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Full Length Research Paper

Evaluation of *in vitro* antioxidant activity of bark extracts of *Terminalia arjuna*

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To uncover the antioxidant and free radical scavenging activity, five different extracts of *Terminalia arjuna* bark were examined. In the present study, the free radical scavenging potential of five extracts of the bark of *Terminalia arjuna* was assessed by measuring its capability for scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, hydrogen peroxide radical, nitric oxide radicals (NO), as well as its ability in reducing power capacity assessment, cupric reducing antioxidant capacity, using appropriate assay systems compared to natural and synthetic antioxidants. Total antioxidant capacity, phenolic and flavonoid contents were determined spectrophotometrically. In DPPH free radical scavenging activity, the highest IC_{50} value was showed by methanol extract with a value of 6.34 µg/ml followed by ethanol and petroleum ether having value of 7.76 and 25.63, respectively, as opposed to that of the scavenging effects of ascorbic acid and butylated hydroxytoluene (BHT) of 5.698 and 8.816, respectively. Methanol extract showed highest activity having IC_{50} value of 14.436 and 25.184 µg/ml in hydrogen peroxide and nitric oxide scavenging assay, respectively. All the five fractions showed good reducing power and cupric reducing capacity with increasing concentration again taking methanol extract to the top position. The methanol extract yielded 817.488 ± 8.108 mg/g gallic acid equivalent phenolic content and 199.122 ± 8.282 mg/g Quercetin equivalent flavonoid content that was highest among five extracts. Methanol extract of *T. arjuna* was found to possess the highest total antioxidant capacity (415.925 ± 2.291) followed by ethanol (377.675 ± 1.889) mg/g Ascorbic Acid Equivalent, respectively. A linear correlation appeared between the total antioxidant capacity and the total phenolic contents of the extracts with good correlation coefficient (R^2 = 0.891). n-Hexane and chloroform extract showed least activity in all the measures. The results obtained beacon that *T. arjuna* is a potential source of antioxidants and thus could prevent many radical related diseases.

Key words: *Terminalia arjuna,* n-hexane extract, petroleum ether extract, chloroform extract, ethanol extract, methanol extract, *in vitro* antioxidant activity.

INTRODUCTION

Terminalia arjuna (Bengali name: Arjun gachh, English name: Arjuna myrobalam) from Combretaceae family is a large tree which is found throughout the South Asian region. This tree is usually an evergreen tree with new leaves appearing in the hot season (February to April) before leaf fall. This tree is an exotic tree in Bangladesh. It is one of the most versatile medicinal plants having a wide spectrum of biological activity. *T. arjuna* is an important cardiotonic plant described in the Ayurveda (Tripathi et al., 1996). Bark of *T. arjuna* contains phenols, flavonoids, tannin, saponin, alkaloids, glycosides, phytosterols and carbohydrate (Ghani, 2003).

Epidemiological studies have shown that foods rich in vitamins provide protection against degenerative diseases including cancer, coronary heart disease and even Alzheimers' disease (Ames et al., 1993). Literature survey revealed that the plants which containing antioxidants like vitamin C, vitamin E, carotenes, polyphenols, and many other compounds reduce these disease risks.

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Most of the antioxidant compounds in a typical balanced diet are derived from plant sources with a wide variety of biological and chemical properties (Scalbert et al., 2005). Synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as food additives; but recent reports have expressed safety concerns allowing natural antioxidant to be the focus of intense interest (Sun and Ho, 2005; Wilson, 1999). Plants are rich sources for natural antioxidants, the best known are tocopherols, flavonoids, vitamin C and other phenolic compounds (Landrault et al., 2001). Other contributors to the antioxidant activity include alkaloids, proteins, minerals and other vitamins such as the carotenoids and vitamin B_6, B_{12} and K (Smolin and Grosvenor, 2007). Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide of lipid hydroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Subramanion et al., 2011).There are a number of clinical studies confirming the powerful anti-cancerous and anti heart disease properties of polyphenols (Prakash et al., 2007; Bajpai et al., 2005; Siddhuraju and Becker, 2003).

In oxidation process, highly reactive and harmful chain reactions of oxygen species are generated, which cause damage to living organism. The oxygen centered free radicals and other reactive oxygen species, which are continuously produced, resulted in cell death or tissue damage. This oxidative damage caused by free radical is related to pathogenesis of many chronic degenerative diseases like cancer, diabetes, neurodegenerative disease, atherosclerosis, cirrhosis, malaria and AIDS (Azizova et al., 2002; Sian et al., 2003; Quintero et al., 2006; Nagler et al., 2006). Reactive oxygen species (ROS), including superoxide free radical, hydrogen peroxide, hydroxyl free radical and singlet oxygen play a key role in the oxidative damage of these diseases (Vertuani et al., 2004).

Antioxidant is a molecule, which terminate the chain reaction by removing free radical intermediates. Plants and animals maintain complex system of multiple type of antioxidant; the natural plant based antioxidants are playing an important role in the maintenance of human health for the past three decades (Devasagayam et al., 2004). There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources (Abdalla and Roozen, 1999). There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants (Sa'nchez-Moreno, 2002). Therefore, the present study was undertaken to evaluate and compare the antioxidative activities of different solvent extracts of *T. arjuna* in different methods.

MATERIALS AND METHODS

Plant material

Plant sample of *T. arjuna* was collected from Savar in July 2011 and a plant sample was submitted to The National Herbarium, Mirpur for identification (DACB; Accession number 35904; dated on 14.09.2011; Sadika Akter, no.SA-01).

Sample preparation

Barks were sun dried for four days and then dried in oven at 40°C for about three days. The dried barks were then ground in coarse powder using high capacity grinding machine which was then stored in air-tight container with necessary markings for identification and kept in cool, dark and dry place for the investigation. The powdered plant material was successively extracted in a Soxhlet extractor at elevated temperature using n-hexane which was followed by petroleum ether, chloroform, ethanol and methanol. All extracts were filtered individually through filter paper and poured on Petri dishes to evaporate the liquid solvents from the extract to get dry extracts. After drying, crude extracts were stored in stock vials and kept in refrigerator for further use.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The free radical scavenging capacity of the extracts was determined using DPPH (Braca et al., 2001). Freshly prepared DPPH solution was taken in test tubes and extracts were added followed by serial dilutions (15.625 to 250 µg/ml) to every test tube so that the final volume was 5 ml and after 30 min, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid and BHT was used as standard. Control sample was prepared containing the same volume without any extract and standard, and the absorbance was read at 517 nm using a spectrophotometer. Methanol was served as blank. % inhibition of the DPPH free radical was measured using the following equation:

$$\%\ inhibition = (1 - \frac{A_1}{A_0}) \times 100$$

Where, A_I = Absorbance of the extract or standard and A_0 = Absorbance of the control.

Scavenging of hydrogen peroxide

Scavenging activity of extract and its sub-fractions were evaluated by hydrogen peroxide (Jayaprakasha et al., 2004). 1 ml of various concentrations of the extract, sub-fractions and standards in ethanol was added to 2 ml of hydrogen peroxide solution in phosphate buffered saline (PBS, pH 7.4). Then finally the absorbance was measured at 230 nm after 10 min. Ascorbic acid and BHT were used as standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 230 nm using a spectrophotometer. The percentage inhibition was calculated according to the following equation:

$$\%\ inhibition = (1 - \frac{A_1}{A_0}) \times 100$$

Where, A_o = absorbance of the control and A_1 = absorbance in the presence of the sample of extract and standard.

Nitric oxide scavenging capacity assay

Nitric oxide scavenging assay was carried out using sodium nitroprusside (Sreejayan and Rao, 1997). This can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract/sub-fraction at various concentrations and the mixture was incubated at 25°C for 150 min. From the incubated mixture, 0.5 ml was taken out and added into 1.0 ml sulphanilamide solution (0.33% in 20% glacial acetic acid) and further incubated at room temperature for 5 min. Finally, 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and maintained at room temperature for 30 min. The absorbance was measured at 546 nm. A typical blank/control solution contained the same solution mixture without plant extract or standard. The absorbance of the blank/control solution was measured at 546 nm. The percentage inhibition was calculated according to the following equation:

$$\% \, inhibition = (1 - \frac{A_1}{A_0}) \times 100$$

Where, A_1 = Absorbance of the extract or standard and A_0 = Absorbance of the control.

Reducing power capacity assessment

Assay of reducing power was carried out by potassium ferricyanide method (Yildirim et al., 2001). 1 ml of extract and its sub-fractions (final concentration 5 to 200 µg/ml) were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide. The mixture was then incubated at 50°C for 20 min. To this mixture, 2.5 ml of trichloroacetic acid was added, which was then centrifuged at 3000 rpm for 30 min. Finally, 2.5 ml of the supernatant solution was collected and mixed with 2.5 ml of distilled water and 0.5 ml ferric chloride and absorbance was measured at 700 nm. Ascorbic acid and butylated hydroxy toluene (BHT) were used as standard and phosphate buffer as blank solution.

Cupric reducing antioxidant capacity (CUPRAC)

The assay was conducted as described previously by Resat et al. (2004). 0.5 ml of plant extract or standard of different concentrations solution, 1 ml of copper (II) chloride solution (0.01 M prepared from $CuCl_2.2H_2O$), 1 ml of ammonium acetate buffer at pH 7.0 and 1 ml of neocaproin solution (0.0075 M) were mixed. The final volume of the mixture was adjusted to 4.1 ml by adding 0.6 ml of distilled water and the total mixture was incubated for 1 h at room temperature.

Then the absorbance of the solution was measured at 450 nm using a spectrophotometer against blank. Ascorbic acid, BHT and BHA were used as a standard.

Determination of total antioxidant capacity

The total antioxidant capacity was evaluated by the phosphomolybdenum method (Prieto et al., 1999). 0.3 ml of extract and sub-fraction in ethanol, ascorbic acid used as standard (5 to 200 µg/ml) and blank (ethanol) were combined with 3 ml of reagent mixture separately and incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following equation:

$$A = (c \times V)/m$$

Where, A = total content of antioxidant compounds, mg/g plant extract, in Ascorbic Acid Equivalent, c = the concentration of Ascorbic acid established from the calibration curve, mg/ml, V = the volume of extract (ml), and m = the weight of crude plant extract (g).

Determination of total phenolic content

Total phenolic contents in the extracts were determined by the Folin-Ciocalteu reagent method described by Demiray et al. (2009). 1 ml of the plant extracts/standard of different concentration solution was mixed with 5 ml Folin-Ciocalteu reagent (previously diluted with water 1:10 v/v) and 4 ml (7.5% sodium carbonate) of sodium carbonate. The tubes were vortexed for few seconds and allowed to stand for 30 min at 20°C for color development. Absorbance of samples and standard were measured at 765 nm using spectrophotometer against blank. A typical blank solution contained the solvent used to dissolve the plant extract. The total content of phenolic compounds plant extracts in gallic acid equivalents (GAE) was calculated using the following equation:

$$C = (c \times V)/m$$

Where C = total content of phenolic compounds, mg/g plant extract, in GAE, c = the concentration of gallic acid established from the calibration curve (mg/ml), V = the volume of extract in ml, and m = the weight of crude plant extract in g.

Determination of total flavonoids content

Aluminum chloride colorimetric method was used for flavonoids determination (Wang and Jiao, 2000). 1 ml of the plant extracts/standard of different concentration solution was mixed with 3 ml of methanol, 0.2 ml of aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with spectrophotometer against blank. Methanol served as blank. The total content of flavonoid compounds in plant methanol extracts in quercetin equivalents was calculated by the following equation:

$$C = (c \times V)/m$$

Where C = total content of flavonoid compounds, mg/g plant extract, in quercetin equivalent, c = the concentration of quercetin established from the calibration curve in mg/ml, V = the volume of extract in ml, and m = the weight of crude plant extract in g.

RESULTS AND DISCUSSION

DPPH free radical scavenging assay

The free radical scavenging activity of different extracts of *T. arjuna* was studied by its ability to reduce the DPPH, a stable free radical and any molecule that can donate an electron or hydrogen to DPPH can react with it and thereby bleach the DPPH absorption. DPPH is a purple colour dye having absorption maximal of 517 nm and upon reaction with a hydrogen donor the purple colour fades or disappears due to conversion of it to 2, 2-diphenyl-1-picryl hydrazine resulting in decrease in

Table 1. IC_{50} values of different extracts of *Terminalia arjuna* in DPPH scavenging assay.

Extracts/standard	IC_{50} (µg/ml)
Methanol	6.34
Ethanol	7.76
Pet-ether	25.63
n-hexane	6828.229
Chloroform	61.890
Ascorbic acid	5.698
Butylated hydroxytoluene (BHT)	8.816

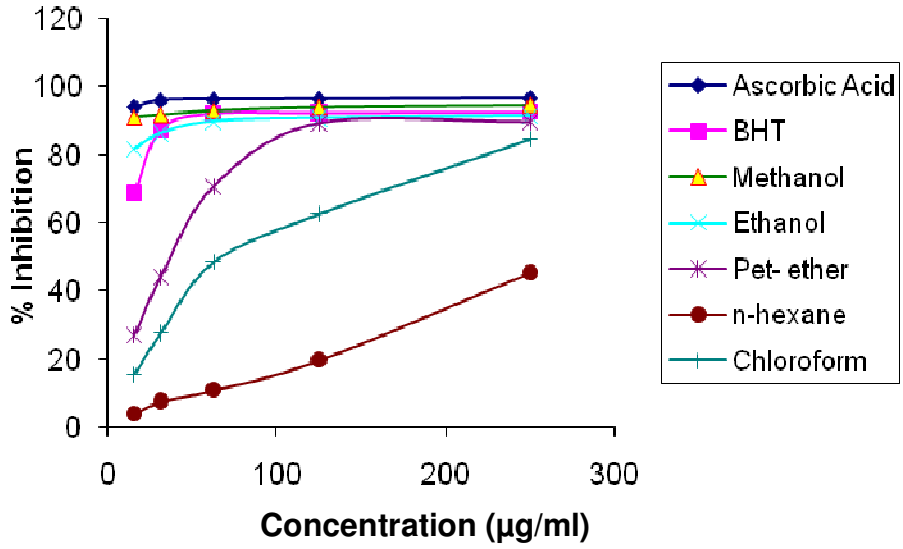

Figure 1. Comparative DPPH radical scavenging activity of *Terminalia arjuna* bark extracts, ascorbic acid and butylated hydroxytoluene (BHT).

absorbance. The methanol and ethanol extracts showed maximum activity of 94.72 and 91.48%, respectively at 250 µg/ml, whereas ascorbic acid and BHT at the same concentration exhibited 96.66 and 92.59% inhibition respectively. Five extracts exhibited considerable DPPH free radical scavenging activity as indicated by their IC_{50} values are shown in Table 1 and Figure 1. IC_{50} indicates the potency of scavenging activity. Standard ascorbic acid and BHT were found to have an IC_{50} of 5.698 and 8.816 µg/ml. In comparison to standard ascorbic acid and BHT, methanol, ethanol, petroleum ether and chloroform extract of *T. arjuna* bark showed IC_{50} of 6.34, 7.76, 25.63 and 61.89, respectively. n-hexane fraction is seen to have the least free radical scavenging activity.

The DPPH test showed the ability of the test compound to act as a free radical scavenger. DPPH assay method is based on the ability of 1, 1-diphenyl-2-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants (Kumarasamy et al., 2007). DPPH, a protonated radical, has characteristic absorbance maximal at 517 nm, which decreases with the scavenging of the proton radical. This property has been widely used to evaluate the free radical scavenging effect of natural antioxidants (Jao and Ko, 2002). The stable free radical DPPH has been widely used to test the free radical-scavenging ability of various dietary antioxidants (Brand--Williams et al., 1995). Because of its odd electron, DPPH gives a strong absorption band at 517 nm in visible spectroscopy. As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes, and the resulting decolourization is stoichiometric with respect to the number of electrons taken up. The bark extracts showed considerable radical scavenging activity in a concentration-dependent manner. Methanol (6.34), ethanol (7.76), petroleum ether (25.63) and chloroform (61.89) extract of the dried bark exhibited a good potential to act as a free radical scavenger with IC_{50} for DPPH inhibition comparable to

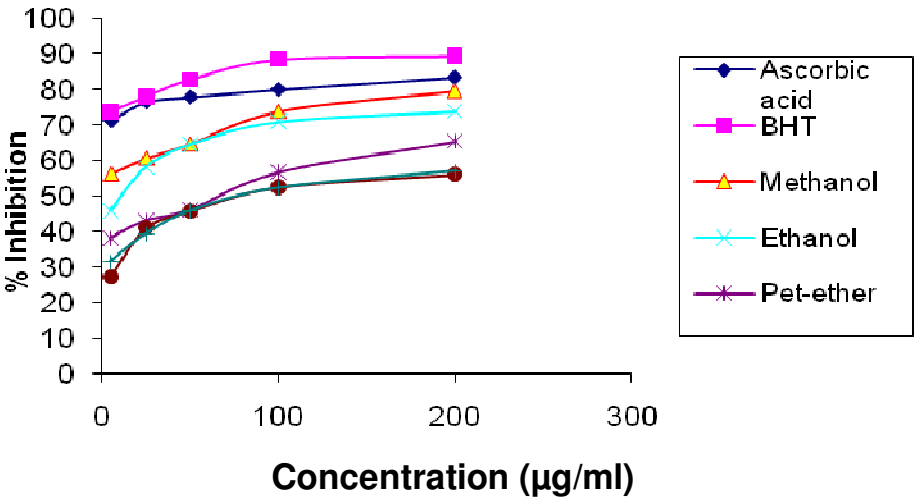

Figure 2. Comparative H_2O_2 scavenging activity of *Terminalia arjuna* bark extracts, ascorbic acid and butylated hydroxytoluene (BHT).

Table 2. IC_{50} values of different extracts of *Terminalia arjuna* in H_2O_2 scavenging assay.

Extracts/standard	IC_{50} µg/ml
Methanol	14.436
Ethanol	18.867
Pet-ether	48.706
n-hexane	78.429
Chloroform	75.907
Ascorbic acid	7.529
Butylated hydroxytoluene (BHT)	6.387

that of ascorbic acid (5.698 µg/ml) and BHT (8.816 µg/ml) which are known free radical scavengers. Figure 1 exhibits the comparative % inhibition among *T. arjuna* bark extracts and standard compounds (ascorbic acid and butylated hydroxytoluene). The highest scavenging effect was showed by methanol extract with a value of 6.34 µg/ml followed by ethanol and petroleum ether having value of 7.76 and 25.63, respectively as opposed to that of the scavenging effects of ascorbic acid and BHT of 5.698 and 8.816, respectively. These results indicated that extract has a noticeable effect on scavenging the free radicals. In fact, IC_{50} value of methanol and ethanol extract appeared to have slightly better than standard BHT at 50% inhibition. However, a maximum inhibition was achieved at a higher concentration of 250 µg/ml compared to 250 µg/ml for both of ascorbic acid and BHT respectively.

Scavenging of hydrogen peroxide

Hydrogen peroxide, although not a radical species play a role to contribute oxidative stress. The generation of even low levels of H_2O_2 in biological systems may be important. Naturally-occurring iron complexes inside the cell believed to react with H_2O_2 *in vivo* to generate highly reactive hydroxyl radicals and this may be the origin of many of its toxic effects (Miller et al., 2000). Scavenging of hydrogen peroxide of different extracts of *T. arjuna* bark is presented in Figure 2. All five extracts compared with standard ascorbic acid IC_{50} value of 7.529 µg/ml and BHT IC_{50} value of 6.387 µg/ml along with methanol and ethanol in highest position in depleting H_2O_2, with an IC_{50} value of 14.436 and 18.867 µg/ml followed by petroleum ether 48.706 µg/ml, respectively (Table 2).

The percentage of H_2O_2 scavenging activity of methanol extract was found to be 79.550% which is highest among five extract at 200 µg/ml compared to antioxidant activity of standard ascorbic acid and BHT was 83.538 and 89.570%, respectively at the same concentration. Ethanol and petroleum ether extract also showed significant activity with a scavenging value of 73.824 and 65.235%, respectively (Figure 2).

Hydrogen peroxide itself is not very reactive, but can sometimes be toxic to cell because it may give rise to hydroxyl radical in the cells (Halliwell et al., 1987). Thus, removal of H_2O_2 is very important for protection of food systems. Scavenging of H_2O_2 by extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. The IC_{50} values were found to be 14.436, 18.867 and 48.706 µg/ml for methanol, ethanol and petroleum ether, in comparison with 7.529 and 6.387 µg/ml for ascorbic acid and BHT, respectively. Chloroform and n-hexane extract showed moderate scavenging activity with an IC_{50} value of 75.907 and 78.429 µg/ml, respectively.

Table 3. IC_{50} values of different extracts of *Terminalia arjuna* bark in Nitric oxide scavenging assay.

Extracts/standard	IC_{50} µg/ml
Methanol	25.184
Ethanol	25.420
Pet-ether	57.141
n-hexane	903.859
Chloroform	181.815
Ascorbic acid	27.685
Butylated hydroxytoluene (BHT)	27.294

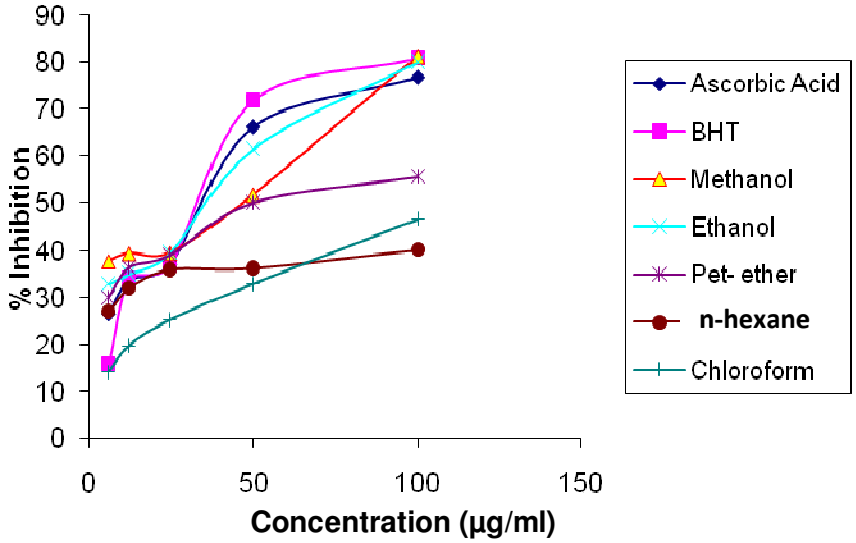

Figure 3. Comparative nitric oxide scavenging activity of *Terminalia arjuna* bark extracts, ascorbic acid and butylated hydroxytoluene (BHT).

Nitric oxide (NO) radical scavenging assay

NO is a very unstable species and reacting with oxygen molecule produce stable nitrate and nitrite which can be estimated by using Griess reagent. In the presence of a scavenging test compound, the amount of nitrous acid will decrease which can be measured at 546 nm. Methanol and ethanol extract of *T. arjuna* bark has potent nitric oxide scavenging activity (IC_{50} value 25.184 and 25.420 µg/ml, respectively) and n-hexane fraction has showed the least nitric oxide scavenging activity (IC_{50} value 903.859 µg/ml) (Table 3).

NO is a potent pleiotropic inhibitor of physiological processses such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodi-latation and antimicrobial and anti-tumor activities (Hagerman et al., 1998). Suppression of released NO may be partially attributed to direct NO scavenging, as the extracts of *T. arjuna* bark decreased the amount of nitrite generated from the decomposition of SNP *in vitro.* The scavenging of NO by the extracts was increased in dose dependent manner. Figure 3 illustrates a decrease in the NO radical due to the scavenging ability of extracts such as percent inhibition of methanol > ethanol > pet-ether > n-hexane > chloroform. The methanol and ethanol extracts showed maximum activity of 81.08 and 79.88%, respectively at 100 µg/ml, whereas ascorbic acid and BHT at the same concentration exhibited 76.65 and 80.84% inhibition, respectively. The IC_{50} values were found to be 25.184, 25.420, 57.141, 27.685 and 27.294 µg/ml for methanol, ethanol, petroleum ether, ascorbic acid and BHT, respectively. In fact, IC_{50} value of methanol (25.184) and ethanol (25.420) extract appeared to be slightly better than standard ascorbic acid (27.685) and BHT (27.294) at 50% inhibition. However, a maximum inhibition was achieved at a higher concentration of 100 µg/ml compared to 100 µg/ml for both ascorbic acid and

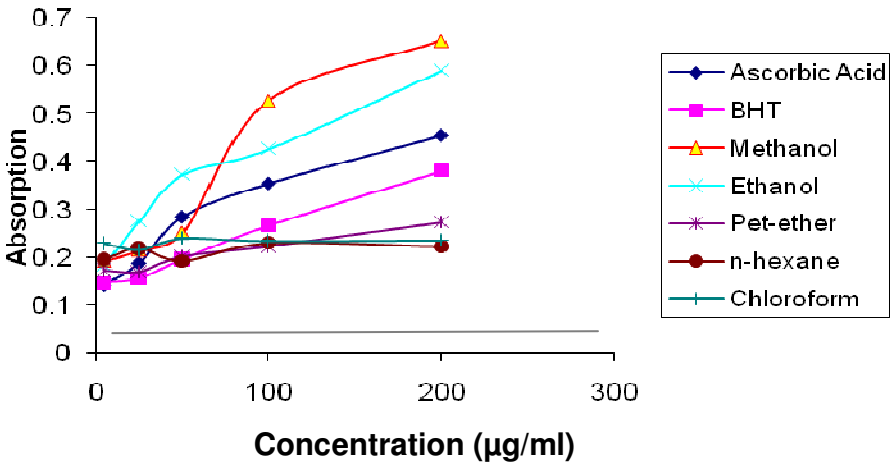

Figure 4. Comparative reducing power of *Terminalia arjuna* bark extracts, ascorbic acid and butylated hydroxytoluene (BHT).

BHT, respectively. Hence, a higher concentration would be required to achieve maximal and a significantly higher content of phenolic compounds compared to chloroform extract and n-hexane extract. NO has shown to play a crucial role in various physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical, which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities (Hagerman et al., 1998). However, excess production of NO is associated with several diseases (Lalenti et al., 1992).

Reducing power capacity assessment

Reducing power of the fractions was assessed using ferric to ferrous reducing activity as determined sectrophotometrically from the formation of Perl's Prussian blue colour complex (Yildirim et al., 2001). Reducing power of different extracts of *T. arjuna* was compared with ascorbic acid and BHT (Figure 4). Among the extracts, methanol and ethanol extract exhibited the most reducing power which was even more than ascorbic acid and BHT. This result indicates that the extracts may consist of polyphenolic compounds that usually show great reducing power. This has been justified by methanol and ethanol extract being the most reducing agent with highest phenolic content (Table 5).

Cupric reducing antioxidant capacity (CUPRAC)

Reduction of Cu^{2+} ion to Cu^{+} was found to rise with increasing concentrations of the different extracts. The standard BHA and BHT showed highest reducing capacity. Among the extracts, methanol and ethanol extracts of *T. arjuna* showed maximum reducing capacity that is comparable to BHA and BHT. But the reducing capacity of methanol and ethanol extracts was more than ascorbic acid (Figure 5).

This method is based on the principle of increase in the absorbance of the reaction mixtures. Increase in the absorbance indicates increase in the antioxidant activity. Increase in absorbance of the reaction mixture indicates the reducing power of the samples (Jayaprakasha et al., 2001). Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity (Oktay et al., 2003). Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Yen and Chen, 1995).

Determination of total antioxidant capacity

Total antioxidant capacity of the different extracts of *T. arjuna* was evaluated by the phosphomolybdenum method and was expressed as ascorbic acid equivalents (AAE) per gram of plant extract. Total antioxidant capacity of the test samples was calculated using the standard curve of ascorbic acid ($y = 0.002x + 0.001$; $R^2 = 0.997$) (Figure 6). Methanol extract of *T. arjuna* was found to possess the highest total antioxidant capacity (Table 4). Total antioxidant capacity of the extracts was found to decrease in the following order: Methanol extract > ethanol extract > petroleum ether extract > chloroform extract > n-hexane extract (Table 4) and a comparative study is shown in Figure 7.

The total antioxidant activity of different extracts of *T. arjuna* was estimated from their ability to reduce Phosphate/Mo (VI) complex to Phosphate/Mo (V). The

Table 4. Total antioxidant capacity of the different extracts of *Terminalia arjuna.*

Extract	Total antioxidant capacity (mg/g, AAE)
Methanol	415.925 ± 2.291
Ethanol	377.675 ± 1.889
Petroleum ether	71.175 ± 16.225
Chloroform	67.675 ± 5.728
n-hexane	47.425 ± 5.826

Values are the mean of duplicate experiments and represented as mean ± SD.

Table 5. Total phenol contents of the different extracts of *Terminalia arjuna.*

Extract	Total phenol contents (mg/g, GAE)
Methanol	817.488 ± 8.108
Ethanol	683.174 ± 19.669
Petroleum ether	86.115 ± 10.427
Chloroform	73.002 ± 10.037
n-hexane	61.728 ± 10.109

Values are the mean of duplicate experiments and represented as mean ± SD.

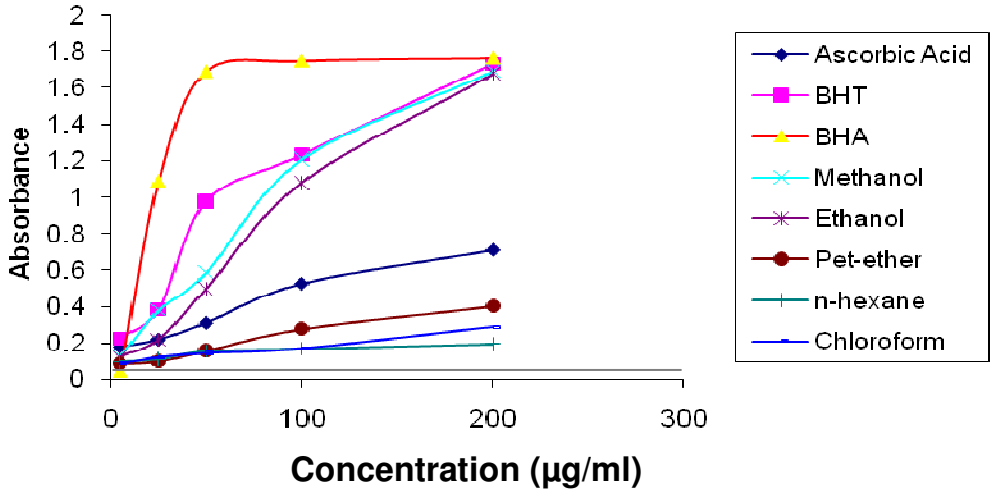

Figure 5. Comparative cupric reducing antioxidant capacity of *Terminalia arjuna* bark extracts, ascorbic acid, BHT and BHA.

assay has been successfully used to quantify vitamin E in seeds; and being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extracts (Prieto et al., 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. According to recent reports, a highly positive relationship between total phenols and antioxidant activity appears to be the trend in many plant species (Oktay et al., 2003). The statement has been justified in the current study where the methanol extract of *T. arjuna* showed maximum total antioxidant capacity (in term of ascorbic acid equivalent) (Table 4) with maximum phenol content (Table 5).

Determination of total phenolic content

Total phenolic content of the different extracts of *T. arjuna* was determined by using the Folin-Ciocalteu reagent and were expressed as GAE per gram of plant extract. The total phenolic contents of the test fractions

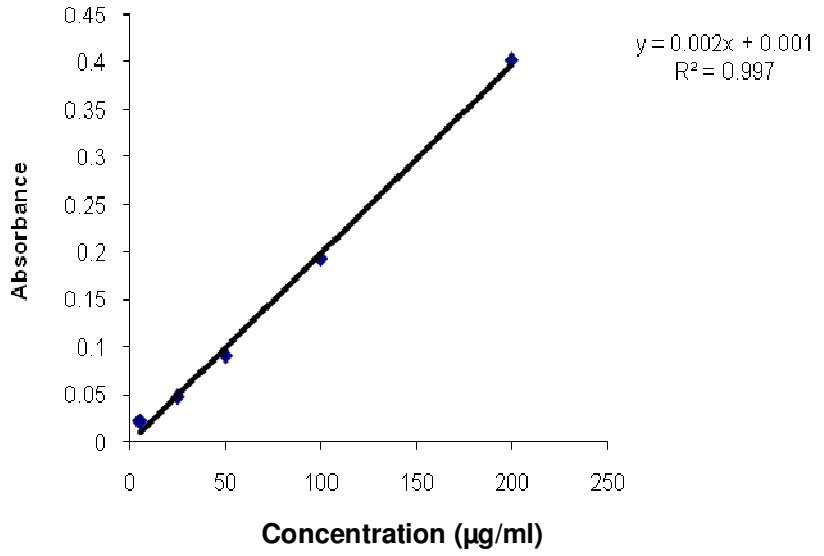

Figure 6. Calibration curve of ascorbic acid.

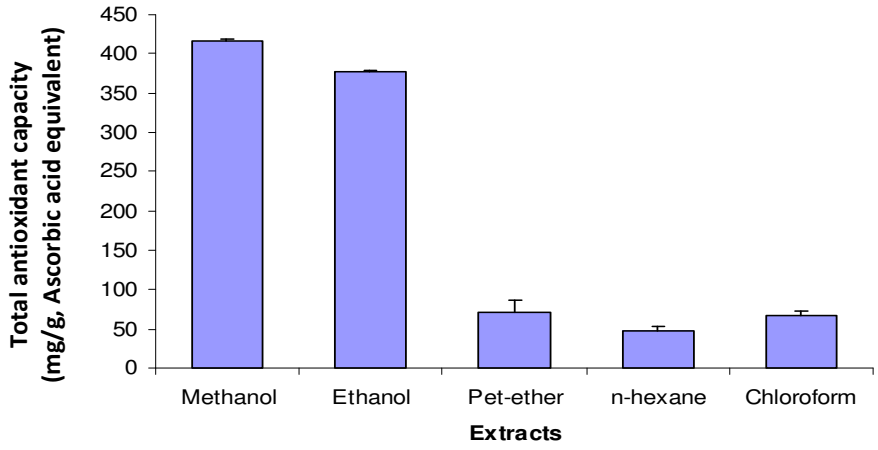

Figure 7. Total antioxidant capacity of the different extracts of *Terminalia arjuna*.

were calculated using the standard curve of gallic acid (y = 0.0136x + 0.0371; R^2 = 0.9948) (Figure 8). Methanol extract of *T. arjuna* was found to contain the highest amount of phenols (Table 5) and a comparative study is shown in Figure 9. Phenol contents of the extracts were found to decrease in the following order: methanol extract > ethanol extract > petroleum ether extract > chloroform extract > n-hexane extract (Table 5).

Phenolic, ubiquitous to the plant kingdom are composed of several classes of compounds including flavonoids (flavones, isoflavones and flavonones), anthocyanins and catechins. They possess an ideal structural chemistry for free radical scavenging activity. Antioxidative properties of polyphenols arise from their high reactivity as hydrogen or electron donors which can stabilize and delocalize the unpaired electron (chain-breaking function) and from their potential to chelate metal ions (termination of the Fenton reaction) (Rice-Evans et al., 1997). The results strongly suggest that phenolics are important components of the tested plant extracts. Among the extracts, the methanol extract of *T. arjuna* showed most potency in phenolic content determination assay (Table 5) and although a crude extract, it possesses considerable amount of gallic acid equivalent substances (polyphenolic compounds).

Relationship between the total antioxidant capacity and the total phenolic content

The extent of antioxidant capacity of extract of *T. arjuna* was correlated with their total phenolic contents. The

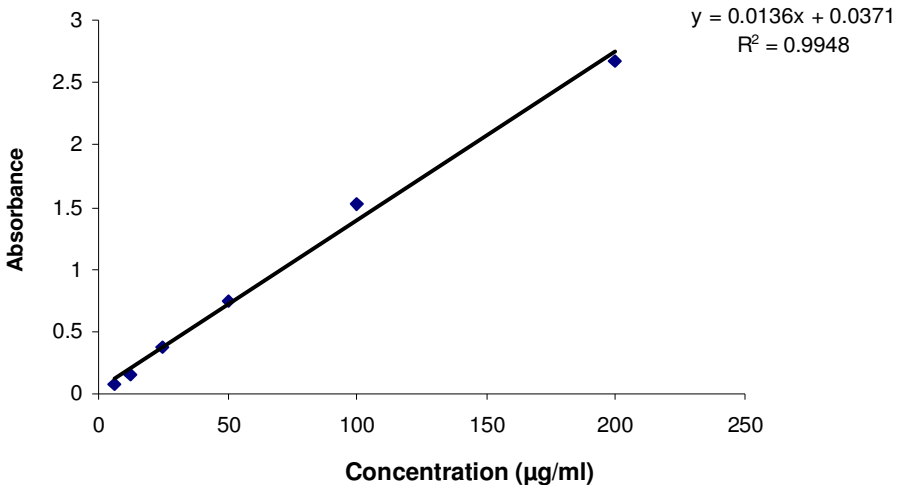

Figure 8. Calibration curve of gallic acid.

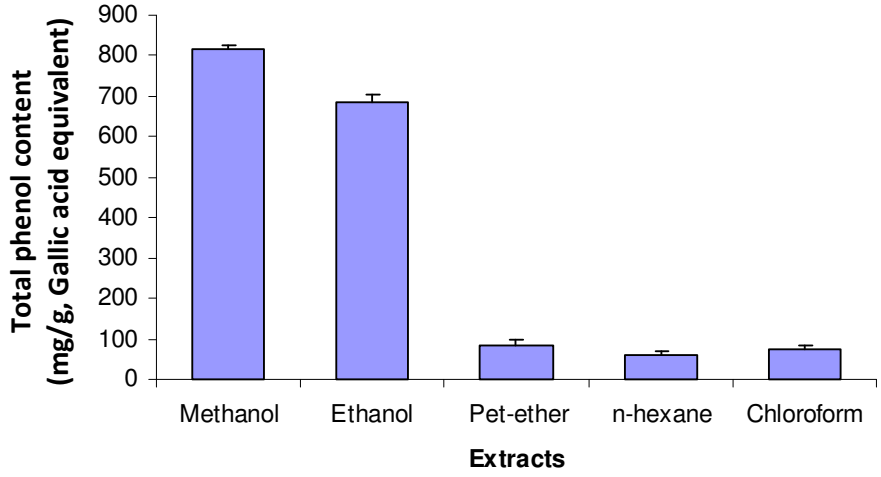

Figure 9. Total phenol contents of the different extracts of *Terminalia arjuna*.

correlation coefficient between the total antioxidant capacity monitored by phosphomolybdenum method and the total phenolics contents of extracts were determined (Figure 10). A linear correlation appeared between the total antioxidant capacity and the total phenolic contents of the extract and fractions with good correlation coefficient (R^2 = 0.995). The results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the *T. arjuna* extracts.

Determination of total flavonoids content

Aluminium chloride colorimetric method was used to determine the total flavonoid contents of the different extracts of *T. arjuna.* Total flavonoid contents was calculated using the standard curve of quercetin (y = 0.0149x - 0.0536; R^2 = 0.9975) (Figure 11) and was expressed as quercetin equivalents (QE) per gram of the plant extract. Methanol extract of *T. arjuna* was found to contain the highest amount of flavonoid (Table 6) and a comparative study is shown in Figure 12. Flavonoid contents of the extracts were found to decrease in the following order: methanol extract > ethanol extract > petroleum ether extract > chloroform extract > n-hexane extract (Table 6).

Flavonoids play an important role in antioxidant system in plants. The antioxidative properties of flavonoids are due to several different mechanisms, such as scavenging of free radicals, chelation of metal ions, such as iron and copper and inhibition of enzymes responsible for free radical generation (Benavente-Garcia et al., 1997). Depending on their structure, flavonoids are able to scavenge practically all known ROS. The methanol extract of *T. arjuna* has been shown to possess the highest flavonoids (Table 6). According to our study, the high contents of these phytochemicals in *T. arjuna* can explain its high radical scavenging activity.

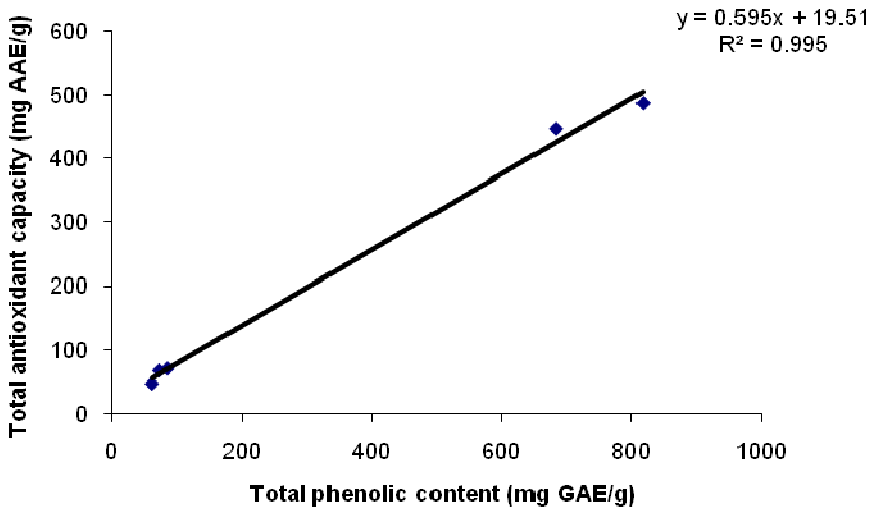

Figure 10. Correlation between the total antioxidant capacity and the total phenolic contents of *Terminalia arjuna* extracts.

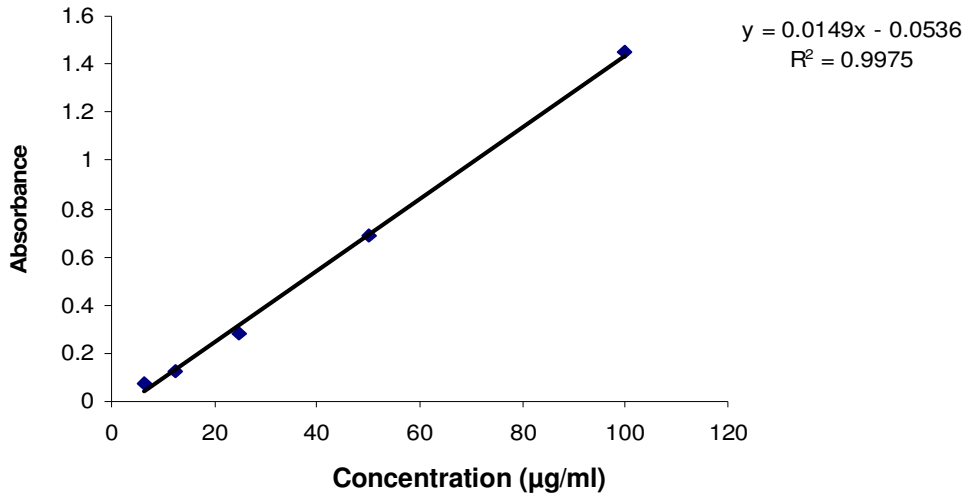

Figure 11. Calibration curve of quercetin.

Table 6. Total flavonoid contents of the different extracts of *Terminalia arjuna*.

Extract	Total flavonoid contents (mg/g, Quercetin equivalent)
Methanol	199.122 ± 8.282
Ethanol	162.072 ± 2.873
Petroleum ether	63.986 ± 8.282
Chloroform	60.721 ± 2.873
n-hexane	51.036 ± 1.921

Values are the mean of duplicate experiments and represented as mean ± SD.

Conclusion

Based on the results of the present study, it can be suggested that the extracts of *T. arjuna* possess antioxidant effects. Almost all extracts exhibited potential antioxidant activity. Methanol and ethanol extracts showed

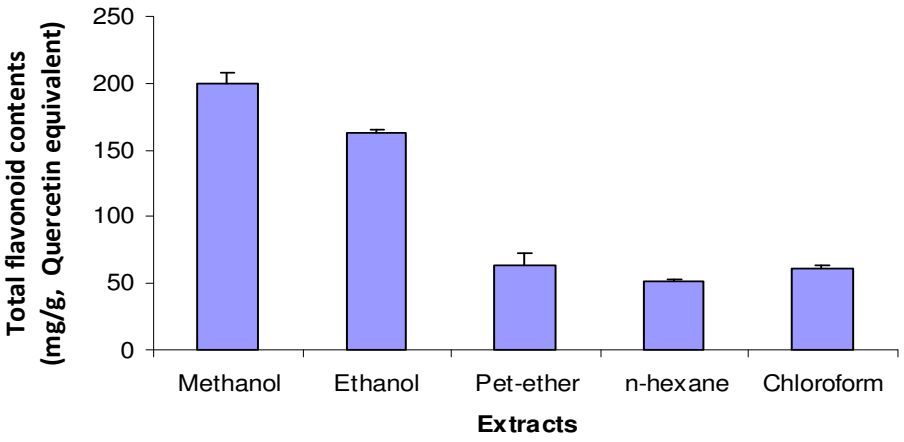

Figure 12. Total flavonoid contents of the different extracts of *Terminalia arjuna*.

highest antioxidant activity.

REFERENCES

Abdalla AE, Roozen JP (1999). Effect of plant extracts on the oxidative stability of sunflower oil and emulsion. Food Chem. 64(3):323–329.

Ames BN, Shigenaga MK, Hagen TM (1993). Oxidants, antioxidants and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA. 90(17):7915-7922.

Azizova OA (2002). Role of free radical processes in the development of atherosclerosis. Biol. Membr, 19(6):451-471.

Bajpai M, Pande A, Tewari SK, Prakash D (2005). Phenolic contents and antioxidant activity of some food medicinal plants. Int. J. Food Sci. Nutr., 56(4):287- 291.

Benavente-Garcia O, Castillo J, Marin FR, Ortuno A, Del-Rio JA (1997). Uses and properties of Citrus flavonoids. J. Agric. Food Chem. 45(12):4505-4515.

Braca A, Tommasi ND, Bari LD, Pizza C, Politi M, Morelli I (2001). Antioxidant principles from *Bauhinia tarapotensis*. J. Nat. Prod., 64(7):892-895.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. Food Sci. Technol., 28(1): 25–30.

Demiray S, Pintado ME, Castro PML (2009). "Evaluation of Phenolic Profiles and Antioxidant Activities of Turkish Medicinal Plants: *Tilia Argentea*, *Crataegi folium* Leaves and *Polygonum bistorta* roots," World Acad. Sci., Eng. Technol. 54:312-317.

Devasagayam TPA, Tilak JC, Boloor KK, Sane KS, Ghaskadbi SS, Lele RD (2004). Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects. J. Assoc. Phys. India 52:794-804.

Ghani A (2003). Medicinal Plants of Bangladesh, 2nd edition, pp. 1-2, 55-57, 402, 500.

Hagerman AE, Riedl KM, Jones GA, Sovik KN, Ritchard NT, Hartzfeld PW, Riechel TL (1998). High molecular weight plant polyphenolics (tannins) as biological antioxidants. J. Agric. Food Chem. 46(5):1887-1892.

Halliwell B, Gutteridge JMC, Aruoma OI (1987). The deoxyribose method: A simple "test-tube" assay for determination of rate constants for reactions of hydroxyl radicals. Anal. Biochem. 165(1):215- 219.

Lalenti A, Lanaro A, Moncada S, Rosa MD (1992). Modulation of acute inflammation by endogenous nitric oxide. Eur. J. Pharmacol. 211(2):177- 182.

Jao CH, Ko WC (2002). 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging by protein hydrolyzates from tuna cooking juice. Fish. Sci. 68(2):430–435.

Jayaprakasha GK, Rao LJ, Sakariah KK (2004). Antioxidant activities of flavidin in different *in vitro* model systems. Bioorg. Med. Chem., 12(19):5141-5146.

Jayaprakasha GK, Singh RP, Sakariah KK (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models *in vitro*. Food Chem. 73(3):285-290.

Kumarasamy Y, Byres M, Cox PJ, Jaspars M, Nahar L, Sarker SD (2007). Screening seeds of some Scottish plants for free-radical scavenging activity. Phytother. Res. 21(7):615-621.

Landrault, N, Poucheret P, Ravel P, Gasc F, Cros G, Teissedre PL (2001). Antioxidant Capacities and Phenolics Levels of French Wines from Different Varieties and Vintages. J. Agric. Food Chem. 49(7):3341-3343.

Miller HE, Rigelhof F, Marquart L, Prakash A, Kanter M (2000). Antioxidant Content of Whole Grain Breakfast Cereals, Fruits and Vegetables. J. Am. Coll. Nutr. 19(3):312S-319S.

Nagler R, Reznick A, Shafir Y, Shehadeh N (2006). Free radical related effects and antioxidants in saliva and serum of adolescents with type 1 diabetes metllitus. Free Radic. Res. 40:S156.

Oktay M, Gulcin I, Kufrevioglu OI (2003). Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT - Food Sci. Technol. 36(2):263-271.

Prakash D, Suri S, Upadhyay G, Singh BN (2007). Total phenol, antioxidant and free radical scavenging activities of some medicinal plants. Int. J. Food Sci. Nutr. 58(1):18-28.

Prieto P, Pineda M, Aguilar M (1999). Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal. Biochem. 269(2):337-341.

Quintero M, Brennan PA, Thomas GJ, Moncada S (2006). Nitric Oxide Is a Factor in the Stabilization of Hypoxia-Inducible Factor-1α in Cancer: Role of Free Radical Formation. Cancer Res. 66(2):770-774.

Resat A, Kubilay G, Mustafa O, Saliha EK (2004). Novel total antioxidant capacity index for diatery polyphenols and vitamins C and E, using there cupric ion reducing capacity in the presence of neocuproine: CUPRAC method. J. Agric. Food Chem. 52:7970-7981.

Rice-Evans C, Miller N, Paganga G (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci. 2(4):152-159.

Sa'nchez-Moreno C (2002). Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci. Technol. Int. 8(3):121-137.

Scalbert A, Johnson IT, Saltmarsh M (2005). Polyphenols: antioxidants and beyond. Am. J. Clin. Nutr. 81(1):215S-217S

Sian BA (2003). Dietary antioxidants past, present and future? Trends Food Sci. Technol. 14(3):93-98.
Siddhuraju P, Becker K (2003). Antioxidant Properties of Various Solvent Extracts of Total Phenolic Constituents from Three Different Agroclimatic Origins of Drumstick Tree (*Moringa oleifera* Lam.) Leaves. J. Agric. Food Chem. 51(8):2144-2155.
Smolin LA, Grosvenor MB (2007). Nutrition: Sci. Appl., Fourth edition.
Subramanion LJ, Zuraini Z, Sasidharan S (2011). Phytochemicals screening, DPPH free radical scavenging and xanthine oxidase inhibitiory activities of *Cassia fistula* seeds extract. J. Med. Plants Res. 5(10):1941-1947.
Sun T, Ho CT (2005). Antioxidant activities of buckwheat extracts. Food Chem. 90(4):743-749.
Sreejayan N, Rao MNA (1997). Nitric Oxide Scavenging by Curcuminoids. J. Pharm. Pharmacol. 49(1):105-107.
Tripathi VK, Singh B, Tripathi RK, Upadhyay G, Pandey VB (1996). *Terminalia arjuna* its present status. Orient. J. Chem. I:1–16.
Vertuani S, Angusti A, Manfredini S (2004). The Antioxidants and Pro-Antioxidants Network: An Overview. Curr. Pharm. Des. 10(14):1677-1694.
Wang SY, Jiao H (2000). Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. J. Agric. Food Chem. 48(11):5672-5676.
Wilson T (1999). Whole foods, antioxidants and health. Antioxidants in human health and disease. pp. 141-150.
Yen GC, Chen HY (1995). Antioxidant Activity of Various Tea Extracts in Relation to Their Antimutagenicity. J. Agric. Food Chem. 43(1):27-32.
Yildirim A, Mavi A, Kara AA (2001). Determination of Antioxidant and Antimicrobial Activities of *Rumex crispus* L. Extracts. J. Agric. Food Chem. 49(8):4083-4089.