 48% (inflorescence) at 1.0 mg/ mL (Hakkim *et al.*, 2006). Scavenging of H₂O₂• by extracts may be attributed to their phenolics, which can donate electrons to H₂O₂•, thus neutralizing it to water (Ebrahimzadeh *et al.*, 2010). However, Yoshiki *et al.*, (2001) illustrated that H₂O₂• scavenging activity corresponded with H₂O₂/ polyphenol levels rather than with only polyphenol levels. This may explain our observed results as no association was found between phenolic contents and H₂O₂• scavenging activity of plants.

The strong correlations between the results of RP, ABTS• scavenging activity and the total phenolic content suggests that phenolic compounds largely contribute to the antioxidant activities of these plants and therefore could play an important role in the beneficial effects of these plants. Though both ABTS• and DPPH• radicals indicate the hydrogen atom donating capacity of extracts, however the reaction kinetics between phenols and ABTS• radical have been different from that between phenols and DPPH• radical over a similar range of sample concentrations, as reactions of phenols with ABTS• radical cation are usually rapid, but the reactions with DPPH• radical differ from compound to compound (Apak *et al.*, 2007). Moreover the high-pigmented and hydrophilic antioxidants are better reflected by ABTS• assay than DPPH• assay (Floegel *et al.*, 2011). The observed negative association between DPPH• scavenging activity and LPO inhibition is supported by Apak *et al.* (2007) as many antioxidants that react quickly with peroxy radicals may react slowly or may even be inert to DPPH•. Since

DPPH• is long-lived nitrogen radical, which bears no similarity to the highly reactive and transient peroxy radicals involved in LPO, indicating extracts different mode of action in LPO inhibition and DPPH radical scavenging. The observed discrepancy in the phenolics-antioxidant association in some plants like *T. arjuna*, *S. reticulata* and *G. glabra*, *C. mukul*, *P. crispum* and *W. somnifera* meets the general agreement that the extracts of medicinal plants often contain complex mixtures of different kinds of active compounds and the contribution from compounds other than phenolics should not be neglected.

Conclusion

In this study, antioxidant potential and total phenolic contents of 19 selected medicinal plants were evaluated. In summary, our results support the view that (a) antidiabetic plants are promising sources of natural antioxidants, (b) antioxidant potential varies considerably among different assays, hence it is imperative to resort to multiple antioxidant measurements for appropriate interpretation, (c) significant correlation exists between total phenolic compounds and RP as well as ABTS• scavenging activity, (d) strongest antioxidant potential was observed in *T. chebula* since it exhibited abundant phenolic content, outstanding reducing power and highest radical scavenging activities. However few plants with less total phenolic content showed good antioxidant potential like *C. mukul*, *P. crispum* and *W. somnifera* while some plants such as *T. arjuna*, *S. reticulata* and *G. glabra* which exhibited relatively high phenolic content, but devoid of comparable antioxidant potential. It is therefore reasonable to conclude that in addition to phenolics, there could be other bioactive compounds present in plants which contribute to antioxidant potential. These results also provide a biochemical rationale which can be very useful for further planning of clinical nutrition and epidemiological diabetic research studies on bioactive compounds of plants and development of antidiabetic dietary products.

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