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Antioxidant and Free Radical Scavenging Activity of Certain Dietary and Medicinal Plant Extracts

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ABSTRACT

The antioxidant and free radical scavenging activity of the ethanol extracts of *Melia dubia* Hiern., *Albizia lebbeck* (L.) Benth., *Brassica oleracea* L. var. *botrytis* (broccoli) and *Glycyrrhiza glabra* L. were evaluated. The total phenolic content of the extracts was found to be higher in *A. lebbeck* (33.6%) and lower in broccoli (16.9%). The antioxidant activity of the extract was evaluated through different chemical assays and *in vitro* methods such as reducing power, $DPPH^{\bullet}$, $ABTS^{\bullet+}$ and $^{\bullet}OH$ radical scavenging activities, peroxidation inhibition in linoleic acid emulsion system and antihemolytic activity. The increasing concentrations of the extract exhibited nonlinear increases in the reducing power. Extract of *A. lebbeck* was found to have more hydrogen donating ability. Similar line of dose dependant activity has been observed in $DPPH^{\bullet}$ and $^{\bullet}OH$ radical scavenging systems. Further, *A. lebbeck* extracts exhibited strong antioxidant activity equivalent to trolox (6423.04 μmol g^{-1}) in ABTS radical cation scavenging system. All the extracts exhibited notable activity against linoleic acid emulsion system (35.5 to 40.2%) and antihemolytic activity (29.8 to 46.6%).

Keywords: *Albizia lebbeck*, *Brassica oleracea* var. *botrytis*, *Glycyrrhiza glabra*, *Melia dubia*, total phenolics

INTRODUCTION

Reactive oxygen species (ROS) from both exogenous and endogenous sources are implicated in many degenerative or pathological processes such as aging, cancer, cardiovascular disease, atherosclerosis, diabetes and arthritis (Aruoma 1998). They can also affect food/ feed quality, reducing its nutrient content and promoting deterioration (Anagnostopoulou *et al*. 2006). Antioxidant molecules, a potent scavenger of these species may serve as a possible preventive intervention for ROS-mediated diseases (Ames *et al*. 1995) and are considered as useful additives in the food/feed industry. Though numerous antioxidants have been developed, only synthetic antioxidants such as BHA (butylated hydroxyl anisole), BHT (butylated hydroxyl toluene) and the natural antioxidants as tocopherol or rosemary extracts are considered to be very effective and used for industrial purpose. However, the use of synthetic antioxidants is suspected to possess some side effects and toxic properties to human health (Tepe *et al*. 2005; Anagnostopolou *et al*. 2006). On the other hand tocopherols, widely used as safe natural antioxidants, are not as effective as synthetic antioxidants and their manufacturing cost is also high. For this circumstance, investigation of non-nutrient components, especially phenolics from fruits, vegetables, spices and herbs have been demonstrated to play a protective role in the pathogenesis of life threatening human diseases (Aruoma 1993). This has led to further intensified search for such naturally occurring antioxidants, especially of plant origin as food/ feed additives (Manian *et al*. 2008).

Glycyrrhiza glabra L. (Fabaceae) also known as licorice or sweetwood, is frequently used in traditional medicine to treat inflammatory, ulcer and allergic diseases. It has a broad range of pharmacological activities including anticancer (Sheela *et al*. 2006), hypocholesterolemic (Visavadivya *et al*. 2006) and antidepressant-like (Dhingra and Sharma 2006) activities. In addition to the presence of compounds like glycyrrhizin, liquiritin and isoliquiritin, Chin *et al*. (2007) isolated antioxidant phenolic compounds from the roots and stolons of licorice which include hispaglabridin, isoliquiritigenin, paratocaspin, formononetin and glabridin. Broccoli (*Brassica oleracea* L. var. *botrytis*) (Cruciferaceae) is one of the widely consumed green vegetables that has a characteristic pungent odor because of sulphated compounds. Generally, consumption of *Brassica* vegetables is associated with reduced risk of cancer of the alimentary tract in human populations (Smith *et al*. 2005). Nonetheless the dietary broccoli sprouts and their component glucosinolates and isothiocynates (sulforaphane) induce phase 2 enzymes and afford protection against chemically induced tumors (Fahey and Talalay 1999; Johnston 2004). This vegetable has a strong antioxidant property that can prevent oxidative stress (Blomhoff 2005).

Albizia lebbeck (L.) Benth. (Mimosaceae), commonly called Indian siris or East Indian walnut, is one of the most promising fodder trees for semi-arid regions. It is used in folk remedies for abdominal tumors, leprosy and lung ailments. The pharmacological effects of *A. lebbeck* have extensively been reviewed, including its anticonvulsant (Kasture *et al*. 2000), antiallergic rhinitis (Pratibha *et al*. 2004) and antifertility (Gupta *et al*. 2004) effects. Some of the active compounds including albiziahexosides, kaempferol and quercetin 3-*O*-α-rhamnopyranosyl(1→6)-β-glucopyranosyl(1→6)-β-galactopyranosides were isolated and identified by El-Mousallamy (1998) and Ueda *et al*. (2003). Recently Resmi *et al*. (2006) reported the antioxidant effects of the aqueous extract of *A. lebbeck* in alloxan induced diabetic rats. *Melia dubia* Hiern. (Meliaceae) is extensively used in traditional folk medicine in India to treat hysteria, leprosy, splenomegaly, cardiac diseases, bronchitis, inflammation and scabies. The plant has been reported to possess antiviral (Petrera and Coto 2006), larvicidal (Wandscheer *et al*. 2004), bacteristatic (Nagalakshmi *et al*. 2003) and antimalarial (Ofulla *et al*. 1995) activities which are attributed to an array of biologically active phytoconstituents including sendanins, azadirachins, meliacarpins, nimbolins and

Received: 25 November, 2008. Accepted: 29 May, 2009.

Original Research Paper

other type of limonoids (Huang *et al*. 1994). However, information regarding the antioxidant properties of the above described plants is meager.

Biological activities such as anticancer, anti-inflammatory, hypocholesterolemic and antidiabetic exhibited by plants are often related to their antioxidant and free radical scavenging abilities. Antioxidant activity of the multifunctional plant extracts cannot be evaluated by a unidimensional method due to complex nature of the phytochemicals present in them (Frankel and Meyer 2000). Therefore, the antioxidant activity is generally evaluated by a number of methods to explain the different mechanisms of antioxidant function. In view of this, the antioxidant potential of ethanol extracts of *Melia dubia*, *Albizia lebbeck*, broccoli sprouts and *Glycyrrhia glabra* were evaluated by employing different chemical assays and *in vitro* methods.

MATERIALS AND METHODS

Chemicals

Potassium ferricyanide, ferric chloride, 2,2-diphenyl-1-picrylhydrazyl, potassium persulfate, 2,2'-azinobis(3-ethylbenzothiozoline-6-sulfonic acid)diammonium salt, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate and ethylenediamine tetraacetic acid (EDTA) disodium salt were obtained from Himedia, Merck or Sigma. All other reagents used were of analytical grade.

Plant materials

The fresh leaves of *Melia dubia* Hiern. and *Albizia lebbeck* (L.) Benth. were collected from Dindigul, Tamil Nadu, India. The green vegetable broccoli sprouts (*Brassica oleraceae* L. var. *botrytis*) and root powder of *Glycyrrhiza glabra* L. were procured from the market, Coimbatore. Voucher specimens are deposited in the Department of Botany Herbarium, Bharathiar University, and identified by Dr. V. S. Ramachandran. All the plant materials were collected/procured in December 2006, and were cleaned to remove adhering dust, air dried under shade, powdered in a Willy mill to 60-mesh size and used for solvent extraction.

Solvent extraction

The dried, powdered plant samples (25 g) were extracted with 250 mL of ethanol by shaking for 24 h and filtering through Whatman No. 4 filter paper. The residues were reextracted with an additional 100 mL of ethanol as described above for 3 h. The extracts were concentrated under reduced pressure (Yamato BO410, Japan), dried and weighed to determine the yield of soluble components. The percentage yield was expressed in terms of air dried weight of plant material. The extracts thus obtained were used directly for the estimation of total phenolics and assessment of antioxidant property through various chemical and *in vitro* assays.

Determination of total phenolic content

The concentration of phenolics in the ethanol extract of *M. dubia*, *A. lebbeck*, broccoli and *G. glabra* was determined according to the method described by Siddhuraju and Becker (2003). Ten microlitres of aliquot of the extracts (1 mg/mL) were taken in test tubes and made up to the volume of 1 mL with distilled water. Then 0.5 mL of Folin-Ciocalteu phenol reagent (1: 1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank in a spectrophotometer (Shimadzu UV-1601, Australia). The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents.

Antioxidant activity assays

1. Reducing power

The reducing power of the ethanolic extract of each sample was determined by the method reported by Siddhuraju *et al*. (2002). 20-100 µg of extract lyophilized was suspended in 1 mL of phosphate buffer. Subsequently 5 mL of 0.2 M phosphate buffer (pH 6.6) and 5 mL of 1% potassium ferricyanide solution were added and incubated at 50°C for 20 min. After the incubation, 5 mL of 10% TCA was added. The content was then centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5 mL) was mixed with 5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. The absorbance of the reaction mixture was determined at 700 nm using spectrophotometer.

2. Free radical scavenging activity on DPPH $^{\bullet}$

The free radical scavenging activity of the ethanolic extract of the samples was determined using the stable DPPH radical, according to the method of Blios (1958). A methanol solution of the sample extracts at various concentrations (20-90 µg) was added to 5 mL of 0.1 mM methanolic solution of DPPH$^{\bullet}$ and allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibittion percentage of free radical by the sample and was calculated as follows:

% DPPH radical scavenging activity = (Control OD-Sample OD / Control OD) × 100

3. Antioxidant activity by radical cation (ABTS $^{\bullet+}$)

The total antioxidant activity of the samples was measured by ABTS radical cation decolorization assay according to the method of Re *et al*. (1999) described by Siddhuraju and Manian (2007). ABTS$^{\bullet+}$ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1: 89, v/v) and equilibrated at 30°C to give an absorbance of 0.700 ± 0.02 at 734 nm. The stock solution of the sample extracts were diluted such that after introduction of 10 µL aliquots into the assay, they produced between 20-80% inhibition of the blank absorbance. After the addition of 1 mL of diluted ABTS$^{\bullet+}$ solution to 10 µL of sample or Trolox standards (final concentration 0-15 µM) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated of the blank absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter basis.

4. Hydroxyl radical scavenging activity

The scavenging activity of the ethanol extracts of the samples on hydroxyl radical was measured according to the method of Klein *et al*. (1991). Various concentrations (100, 200, 300 and 400 µg) of extracts were added with 1mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1 mL of DMSO (0.85%, v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL of ascorbic acid (0.22%) and incubated at 80-90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1mL of ice-cold TCA (17.5%, w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and raised to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity is calculated by the following formula:

%HRSA = [1-(Difference in absorbance of sample/Difference in absorbance of blank)] ×100

5. Antioxidant activity in linoleic acid emulsion system

The antioxidant activity of the ethanolic extracts was determined using the thiocyanide method (Kikuzaki and Nakatani 1993). Each sample (250 µg) in 0.5 mL of absolute ethanol was mixed with 0.5 mL of 2.51% linoleic acid in absolute ethanol, 1 mL of 0.05 M phosphate buffer (pH 7), and 0.5 mL of distilled water and placed in a screw capped tube. The reaction mixture was incubated in dark at 40°C in an oven. Aliquots of 0.1 mL were taken at every 12 h during incubation and the degree of oxidation was measured by sequentially adding ethanol (9.7 mL, 75%), ammonium thiocyanate (0.1 mL, 30%) and ferrous chloride (0.1 mL, 0.02 M in 3.5% HCl). After the mixture rested for 3 min, the peroxide value was determined by monitoring absorbance at 500 nm until the absorbance of the control reached the maximum. The antioxidant activity (AA) was calculated as percentage of inhibition relative to the control:

AA = [100 - (Sample absorbance at 48 h - Sample absorbance at 0 h /Control absorbance at 48 h - Control absorbance at 0 h)] ×100

6. Antihemolytic activity

Antihemolytic activity of the extracts was assessed as described by Naim *et al*. (1976). The erythrocytes from cow blood were separated by centrifugation and washed with phosphate buffer (pH 7.4). The erythrocytes were then diluted with phosphate buffered saline to give 4% suspension. 500 µg of plant extract/ mL of saline buffer were added to 2 mL of the erythrocyte suspension and the volume was made up to 5 mL with saline buffer. The mixture was incubated for 5 min at room temperature and then 0.5 mL of H_2O_2 solution in saline buffer was added to induce the oxidative degradation of the membrane lipids. The concentration of H_2O_2 in the reaction mixture was adjusted to bring about 90% hemolysis of blood cells after 240 min. After incubation the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.

RESULTS AND DISCUSSION

Recovery percent and total phenolic content of extracts

The yield of ethanolic extracts from *Melia dubia*, *Albizia lebbeck*, broccoli and *Glycyrrhiza glabra* and total phenolic content of the respective extracts are shown in **Table 1**. The extracts of broccoli sprouts and *M. dubia* gave the maximum yield followed by *A. lebbeck* and the yield was minimum in *G. glabra*. However the phenolic content of the extracts was maximum in *A. lebbeck* (33.6%) followed by *M. dubia* (24%), *G. glabra* (21.8%) and broccoli sprouts (16.9%) in terms of tannic acid equivalents. Though the broccoli registered higher extract yield, the phenolic content was found to be very low when compared with the other plant extracts.

Table 1 Yield recovery percent, total phenolics (Folin method), ABTS radical cation scavenging activity and antihemolytic activity of ethanolic extracts of *Melia dubia*, *Albizia lebbeck*, broccoli sprouts and *Glycyrrhiza glabra*.

Sample (%)	Yield (%)	Total phenolics (%)	TAA[a] (µmol g $^{-1}$ DM)	Antihemolytic activity (%)
MDE	12.6	23.9 ± 1.5	5798.0 ± 436.5	37.7 ± 5.6
ALE	7.4	33.6 ± 4.0	6423.0 ± 801.7	46.6 ± 2.1
BSE	12.7	16.9 ± 0.1	5826.9 ± 557.1	29.8 ± 6.2
GGE	4.2	21.8 ± 2.5	5923.0 ± 305.7	39.7 ± 6.3

[a]Total antioxidant activity (µmol equivalent Trolox performed by using ABTS radical cation). MDE, ethanol extract of *M. dubia*; ALE, ethanol extract of *A. lebbeck*; BSE, ethanol extract of broccoli sprouts; GGE, ethanol extract of *G. glabra*.
Values are means of three independent analyses of the extract ± standard deviation (n=3)

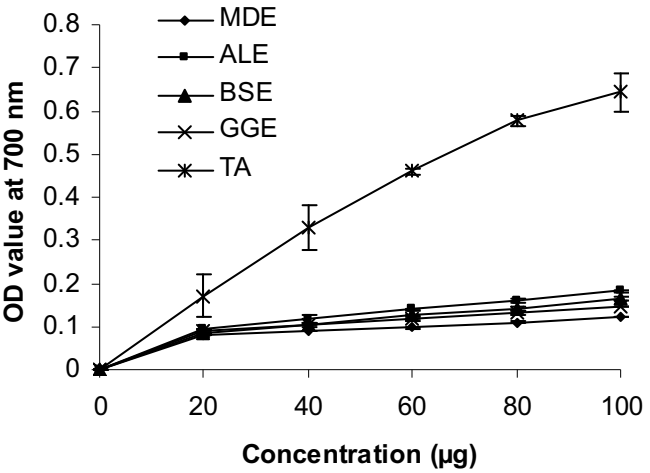

Fig. 1 Reducing power of ethanol extract of *M. dubia*, *A. lebbeck*, broccoli sprouts and *G. glabra* and tannic acid. Values are means of three independent analyses of the extract (n=3) ± standard deviation. MDE, ethanol extract of *M. dubia*; ALE, ethanol extract of *A. lebbeck*; BSE, ethanol extract of broccoli sprouts; GGE, ethanol extract of *G. glabra*; TA, tannic acid.

Reducing power assay

The reducing capability of the ethanol extract of *M. dubia*, *A. lebbeck*, broccoli and *G. glabra* in comparison with tannic acid was evaluated using potassium ferricyanide reduction method (**Fig. 1**). The reducing properties are associated with the presence of reductants, which have been shown to exert antioxidant action by breaking free radical chain through the donation of hydrogen atom (Gordon 1990). Though the increased concentrations of the extracts showed higher reducing activity, the relationship was not found to be linear. This might be attributed to the uneven distribution of the bioactive constituents in the crude extract. Among the samples, at the concentration of 100 µg in the reaction mixture, *A. lebbeck* exhibited the maximum reducing power and *M. dubia* was found to have the least when compared to other extracts. The antioxidant activity might be directly correlated to the phenolic content of various plant extracts, thus the ethanol extract of *A. lebbeck* showed higher reducing power. Therefore all these extracts are electron or hydrogen atom donors and can react with free radicals to convert them to more stable products and terminate radical chain reaction. Similar observations between the total phenolic constituents and dose dependant reducing power activity have been reported for several plant extracts (Amarowicz *et al*. 2004).

DPPH radical scavenging activity

The free radical scavenging potential of the extracts was determined by DPPH$^{\bullet}$ assay. Since the DPPH$^{\bullet}$ assay is sensitive enough to detect natural compounds at low concentrations, it has been extensively used for evaluating the free radical scavenging potential of natural antioxidants (Porto *et al*. 2000). DPPH$^{\bullet}$ is a stable free radical and accepts an electron or hydrogen by reacting with antioxidants to become a stable diamagnetic molecule (Blios 1958). **Fig. 2** illustrates the DPPH free radical scavenging activity of the ethanolic extracts of each sample at 3 different concentrations, 20, 40 and 60 µg in the reaction mixture. All the extracts showed dose dependant increase in activity. Extract of *G. glabra* exhibited strong scavenging activity at the concentration of 60 µg. The scavenging activity of *A. lebbeck* and *M. dubia* were comparable with each other. The effect of broccoli was relatively lower in accordance with other samples and this might be due to the presence of relatively low concentration of polyphenolic substances (**Table 1**). In general the radical scavenging activity of extracts could be

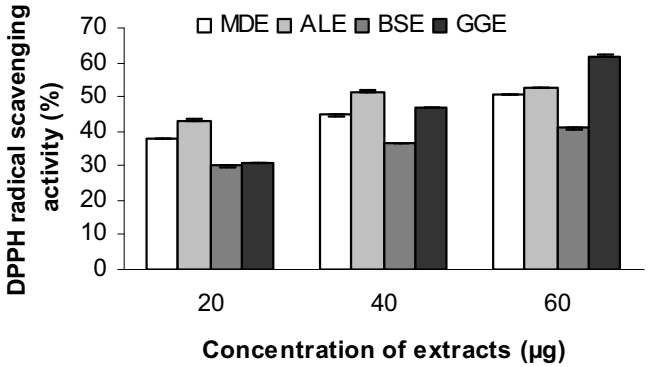

Fig. 2 DPPH free radical scavenging activity of *M. dubia*, *A. lebbeck*, broccoli sprouts and *G. glabra*. Values are means of three independent analyses of the extract (n=3) ± standard deviation. MDE, ethanol extract of *M. dubia*; ALE, ethanol extract of *A. lebbeck*; BSE, ethanol extract of broccoli sprouts; GGE, ethanol extract of *G. glabra.*

related to the nature of phenolics and their hydrogen donating ability (Shimada *et al*. 1992).

ABTS $^{\bullet+}$ radical scavenging activity

The effect of ethanol extracts of *M. dubia*, *A. lebbeck*, broccoli and *G. glabra* on ABTS radical cation scavenging activity is presented in **Table 1**. It reflects the ability of hydrogen donating antioxidants in the extract to scavenge the ABTS radical cation, compared with that of Trolox. The activity of the tested sample extracts was expressed as Trolox equivalent- the micromolar Trolox solution having an antioxidant capacity equivalent to 1 g dry matter of the substance under investigation. Though all the samples exhibited strong ABTS$^{\bullet+}$ radical scavenging activity, *A. lebbeck* exhibited strong TAA (6423.04 µmolg^{-1}). However, the TAA values for *M. dubia*, broccoli and *G. glabra* were comparable with each other (5798.04, 5826.89 and 5923.04 µmolg^{-1}, respectively). These results suggests that the investigated samples may prevent or retard the *in vitro* formation of radical species related with oxidative stress, and may play an important role in protecting against damage to membrane functions. Similar observations between the phenolic constituents and ABTS$^{\bullet+}$ scavenging activity have been reported for several plant extracts including broccoli (Proteggente *et al*. 2002; Puertas *et al*. 2005).

Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as highly damaging to almost every molecule found in living cells. This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutation and cytotoxicity (Moskovitz 2002). Further this species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids (Gordon 1990). Hydroxyl radical scavenging activity was assessed by generating the hydroxyl radicals using ascorbic acid-iron-EDTA. The hydroxyl radicals formed by the oxidation, reacts with dimethyl sulfoxide to yield formaldehyde, which provides a convenient method to detect hydroxyl radicals by treatment with Nash reagent. In the present investigation, a concentration dependant inhibition against hydroxyl radical was observed in ethanol extract of *M. dubia*, *A. lebbeck*, broccoli and *G. glabra* (**Table 2**). All the samples exhibited between 27.7 and 35.1% hydroxyl radical scavenging activity at 400 µg concentration in the reaction mixture. The ability of the above mentioned extracts to quench hydroxyl radicals seems to be directly related to the prevention of propagation of the process of lipid peroxidation and seems to be good scavenger of active oxygen species, thus reducing the rate of chain reaction.

Table 2 Hydroxyl radical scavenging activity of ethanol extract of *Melia dubia*, *Albizia lebbeck*, broccoli sprouts and *Glycyrrhiza glabra*.

Sample	Hydroxyl radical scavenging activity (%)			
	100 µg	200 µg	300 µg	400 µg
MDE	16.6 ± 0.5	21.9 ± 1.1	26.7 ± 1.8	31.0 ± 3.1
ALE	15.9 ± 0.8	20.4 ± 1.6	24.9 ± 2.0	27.7 ± 1.3
BSE	12.7 ± 0.8	19.3 ± 1.5	23.2 ± 2.2	29.4 ± 2.5
GGE	24.5 ± 4.4	27.3 ± 1.3	28.7 ± 8.3	35.1 ± 2.5

MDE, ethanol extract of *M. dubia*; ALE, ethanol extract of *A. lebbeck*; BSE, ethanol extract of broccoli sprouts; GGE, ethanol extract of *G. glabra.*
Values are means of three independent analyses of the extract ± standard deviation (n=3)

Antioxidant activity in linoleic acid emulsion system

Lipid peroxidation has been defined as the oxidative deterioration of polyunsaturated lipids. Initiation of a peroxidation sequence in a membrane or polyunsaturated fatty acid is due to abstraction of a hydrogen atom from the double bond in the fatty acid. The free radical tends to be stabilized by a molecular rearrangement to produce a conjugated diene which then easily reacts with an oxygen molecule to give a peroxyl radical (Gordon 1991). Peroxy radicals can abstract hydrogen from another molecule or they can abstract a hydrogen atom to give a lipid hydroperoxide R-OOH. The antioxidant activity of ethanol extract of *M. dubia*, *A. lebbeck*, broccoli and *G. glabra* was determined by peroxidation of linoleic acid using the thiocyanide method. During linoleic acid peroxidation, peroxides are formed and these compounds oxidizes Fe^{2+} to Fe^{3+}. Fe^{3+} ion forms a complex with SCN^-, which has a maximum absorbance at 500 nm. High absorbance is an indicator of high concentration of peroxide formed during the incubation. In the present study, all the extracts (250 µg/ 2.5 mL reaction mixture) inhibited 35.5 to 40.2% (**Fig. 3**) peroxidation of linoleic acid after incubation for 48 h. Further, these values were comparable to that of the natural antioxidant α-tocopherol (35.07%). The efficacy of the tested sample extracts to inhibit peroxidation of linoleic acid emulsion is a reflection of the complexity of the extract composition (aqueous versus hydrophobic nature of compounds) as well as potential interaction between the extract and emulsion compound oil, water or lipid: air interfaces (Koleva *et al*. 2002). Similar peroxidation inhibiting activity was reported in various solvent extracts of different plant samples (Siddhuraju *et al*. 2002; Zhou *et al*. 2004).

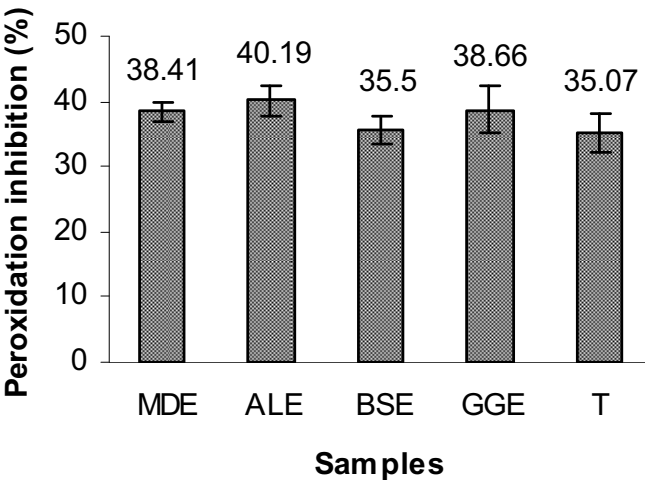

Fig. 3 Peroxidation inhibition property of ethanol extract of *M. dubia*, *A. lebbeck*, broccoli sprouts and *G. glabra* at a concentration of 250 µg in the reaction mixture. Values are means of three independent analyses of the extract (n=3) ± standard deviation. MDE, ethanol extract of *M. dubia*; ALE, ethanol extract of *A. lebbeck*; BSE, ethanol extract of broccoli sprouts; GGE, ethanol extract of *G. glabra*; T, α-tocopherol.

Antihemolytic activity

Antihemolytic activity of ethanol extract of *M. dubia*, *A. lebbeck*, broccoli and *G. glabra* was determined and the results are shown in **Table 1**. The red blood cell (RBC) hemolysis is a more sensitive and more clinically relevant system than other common assays because it can detect several classes of antioxidants (Djeridane *et al*. 2006). In this study, we used a biological test based on free radical-induced erythrocyte lysis in cow blood. Lipid peroxidation of cow blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. All the extracts showed antihemolytic activity in terms of percentage inhibiting activity ranging from 29.8 to 46.6%. *A. lebbeck* which exhibited higher amount of phenolics induced strong delay of free radical induced RBC hemolysis. Similarly, two different solvent extracts from *Ficus* species exhibited potential antihemolytic activity (Manian *et al*. 2007). This effect could be related to possible coenzymatic activities which cannot be noticed in chemical tests (Stocker *et al*. 2003) although the antioxidant mechanisms which rule their action are not well established yet. In our study, the concentration of the various extracts needed to inhibit erythrocyte hemolysis was much higher than those used to test the reducing power and DPPH radical scavenging activity. The higher concentrations needed to inhibit oxidation in the erythrocyte membranes may be due to the ability of polyphenols to readily bind to proteins through hydrophobic interactions and hydrogen bonding.

In the present investigation, all the tested plant extracts showed antioxidant and free radical scavenging activity at different magnitude of potency. Further investigations on the isolation and characterization of individual phenolic compounds in the investigated plants will be required to elucidate their different antioxidant mechanism and the existence of possible synergism among the compounds. However, *in vivo* studies are needed to confirm their impact on various health improvement/control of free radical-mediated diseases and act as food and feed additives.

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