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The antioxidant activity and free radical scavenging potential of two different solvent extracts of *Camellia sinensis* (L.) O. Kuntz, *Ficus bengalensis* L. and *Ficus racemosa* L.

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Abstract

The stem bark and fruits of *Ficus bengalensis* L. and *Ficus racemosa* L. are used in India for the treatment of diabetes and a number of other diseases. Since these effects may be correlated with the presence of antioxidant compounds, methanol and 70% acetone (acetone:water, 70:30) extracts of *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) were evaluated for their antioxidant activity and radical scavenging capacity in comparison with *Camellia sinensis* (L.) O. Kuntz (green tea). Methanol extracts of green tea and *F. bengalensis* and 70% acetone extract of *F. racemosa* contained relatively higher levels of total phenolics than the other extracts. The antioxidant potential of the extracts were assessed by employing different *in vitro* assays such as reducing power assay, DPPH$^{\cdot}$, ABTS$^{\cdot+}$ and $^{\cdot}$OH radical scavenging capacities, peroxidation inhibiting activity through linoleic acid emulsion system, antihemolytic assay by hydrogen peroxide induced method and metal ion chelating ability. Though all the extracts exhibited dose dependent reducing power activity, methanol extracts of all the samples were found to have more hydrogen donating ability. Similar line of dose dependent activity has been maintained in all the samples in DPPH$^{\cdot}$ and $^{\cdot}$OH scavenging systems. All the extracts exhibited antioxidant activity against the linoleic acid emulsion system (34–38%). The potential of multiple antioxidant activity was evident as it possessed antihemolytic activity and metal ion chelating potency.

Keywords: *Camellia sinensis*; *Ficus bengalensis*; *Fucus racemosa*; Polyphenols; Antioxidant activity; Free radicals; Reducing power

1. Introduction

Oxidative stress is an important contributor to the pathophysiology of a variety of pathological conditions including cardiovascular dysfunctions, atherosclerosis, inflammation, carcinogenesis, drug toxicity, reperfusion injury and neurodegenerative diseases (Aruoma, 1998). Human body has multiple mechanisms especially enzymatic and nonenzymatic antioxidant systems to protect the cellular molecules against reactive oxygen species (ROS) induced damage (Anderson, 1999). However the innate defense may not be enough for severe or continued oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS in human body. Many synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are very effective and are used for industrial processing but they may possess some side effects and toxic properties to human health (Anagnostopoulou, Kefalas, Papageorgiou, Assimepoulou, & Boskou, 2006). Hence, compounds especially from natural sources capable of protecting against ROS mediated damage may have potential application in prevention and/or curing of diseases.

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Green tea (*Camellia sinensis* L. Kuntz), a beverage commonly used in Asian countries contains compounds having strong antioxidant capacities. It is a significant source of polyphenols including (−)-epigallocatechingallate (EGCG), (−)-epigallocatechin, (−)-epicatechingallate and (−)-epicatechin. These polyphenols have recently attracted the medicinal attention as bioactive agents with anticancer, antidiabetic, antiviral, antimalarial, hepatoprotective, neuroprotective and cardioprotective effects (Adhami et al., 2007; Anderson & Polansky, 2002; Noonan, Benelli, & Albini, 2007). According to Ayurvedic system of medicine, bark, aerial root and fruits of *Ficus bengalensis* L. (banyan tree) and bark and fruits of *Ficus racemosa* L. (Indian fig) are well known to be useful in diabetes. Bhardwaj et al. (2000) found that fistein and resveratol were as effective as vitamin E in their inhibitory action on lipid peroxidation. A *Ficus* flavonoid leucopelargonin and its derivative isolated from *F. bengalensis* were proved as good hypoglycemic agents and antioxidants (Daniel, Devi, Augusti, & Sudhakaran Nair, 2003). *F. racemosa* showed significant anticancer (Rubnov, Kashman, Rabinowitz, Schlesinger, & Mechoulam, 2001) and antihelminthic activities (Hansson et al., 1986). The bark is antiseptic, antipyretic, vermicidal and a decoction of the bark is used in treating various skin diseases and ulcers. It is used as a plaster in inflammatory swellings and boils. It is also effective in the treatment of piles, dysentery, asthma, gonorrhea, hemoptysis and urinary diseases. However, information pertaining to the systematic studies on the antioxidant properties of *F. bengalensis* and *F. racemosa* in comparison with the green tea polyphenols is lacking. In view of the above fact, in the present study the possible antioxidant activity of the methanol and 70% acetone extracts of *C. sinensis* (green tea), stem bark of *F. racemosa* and aerial root of *F. bengalensis* were investigated by employing different *in vitro* models.

2. Materials and methods

2.1. Chemicals

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

2.2. Plant material

The fresh harvestable shoots of *C. sinensis* were collected during the month of January, 2007 from Parry Agro Estates, Tamil Nadu, India. The aerial roots of *F. bengalensis* and the stem bark of *F. racemosa* were collected during the month of January, 2007 from Coimbatore, Tamil Nadu, India. Freshly collected plant materials were cleaned to remove adhering dust and then dried under shade. The dried samples were powdered in a Willy Mill to 60-mesh size and used for solvent extraction.

2.3. Solvent extraction

The air dried powdered plant samples were extracted in soxhlet extractor successively with methanol followed by 70% acetone. Each time before extracting with the next solvent, the material was dried in hot air oven at 40 °C. The extracts were concentrated by rotary vacuum evaporator and then dried. The dry extract obtained with each solvent was weighed. 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 phenolic and also for the assessment of antioxidant potential through various chemical assays.

2.4. Determination of total phenolic content

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003). Ten microliters of aliquot of the extracts (2 mg/2 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. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents.

2.5. Total antioxidant activities

2.5.1. Reducing power

The reducing power of the methanolic and 70% acetone extracts of each sample was determined by the method reported by Siddhuraju, Mohan, and Becker (2002). 20–100 μg of extracts in 1 ml of phosphate buffer with 5 ml of 0.2 M phosphate buffer (pH 6.6) and 5 ml of 1% potassium ferricyanide solution were 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. Then the absorbance of the reaction mixture was read spectroscopically at 700 nm.

2.5.2. Free radical scavenging activity on DPPH

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH, according to the method of Blios (1958). A methanol solution of the sample

extracts at various concentrations (12.5–50 μg) was added to 5 ml of a 0.1 mM methanolic solution of DPPH 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 inhibition percentage of free radical by the sample and was calculated using the formula

$$\%\ \text{DPPH radical scavenging activity} = (\text{control OD} - \text{sample OD}/\text{control OD}) \times 100$$

2.5.3. Antioxidant activity by radical cation ($ABTS^{\cdot+}$)

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). $ABTS^{\cdot+}$ 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 at 734 nm of 0.700 ± 0.02. The stock solution of the sample extracts were diluted such that after introduction of 10 μl aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1 ml of diluted ABTS 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.

2.5.4. Hydroxyl radical scavenging activity

The scavenging activity of the methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) on hydroxyl radical was measured according to the method of Klein, Cohen, and Cederbaum (1991). Various concentrations (100, 150, 200 and 250 μg) of extracts were added with 1.0 ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of EDTA solution (0.018%), and 1.0 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 1.0 ml of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0 g of ammonium acetate, 3.0 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

$$\%\ \text{HRSA} = 1 - (\text{difference in absorbance of sample}/\text{difference in absorbance of blank}) \times 100$$

2.5.5. Antioxidant activity in linoleic acid emulsion system

The antioxidant activity of the extracts was determined using the thiocyanide method (Kikuzaki & 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 was 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 was calculated as percentage of inhibition relative to the control.

$$\text{AA} = 100 - (\text{sample absorbance at 48h} - \text{sample absorbance at 0 h}/\text{control absorbance at 48 h} - \text{control absorbance at 0 h}) \times 100$$

2.5.6. Antihemolytic activity

Antihemolytic activity of the extracts was assessed as described by Naim, Gestener, Bondi, and Birk (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 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 extend of hemolysis was determined by measuring the absorbance at 540 nm corresponding to hemoglobin liberation.

2.5.7. Chelating capacity

Chelating property of the two different solvent extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) was assessed by bipyridyl assay (Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000). The reaction mixture contained 0.25 ml of 1mM $FeSO_4$ solution, 0.25 ml of extract, 1 ml of 0.2 M Tris-HCl buffer (pH 7.4), 1 ml of 2,2′ bipyridyl solution, 0.4 ml of 10% hydroxylamine-HCl and

2.5 ml of ethanol. The final volume was made up to 5 ml with deionized water and the absorbance was determined at 522 nm. The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as mg EDTA equivalent/g sample extracts.

3. Results and discussion

3.1. Recovery percent and phenolic content of extracts

The yield percent and total phenolic content of extracts obtained from green tea (*C. sinensis* L. Kuntz), *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) using methanol and 70% acetone solvents are shown in Table 1. The maximum recovery percentage and extractable total polyphenolic content were recorded in methanolic extract of green tea whereas the methanol extracts of both *F. bengalensis* and *F. racemosa* samples were found to contain comparable level of extracts recovery percentage. Since the 70% acetone was a subsequent solvent to methanol, the extract yield percentage of all the samples were much lower. However, the total phenolic content of the respective extracts were found to be higher. Generally, the higher contents of total phenolic in green tea might be contributed by the presence of catechins such as catechin, gallocatechin, gallocatechin gallate, epigallocatechin, epicatechin gallate, and epigallocatechin gallate (Yen & Chen, 1995). The major contributor of the phenolics in *Ficus* spp. might be pelargonidin and leucopelargonin derivatives, flavonoids and high molecular tannins (Cherian & Augusti, 1993).

3.2. Reducing power assay

Antioxidant potential of the methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) was estimated using potassium ferric cyanide reduction method (Fig. 1). The yellow color of the test solution changes to various shades of green and blue depending upon the reducing power of each extract. The presence of reductants (antioxidants) in the herbal extracts causes the reduction of Fe^{3+}/Ferric cyanide complex to ferrous form. Therefore the Fe^{2+} complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm (Chung, Chang, Chao, Lin, & Chou, 2002). Methanol extracts of green tea and *F. bengalensis* aerial root showed the highest reducing power and the values were comparable to that of tannic acid. In *F. racemosa*, both methanol and 70% acetone extracts exhibited similar reducing power activity at the concentration of 100 μg in the reaction mixture. Polyphenolic contents of all the sample extracts appear to function as good electron and hydrogen atom donors and therefore should be able to terminate radical chain reaction by converting free radicals and reactive oxygen species to more stable products. Similar observation between the polyphenolic constituents in terms of dose dependent and reducing power activity have been reported for several plant extracts including tea (Amarowicz, Pegg, Raim-Mohaddam, Bral, & Weil, 2004; Yen & Chen, 1995; Zhu, Hackman, Ensunsa, Holt, & Keen, 2002).

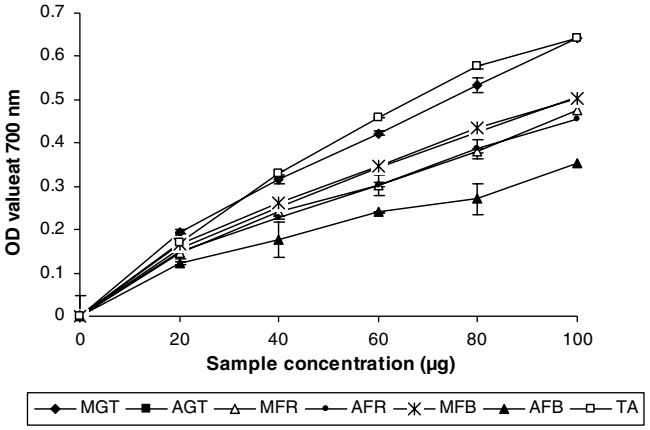

Fig. 1. Reducing power of methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root), *F. racemosa* (stem bark) and tannic acid. Values are means of triplicate determinations ($n = 3$) ± standard deviation. MGT – methanol extract of green tea, AGT – 70% acetone extract of green tea, MFB – methanol extract of *F. bengalensis* (aerial root), AFB – 70% acetone extract of *F. bengalensis* (aerial root), MFR – methanol extract of *F. racemosa* (stem bark), AFR – 70% acetone extract of *F. racemosa* (stem bark).

Table 1
Yield percent and total phenolics of methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark)

Sample	Yield (%)	Total phenolics (%)
MGT	45.5	72.4 ± 7.8
AGT	2.8	47.6 ± 3.3
MFB	16.5	39.5 ± 7.1
AFB	3.8	60.3 ± 4.5
MFR	16.2	59.6 ± 5.9
AFR	2.4	54.8 ± 1.2

Values are mean ± standard deviation ($n = 3$). MGT – methanol extract of green tea, AGT – 70% acetone extract of green tea, MFB- methanol extract of *F. bengalensis* (aerial root), AFB – 70% acetone extract of *F. bengalensis* (aerial root), MFR – methanol extract of *F. racemosa* (stem bark), AFR – 70% acetone extract of *F. racemosa* (stem bark).

3.3. DPPH radical scavenging activity

The free radical scavenging activity of the methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) were determined by the DPPH method and the results are shown in Figs. 2–4. The DPPH radical has been widely used to test the ability of compounds as free-radical scavengers or hydrogen donors and to evaluate the antioxidative activity of plant extracts and foods (Porto, Calligaris, Celloti, & Nicoli, 2000; Soares, Dinis, Cunha, & Almeida, 1997). Percent DPPH radical scavenging activities of all the extracts were dose dependent. Similar to the reducing power, the amount

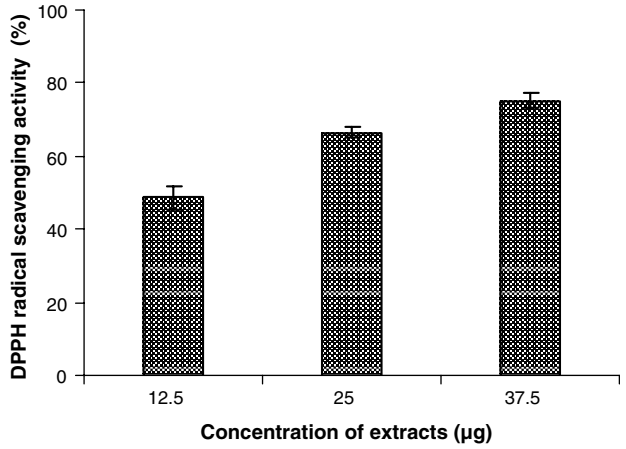

Fig. 2. DPPH free radical scavenging activity of methanol extract of green tea. Values are means of triplicate determinations ($n = 3$) ± standard deviation.

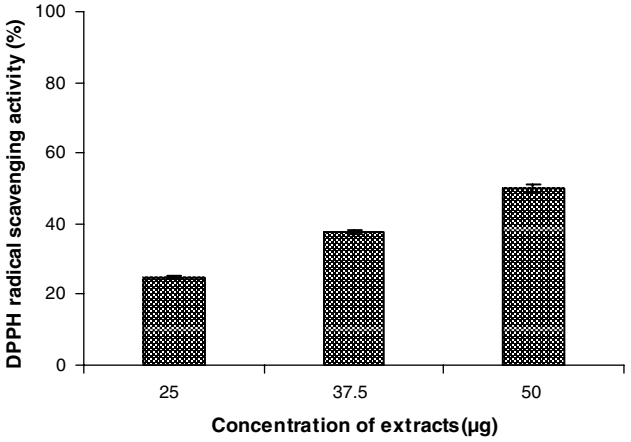

Fig. 3. DPPH free radical scavenging activity of 70% acetone extract of green tea. Values are means of triplicate determinations ($n = 3$) ± standard deviation.

of DPPH scavenging activity appeared to depend on the phenolic concentration of the extracts except methanolic extracts of *F. racemosa* sample. The highest DPPH scavenging activities were shown by methanol extracts of green tea and stem bark of *F. racemosa* (19.50 µg and 21.50 µg/50% DPPH scavenging activity under the experimental conditions, respectively) and these values were comparable to each other. In *F. bengalensis* sample, both solvent extracts on DPPH activity values were found to be nearer to each other (for 50% activity of DPPH, the required extracts concentration under the experimental condition were 39.3 µg and 46.79 µg, respectively). The strong DPPH scavenging activity of tea could be attributed in part to the tea catechins and some low molecular polyphenols (Zhu et al., 2002). In the present study, the order of scavenging activity of sample extracts is FRM > GTM > FRA > FBM > FBA > GTA. This radical scavenging activity of extracts could be related to the nature of phenolics, thus

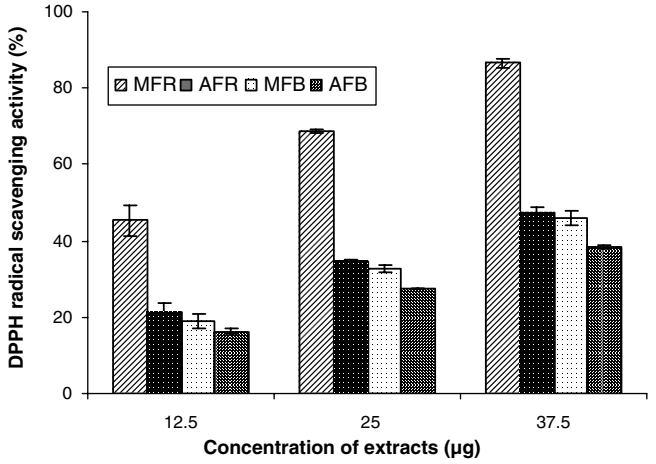

Fig. 4. DPPH radical scavenging activity of methanol and 70% acetone extracts of *F. bengalensis* (aerial root) and *F. racemosa* (stem bark). Values are means of triplicate determinations ($n = 3$) ± standard deviation. MFB – methanol extract of *F. bengalensis* (aerial root), AFB – 70% acetone extract of *F. bengalensis* (aerial root), MFR – methanol extract of *F. racemosa* (stem bark), AFR – 70% acetone extract of *F. racemosa* (stem bark).

contributing to their electron transfer/hydrogen donating ability.

3.4. ABTS radical cation scavenging activity

The effect of methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) on ABTS radical cation scavenging activity is presented in Table 2. In ABTS radical cation scavenging assay, 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. Even though the samples exhibited good ABTS radical scavenging activity, the 70% acetone extracts of green tea and 70% acetone extracts of *F. racemosa* stem bark, of all the samples, showed the highest TAA (13500 and 10885 µmol g^{-1}, respectively). However, the TAA values for methanol extracts of green tea and *F. racemosa* were 8077 and 8615 µmol g^{-1}, respectively. The lowest TAA values were obtained for both methanol and 70% acetone extracts of *F. bengalensis* sample (6096 and 6183 µmol g^{-1}, respectively). The extensive investigations on antiradical and antioxidant activities of small phenolics, including flavonoids and phenolic acids have been reported (Heim, Tagliaferro, & Bobilya, 2002). Apart from these, Hagerman et al. (1998) have reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^{\cdot+}$) and their effectiveness depends on the molecular weight, the number of aromatic rings and nature of hydroxyl group's substitution than the specific functional groups. Free radical ($ABTS^{\cdot+}$) scavenging activity of green tea, *F. racemosa* and *F. bengalensis* samples might be due to the presence of high molecular phenolics such as catechins, pelargonin

Table 2
ABTS radical cation scavenging activity, antihemolytic activity and metal chelating activity of methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark)

Sample	TAA* (μmol g^{-1} DM)	Antihemolytic activity (%)	Metal chelating activity (mg EDTA/g sample)
MGT	8076.9 ± 1364.0	40.1 ± 14.5	11.3 ± 1.8
AGT	13499.9 ± 1586.3	68.0 ± 0.0	22.3 ± 2.8
MFB	6096.1 ± 483.8	70.5 ± 1.2	7.4 ± 0.3
AFB	6182.7 ± 661.8	75.0 ± 0.9	19.9 ± 6.4
MFR	8615.3 ± 697.9	79.4 ± 5.8	14.7 ± 1.7
AFR	10884.6 ± 989.4	68.9 ± 5.5	17.9 ± 2.5

Values are means of triplicate determinations ($n = 3$) ± standard deviation.MGT – methanol extract of green tea, AGT – 70% acetone extract of green tea, MFB – methanol extract of *F. bengalensis* (aerial root), AFB – 70% acetone extract of *F. bengalensis* (aerial root), MFR – methanol extract of *F. racemosa* (stem bark), AFR – 70% acetone extract of *F. racemosa* (stem bark).
* Total antioxidant activity (μmol equivalent Trolox performed by using ABTS radical cation).

and leucopelargonin derivatives in addition to the flavonoids.

3.5. Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells (Hochestein & Atallah, 1988). This radical has the capacity to join nucleotides in DNA and cause strand breakage, which contributes to carcinogenesis, mutagenesis and cytotoxicity. In addition this species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids. The hydroxyl radical scavenging activity of the two different solvent extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) is shown in Table 3. In the present investigation, all the extracts of samples exhibited between 24.2% and 43.9% hydroxyl radical scavenging activity at 250 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 3
Hydroxyl radical scavenging activity of methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark)

Sample	Hydroxyl radical scavenging activity (%)			
	100 μg	150 μg	200 μg	250 μg
MGT	21.9 ± 2.2	29.5 ± 1.2	37.7 ± 3.4	44.0 ± 9.2
AGT	18.0 ± 4.1	23.6 ± 1.4	28.1 ± 1.2	35.3 ± 0.6
MFB	20.0 ± 8.0	25.4 ± 1.3	27.5 ± 2.5	32.4 ± 4.0
AFB	17.2 ± 1.7	19.1 ± 1.0	23.8 ± 1.7	24.2 ± 3.6
MFR	22.4 ± 2.6	22.2 ± 3.6	26.7 ± 2.3	29.0 ± 6.0
AFR	23.6 ± 1.2	29.3 ± 3.7	34.1 ± 3.4	37.2 ± 0.9

Values are means of triplicate determinations ($n = 3$) ± standard deviation. MGT – methanol extract of green tea, AGT – 70% acetone extract of green tea, MFB – methanol extract of *F. bengalensis* (aerial root), AFB – 70% acetone extract of *F. bengalensis* (aerial root), MFR – methanol extract of *F. racemosa* (stem bark), AFR- 70% acetone extract of *F. racemosa* (stem bark).

3.6. Antioxidant activity in linoleic acid emulsion system

The antioxidant activity in terms of peroxidation inhibition of both methanol and 70% acetone extracts of green tea, *F. racemosa* (stem bark) and *F. bengalensis* (aerial root) and α-tocopherol were investigated and the results are presented in Fig. 5. At a concentration of 250 μg in the final reaction mixture, all the extracts inhibited 34.3% to 38.3% peroxidation of linoleic acid after incubation for 48 h. Further, those values were comparable to that of the natural antioxidant, α-tocopherol (35.0%). Thus the results indicated that the equivalent antioxidant activity of *F. racemosa* and *F. bengalensis* extracts comparable with green tea extracts could open an avenue for exploitation of cost effective natural antioxidants, antidiabetic and hypo-

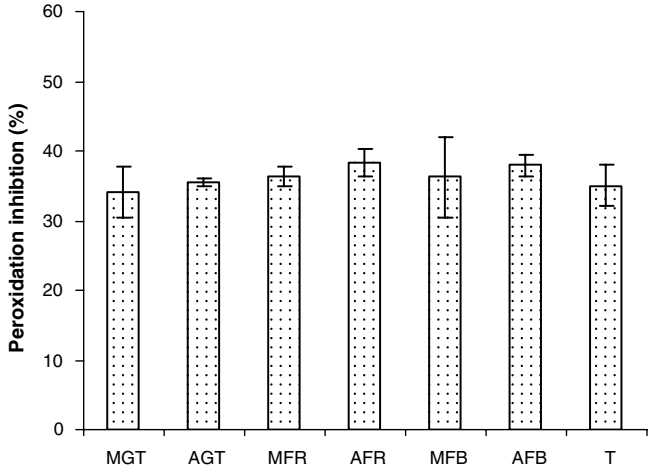

Fig. 5. Peroxidation inhibiting property of methanol and 70% acetone extracts of green tea, *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) at a concentration of 250 μg in the reaction mixture. MGT – methanol extract of green tea, AGT – 70% acetone extract of green tea, MFB-methanol extract of *F. bengalensis* (aerial root), AFB – 70% acetone extract of *F. bengalensis* (aerial root), MFR – methanol extract of *F. racemosa* (stem bark), AFR – 70% acetone extract of *F. racemosa* (stem bark), T-α Tocopherol.

lipidemic components. Peroxidation inhibiting activity was reported in various solvent extracts of different parts of *Cassia fistula* (Siddhuraju et al., 2002). The efficacy of green tea, *F. racemosa* and *F. bengalensis* extracts to inhibit oxidation 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 component, oil, water or lipid: air interfaces (Koleva, Van Beck, Linsen, de Groot, & Evstatieva, 2002).

3.7. Antihemolytic activity

Hemolysis has a long history of use in measuring free radical damage and its inhibition by antioxidants but only few studies have been performed with erythrocytes in whole blood. In this study, we used a biological test based on free radical-induced erythrocytes lysis in cow blood. This assay is useful either for screening studies on various molecules and their metabolites, especially on the one hand molecules having an oxidizing or antioxidizing activity and on the other hand molecule having a long-term action (Djeridane et al., 2006). Lipid oxidation of cow blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently hemolysis. All the extracts except methanol extracts of green tea (40.1%), showed antihemolytic activity in terms of percentage inhibiting activity ranging from 68.0% to 79.4% (Table 2). Zhu et al. (2002) reported that three fractions of Oolong tea were more effective against lipid oxidation in the erythrocytes membranes and fractions were also demonstrated to have dose-dependent inhibition effects toward RBC hemolysis. More over the RBC hemolysis is a more sensitive system for evaluating the antioxidant properties of the phytoceuticals. Similarly, a highly significant efficiency in inhibiting radical-induced red blood cell hemolysis was also observed for *Oudneyna africanan*, *Artemisia arboresens* and *Globularia alpyum* whose activities were nearly similar to caffic acid (Djeridane et al., 2006).

3.8. Metal ion chelating activity

An important mechanism of antioxidant activity is the ability to chelate/deactivate transition metals, which possess the ability to catalyze hydroperoxide decomposition and Fenton-type reactions. Therefore, it was considered of importance to screen the iron (II) chelating ability to the extracts. All the extracts demonstrated the ability to chelate irons (Table 2) and 70% acetone extracts of green tea (22.3 mg/g), *F. bengalensis* (aerial root) (19.9 mg/g) and *F. racemosa* (stem bark) (17.9 mg/g) were found to be higher than chelating activity of methanol extracts of respective samples (11.3, 7.4 and 14.7 mg/g extracts). From the iron (II) chelating data, the extracts may be able to play a protective role against oxidative damage by sequestering iron (II) ions that may otherwise catalyze Fenton-type reactions or participate in metal-catalyzed hydroperoxide decomposition reactions (Dorman, Kosar, Kahlos, Holm, & Hilturien, 2003). On the other hand the iron (II) chelating properties of the additives may be attributed to their endogenous chelating agents, mainly phenolics. Certain phenolic compounds have properly oriented functional groups, which can chelate metal ions (Thompson, Williams, & Elliot, 1976). Chelating agents may serve as secondary antioxidants because they reduce the redox potential thereby stabilizing the oxidized form of the metal ions. Accordingly it is suggested that the low to moderate ferrous ions chelating effects of these fractions would be somewhat beneficial to protect against oxidative damage.

The present investigation suggests that not only the phenolic constituents from green tea but also the substances from the tree species such as *F. bengalensis* (aerial root) and *F. racemosa* (stem bark) possess potential antioxidant activity. Further isolation and preparation of bioactive compounds from the above mentioned *Ficus* samples and their impact on various health improvements/control of free radical mediated diseases through *in vivo* studies are needed. Such identified potential and natural constituents could be exploited as cost effective food/feed additives for human and animal health.

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