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# Hydroalcoholic extracts of Indian medicinal plants can help in amelioration from oxidative stress through antioxidant properties

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# Hydroalcoholic extracts of Indian medicinal plants can help in amelioration from oxidative stress through antioxidant properties

Rhitajit Sarkar and Nripendranath Mandal

## Abstract

The in vitro study of the antioxidant properties of the hydroalcoholic extracts of various Indian medicinal plants can logically help to develop a better and safer way of amelioration from oxidative stress. As aimed, the present study has been done to estimate and thereby conclude regarding the antioxidant activities of a few Indian medicinal plants, viz., *Terminalia chebula, Terminalia belerica, Emblica officinalis, Caesalpinia crista, Cajanus cajan, and Tinospora cordifolia*. The extracts of the plants have been subjected to the evaluation of antioxidant properties through scavenging assays for reactive oxygen species like superoxide, nitric oxide, peroxynitrite, hypochlorous acid, singlet oxygen, etc. and measurement of TEAC values and other phytochemical parameters. The phenolic and flavonoid contents of each plant have been found to be correlated to their individual antioxidant activity. The results showed the hydroalcoholic extracts of the plants were efficient indicators of their antioxidant capacity thus concreting their basis to be used as natural antioxidant.

**KEYWORDS:** Oxidative stress, hydroalcoholic extract, free radicals, antioxidant, iron chelating, phenolic content

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## Introduction

Potentially harmful reactive oxygen species (ROS), which include reactive molecules such as superoxide radical ($O_2^{\cdot-}$), nitric oxide ($NO^{\cdot}$), peroxynitrite radical anion ($ONOO^-$) and singlet oxygen ($^1O_2$) evolved as a consequence of normal aerobic metabolism (Aruoma et al. 1989; Halliwell 1991). ROS directly bring about damage of cellular macromolecules, especially DNA, which undergo strand breakage, change and release of bases as well as modification of sugar moieties (Henle and Linn 1997; Marnett 2000), thus promoting a series of pathological events, viz. cancer and ageing (Retsky et al. 1993). On the other hand ROS along with pro-oxidants give rise to emphysema, cirrhosis, arteriosclerosis, inflammation, genotoxicity and other diseased conditions (Braca et al. 2002). Use of synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) to combat the pathologic conditions have been restricted due to their possible carcinogenic properties (Branen 1975). Thus, major attention is being given to isolation of natural antioxidants. Presence of natural antioxidants from different kinds of plant materials have been reported (Ashokkumar et al. 2008; Owolabi et al. 2008) and it has been found that these phytocompounds are of great interest (Bongiorno et al. 2008; Naik and Panda 2008). Many indigenous herbal plants have been used as medicines in India (Packer and Ong 1998; Jovanovic and Simic 2000).

The fruits of *Terminalia chebula* Retz, *Terminalia belerica* Roxb, and *Emblica officinalis* Gaertn are widely used in the Indian traditional system of medicine. Fruits of *T. belerica* and *T. chebula* are reported to be purgative (Chopra et al. 1956). The fruit of *T. chebula* was traditionally used to cure asthma, urinary disorders and heart diseases (Reddy et al. 1990; Lee et al. 2005). The fruit of *E. officinalis* is a rich source of vitamin C, a well-known antioxidant (Halliwell and Gutteridge 1985). The crude extract of *E. officinalis* was reported to counteract the hepatotoxic and renotoxic effects of metals (Roy et al. 1991) due to antioxidant properties. In Ayurveda, the fruit of *E. officinalis* is used to treat cardiac, cerebral and intestinal disorders, along with cancerous conditions (Aslokar et al. 1992; Rajarama Rao and Siddiqui 1964). *Caesalpinia crista* Linn is used for the traditional treatment of gynaecological disorders, skin diseases, constipation, piles and ulcers (Williamson 2002). *C. crista* is also reported to have anthelmintic (Jabbar et al. 2007), antidiuretic, antibacterial (Neogi and Nayak 1958), antianaphylactic and antidiarrhoeal (Iyenger and Pendse 1965), antiameobic and antiestrogenic (Raghunathan and Mitra 1982), antiviral (Dhar et al. 1968), antidiabetic and hypoglycaemic properties (Rao et al. 1994). *Cajanus cajan* (L.) Millsp leaves have been used in traditional Indian medicinal system for the treatment of jaundice (Morton 1976). The leaves are also used in treatment of sore gums, dysentery and liver & kidney ailments; along with noticeable

hypoglycemic potential (Jaiswal et al. 2008). The flavonoid rich leaves of *C. cajan* are beneficial for human health (Duker-Eshun et al. 2004; Zu et al. 2006). Various pharmacological actions and medicinal uses of the different parts of *Tinospora cordifolia* (Thunb.) Miers are well reported in the ancient literatures (Arya Vaidya Sala 1997; Bentley and Trimen 2004). Immunomodulatory, antihyperglycaemic, hepatoprotective, cardioprotective and cytotoxic activities of the plant have also been studied (Krishna et al. 2009).

Keeping all the information in mind, this study is aimed to find the antioxidant and free radical scavenging properties of the hydroalcoholic (60% ethanolic) extract of six medicinal plants widely used in Indian system of traditional medicine, *Ayurveda*. Not only this will help to enunciate the scientific basis of the traditional medicine system, but also provide impetus for avid use of the plants as natural antioxidants, both as medicine and dietary supplements, thoroughly effective at non-toxic dose levels.

## Materials and Methods

### Chemicals

2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) was obtained from Roche Diagnostics, Mannheim, Germany. 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Fluka, Buchs, Switzerland. Potassium persulfate ($K_2S_2O_8$), ethylenediamine tetraacetic acid (EDTA), ascorbic acid, 2-deoxy-2-ribose, trichloroacetic acid (TCA), mannitol, nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide phosphate (NADH), phenazine methosulfate (PMS), sodium nitroprusside (SNP), sulfanilamide, naphthylethylenediamine dihydrochloride (NED), L-histidine, lipoic acid, quercetin and ferrozine were obtained from Sisco Research Laboratories Pvt. Ltd, Mumbai, India. Hydrogen peroxide, sulfuric acid, potassium hexacyanoferrate, Folin-Ciocalteu reagent, sodium carbonate ($Na_2CO_3$), ammonium iron (II) sulfate hexahydrate ($(NH_4)_2Fe(SO_4)_26H_2O$), butylated hydroxy toluene (BHT), sodium hypochlorite (NaOCl), aluminium chloride ($AlCl_3$), potassium nitrite ($KNO_2$) and *N*,*N*-dimethyl-4-nitrosoaniline were obtained from Merck, Mumbai, India. Gallic acid and curcumin were obtained from MP Biomedicals, France. HEPES buffer and catalase were obtained from HiMedia Laboratories Pvt. Ltd, Mumbai, India. Evans Blue was purchased from BDH, England. Manganese dioxide was obtained from SD Fine Chemicals, Mumbai, India. Diethylene-triamine-pentaacetic acid (DTPA) was obtained from Spectrochem Pvt. Ltd, Mumbai, India. Sodium nitrite was obtained from Qualigens Fine Chemicals, Mumbai, India.

## Collection & Authenticity of Medicinal Plants

The plant materials were collected from Bankura district of West Bengal, India and the genus and species of the medicinal plants are authenticated through the Central Research Institute (Ayurveda), Kolkata, India, where specimens for each plant were deposited. A list of the specimen numbers for the plants is provided below:

| Serial No. | Name of the plant | Specimen No. | Plant part used |
|---|---|---|---|
| 1 | *Terminalia chebula* | CRHS 113/08 | Fruit |
| 2 | *Terminalia belerica* | CRHS 114/08 | Fruit |
| 3 | *Emblica officinalis* | CRHS 115/08 | Fruit |
| 4 | *Caesalpinia crista* | CRHS 121/08 | Leaf |
| 5 | *Cajanus cajan* | CRHS 119/08 | Leaf |
| 6 | *Tinospora cordifolia* | CRHS 123/08 | Stem |

## Preparation of Crude Extract

The powder (100 g) of the dried fruits of *T. chebula, T. belerica* and *E. officinalis*, leaves of *C. crista* and *C. cajan* or stem of *T. cordifolia* was stirred using a magnetic stirrer with 500 ml mixture of ethanol:water (6:4) (hydro-alcoholic extract) for 15 hours; then the mixture was centrifuged at 2850 x *g* and the supernatant decanted. The process was repeated again with the precipitated pellet. The supernatants were collected, concentrated in a rotary evaporator at room temperature and freeze dried in a lyophilizer. The dried extract was stored at -20°C until use.

## Antioxidant and Phytochemical Studies

*Antioxidant activity*

Antioxidant capacity was measured based on the scavenging of $ABTS^{\cdot+}$ radical cation by the sample in comparison to trolox standard (Hazra et al. 2008). ABTS solution was mixed with potassium persulfate to generate $ABTS^{\cdot+}$ radical cation. Then 10 µl sample solution was mixed with 1 ml $ABTS^{\cdot+}$ solution and the absorbance was measured at 734 nm. All experiments were repeated six times. The percentage inhibition of absorbance was calculated and plotted as a function of concentration of standard and sample to determine the trolox equivalent antioxidant concentration (TEAC).

*Reducing power*

The $Fe^{3+}$-reducing power of the extract was determined by a standard method (Hazra et al. 2008). In a phosphate buffer solution (0.2 M, pH 6.6), different concentrations (0.0-0.4 mg/ml) of the extract were mixed with potassium hexacyanoferrate (0.1%), followed by incubation. After incubation, the upper portion of the solution was diluted, and $FeCl_3$ solution (0.01%) was added. The reaction mixture was left at room temperature for colour development and the absorbance was measured at 700 nm. All tests were performed six times. A higher absorbance of the reaction mixture indicated greater reducing power. Ascorbic acid was used as a positive control.

*Determination of total phenolic content*

The amount of total phenols present in the plant extracts was determined using Folin-Ciocalteu (FC) reagent by a formerly described method (Hazra et al. 2008). The phenolic content was evaluated from gallic acid standard curve ($R^2$=0.955).

*Determination of total flavonoid content*

The amount of total flavonoids was determined with aluminium chloride ($AlCl_3$) according to an earlier method (Hazra et al. 2008). The flavonoid content was calculated from quercetin standard curve ($R^2$=0.994).

REACTIVE OXYGEN SPECIES (ROS) SCAVENGING CAPACITIES

*Superoxide radical*

Measurements of superoxide anion scavenging activities of the sample and standard quercetin were done based on the reduction of NBT according to a previously described method (Hazra et al. 2008). Superoxide radical is generated by a non-enzymatic system of phenazine methosulfate-nicotinamide adenine dinucleotide (PMS/NADH). These radicals reduce nitro blue tetrazolium (NBT) into a purple colored formazan which was measured spectrophotometrically at 562 nm. All tests were performed six times.

*Nitric oxide radical*

Sodium nitroprusside (SNP) gives rise to nitric oxide that under interaction with oxygen produce nitrite ions measured by Griess Illosvoy reaction (Hazra et al. 2008). The chromophore generated was spectrophotometrically measured at 540

nm against blank sample. All tests were performed six times. Curcumin was used as a standard.

*Peroxynitrite scavenging*

Peroxynitrite ($ONOO^-$) synthesis was done 12 hrs before the assay, according to Beckman et al. (1994). Acidic solution (0.6 M HCl) of 5 ml $H_2O_2$ (0.7 M) was mixed with 5 ml of 0.6 M $KNO_2$ on an ice bath for 1 s and 5 ml of ice-cold 1.2 M NaOH was added to the reaction mixture. Excess $H_2O_2$ was adsorbed by granular $MnO_2$ and the reaction mixture was left at -20°C. The concentration of the peroxynitrite solution was measured spectrophotometrically at 302 nm ($\varepsilon = 1670$ $M^{-1}$ $cm^{-1}$).

Evans blue bleaching assay was used to measure the peroxynitrite scavenging activity (Hazra et al. 2008). The percentage of scavenging of $ONOO^-$ was calculated by comparing the results of the test and blank sample. All tests were performed six times. Gallic acid was used as reference compound.

*Singlet oxygen scavenging*

Singlet oxygen ($^1O_2$) production, and at the same time, its scavenging by the sample and the reference compound lipoic acid can be monitored by *N*,*N*-dimethyl-4-nitrosoaniline (RNO) bleaching, using a earlier reported method (Hazra et al. 2008). Singlet oxygen was generated by a reaction between NaOCl and $H_2O_2$ and the bleaching of RNO was read at 440 nm. All tests were performed six times.

*Hypochlorous acid scavenging*

According to a previously described method (Hazra et al. 2008), hypochlorous acid (HOCl) was prepared just before the experiment by adjusting the pH of a 10% (v/v) solution of NaOCl to pH 6.2 with 0.6 M $H_2SO_4$ and the concentration of HOCl was determined by taking the absorbance at 235 nm using the molar extinction coefficient of 100 $M^{-1}$ $cm^{-1}$. The scavenging activities of the plant extract and the standard, ascorbic acid, a potent HOCl scavenger was evaluated by measuring the decrease in the absorbance of catalase at 404 nm. All tests were performed six times.

### Statistical Analysis

All data are reported as the mean ± SD of six measurements. Statistical analysis was performed using KyPlot version 2.0 beta 15 (32 bit). The $IC_{50}$ values were calculated using the formula Y = 100*A1/(X + A1) where A1 = $IC_{50}$, Y = response (Y = 100% when X = 0), X = inhibitory concentration. The $IC_{50}$ values were compared by paired t-Test. The results with a value of $p < 0.05$ were considered significant.

## Results and Discussion

Free radicals are responsible for the damage of cellular bio-molecules such as proteins, enzymes, nucleic acids, lipids and carbohydrates and may adversely affect immune functions. Antioxidants interrupt the production of free radicals and also play a key role to inactivate them. Although, all human cells protect themselves against oxidative damage by some antioxidant mechanism, these sometimes are not sufficient to prevent the free radical damage totally. Different kinds of plant material have already been reported as natural antioxidants, which triggers the interest for the present study.

### Antioxidant and Phytochemical Studies

*Antioxidant activity*

The reaction between ABTS and potassium persulfate results in the production of a blue colored chromophore, $ABTS^{\cdot+}$. After addition of the plant extracts this pre-formed radical cation was converted to ABTS in a dose dependant manner. The results (Figure 1) are compared with trolox and the TEAC value demonstrates the extracts as a potent antioxidant. The respective TEAC values of the plants are provided in Table 1 that followed the order *E. officinalis* > *T. chebula* > *T. belerica* > *C. cajan* > *C. crista* > *T. cordifolia*.

*Reducing power*

As illustrated in Figure 2, $Fe^{3+}$ to $Fe^{2+}$ transformation in the presence of most of the plant extracts and reference ascorbic acid was performed to measure the reductive capability. Most of the plants showed reducing power, which alternatively proved their antioxidant property. The trend of reducing capacity of the plant extracts is as follows: *T. chebula* > *T. belerica* > *E. officinalis* > *C. cajan* > *T. cordifolia* > *C. crista*.

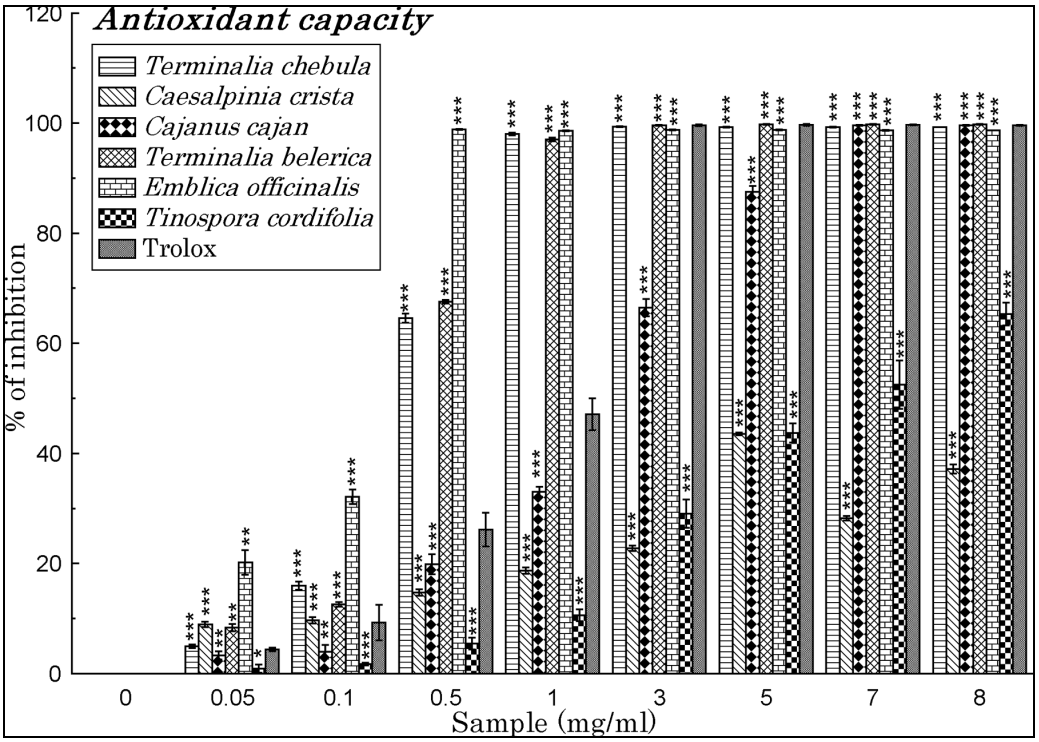


***Figure 1***

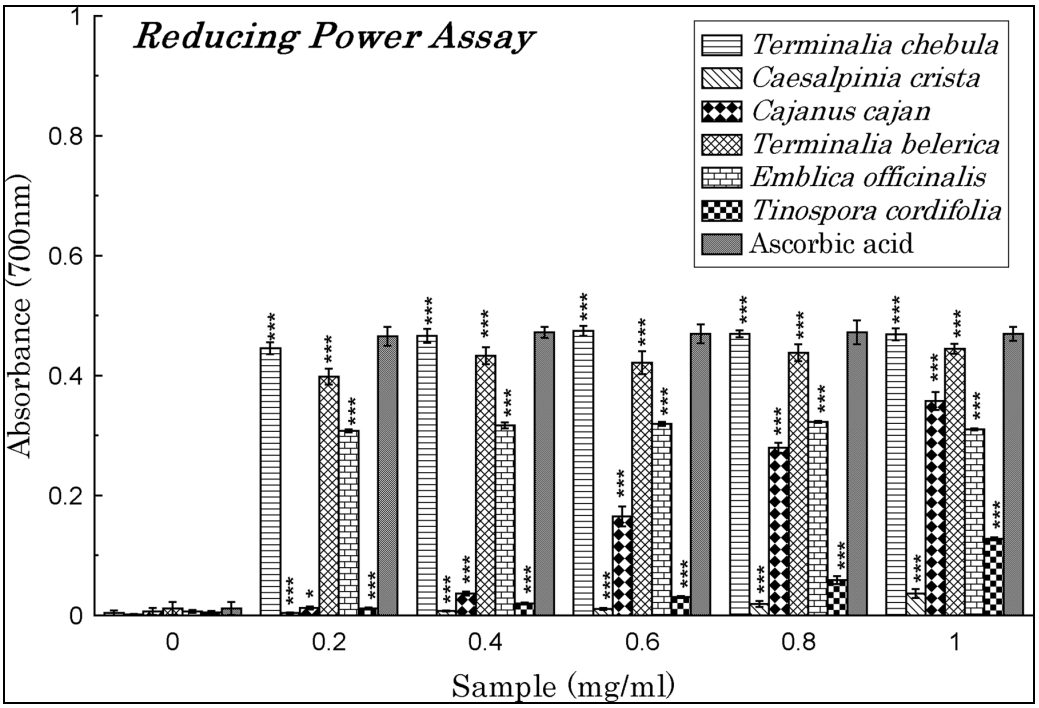


***Figure 2***

*Determination of total phenolic and flavonoid content*

It is also found that the plant extracts showed significant amount of flavonoid and phenolic contents. Flavonoids show their antioxidative action through scavenging or chelating process (Cook and Samman 1996). Phenolic content is also very important plant constituent because of their scavenging ability due to their hydroxyl groups (Diplock 1997). Both of these compounds have good antioxidant potential and their effects on human nutrition and health are significant. The amount of total phenolics and flavonoids present in the plant extracts are given in Table 1.

**Reactive Oxygen Species (Ros) Scavenging Capacities**

*Superoxide radical*

Superoxide anion is also implicated as harmful reactive oxygen species (ROS). It has detrimental effect on the cellular components in a biological system (Halliwell et al. 1987). It indirectly initiates lipid oxidation by generating singlet oxygen. The 60% ethanolic extract of nearly all the plants (except *Tinospora cordifolia*) dose-dependently scavenged superoxide radical, one of the most detrimental ROS, quite successfully (Figure 3), as is also corroborated in their $IC_{50}$ values (Table 1) that followed the order *T. chebula* > *T. belerica* > *E. officinalis* > *C. cajan* > *C. crista* > *T. cordifolia*.

*Nitric oxide radical*

The production of nitric oxide radical at a sustained level results in direct tissue toxicity and contribute to the vascular collapse associated with septic shock, whereas chronic expression of nitric oxide radical is associated with various carcinomas and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (Okuda et al. 1983). The reaction of NO with superoxide radical generates highly reactive peroxynitrite anion (ONOO-) which is highly toxic for living cell (Tylor et al. 1997). The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extracts directly compete with oxygen to react with nitric oxide, thus inhibiting nitrite formation. Nearly all the plants showed (Figure 4) excellent nitric oxide scavenging results in the given manner *T. chebula* > *T. belerica* > *C. crista* > *C. cajan* > *E. officinalis* > *T. cordifolia*; some of them well above than the standard in activity. The $IC_{50}$ values of the respective plants also reflect the fact (Table 1).

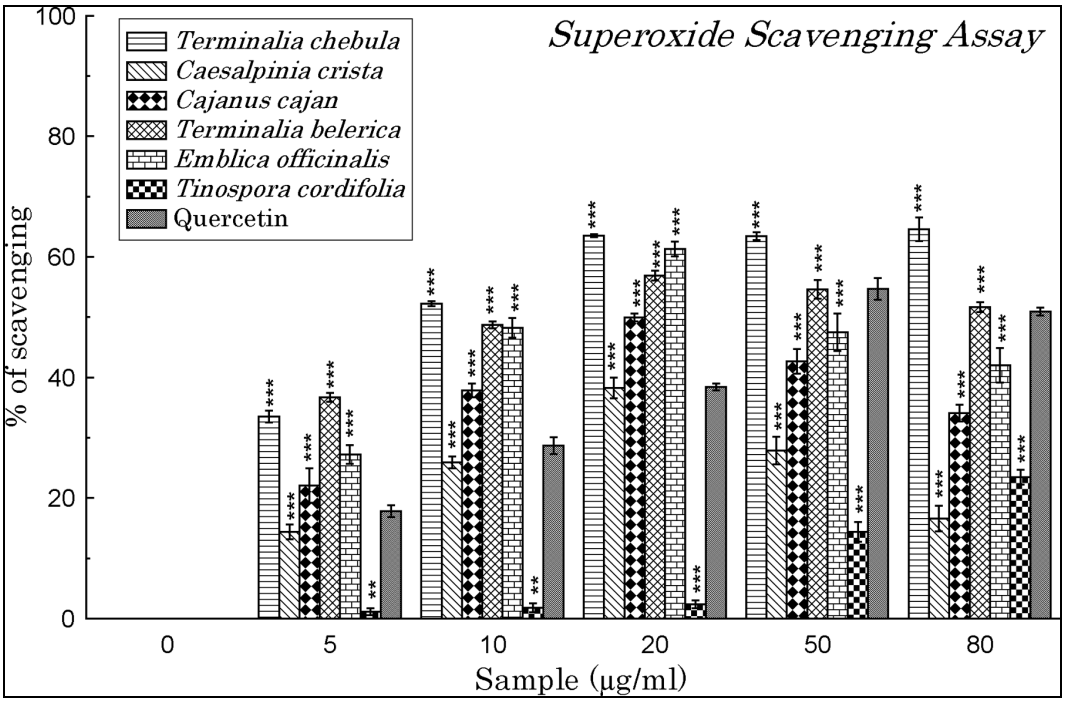


***Figure 3***

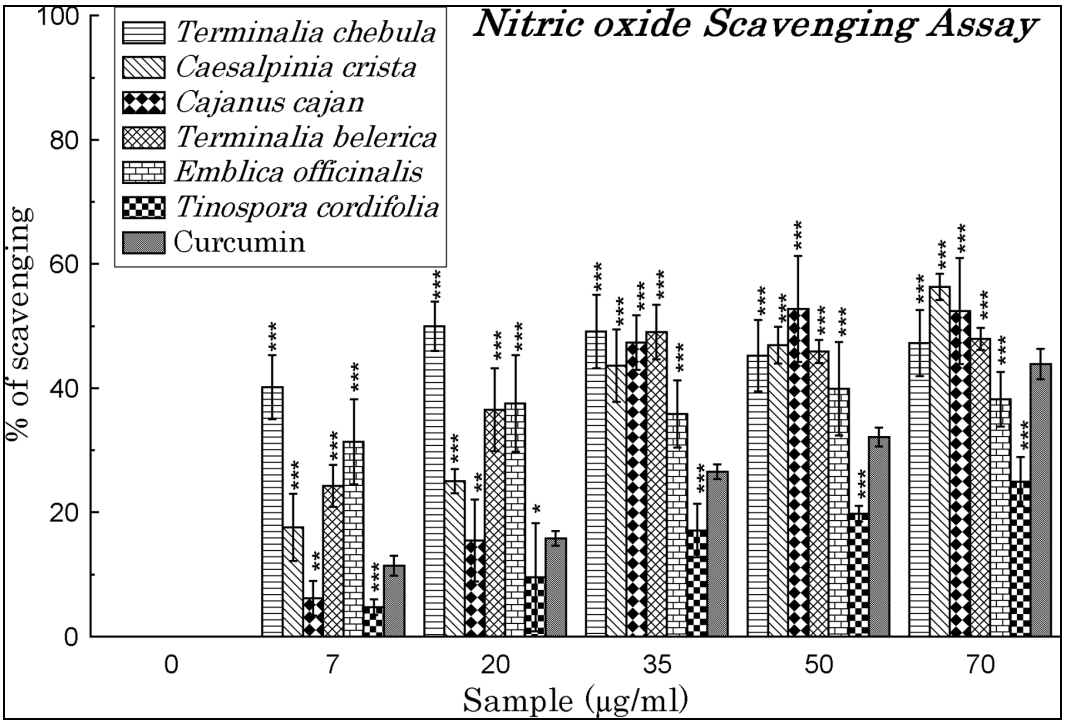


***Figure 4***

*Peroxynitrite scavenging*

Peroxynitrite ($ONOO^-$) is relatively stable amongst all free radicals but once protonated forms highly reactive peroxynitrous acid (ONOOH). The generation of excess $ONOO^-$ leads to oxidative damage and tissue injury. Peroxynitrite bleaches Evans blue by oxidizing it. According to the present results, the extracts inhibit Evans blue bleaching through peroxynitrite scavenging and it is observed that the figures (Figure 5) and the $IC_{50}$ values (Table 1) of the extracts of the plants showed that some of the plants have considerable scavenging ability of peroxynitrite anion, while others have minor thus providing the trend *E. officinalis* > *T. chebula* > *T. belerica* > *C. cajan* > *C. crista* > *T. cordifolia*.

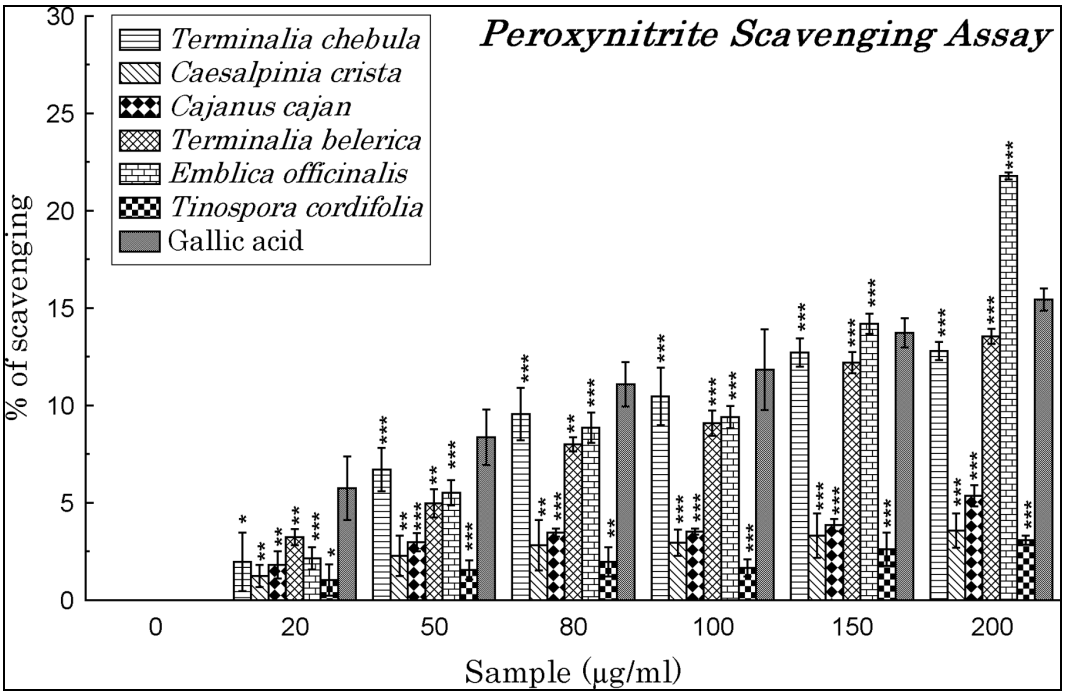


***Figure 5***

*Singlet oxygen scavenging*

Another ROS, singlet oxygen which is a high energy form of oxygen, is generated in the skin upon UV-irradiation. Singlet oxygen induces hyperoxidation, oxygen cytotxicity and decreases the antioxidative activity (Kochever and Redmond 2000). The plant extracts showed considerable activity of the order *E. officinalis* > *T. chebula* > *T. cordifolia* > *T. belerica* > *C. crista* > *C. cajan* in scavenging singlet oxygen species, as is evident from the figures (Figure 6) and from the $IC_{50}$ values of the plants (Table 1).

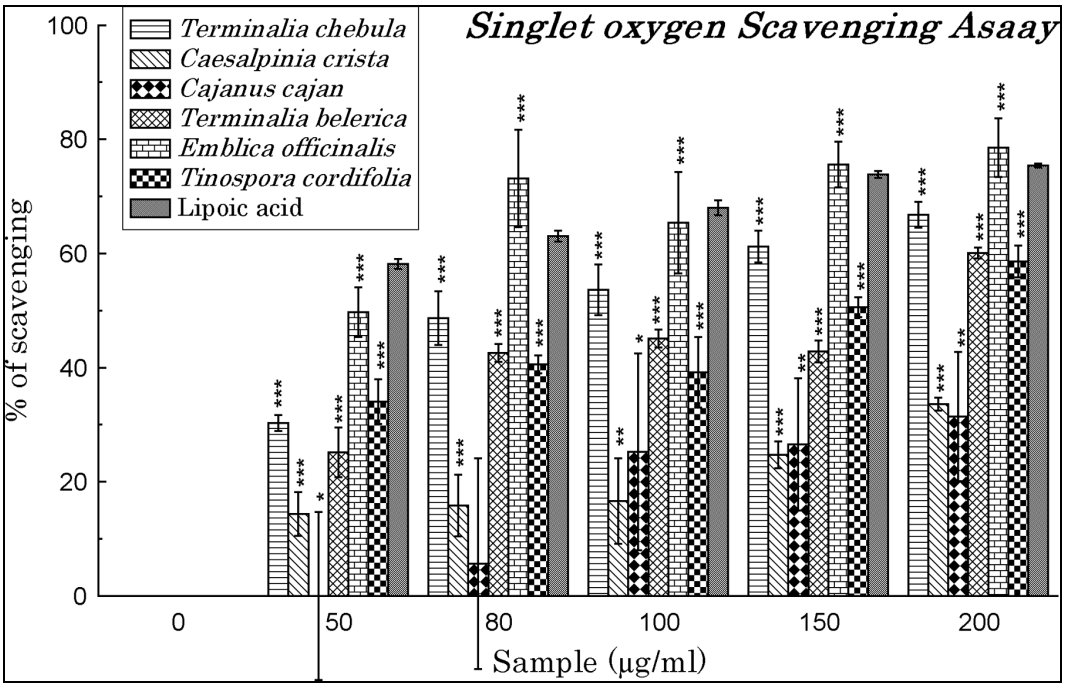


***Figure 6***

*Hypochlorous acid scavenging*

At the sites of inflammation, the oxidation of $Cl^-$ ions by the neutrophil enzyme myeloperoxidase results in the production of another harmful ROS, hypochlorous acid (Aruoma et al. 1989). HOCl has the ability to inactivate the antioxidant enzyme, catalase through break down of heme-prosthetic group. The inhibition of catalase inactivation in the presence of the extracts signifies its HOCl scavenging activity and from the results obtained, it is anticipated that a reasonable dose-dependent efficiency in scavenging the hypochlorous acid is observed for all the plant extracts (*C. cajan* > *T. belerica* > *T. cordifolia* > *T. chebula* > *C. crista* > *E. officinalis*), as is corroborated in the figures (Figure 7) and from the $IC_{50}$ values (Table 1).

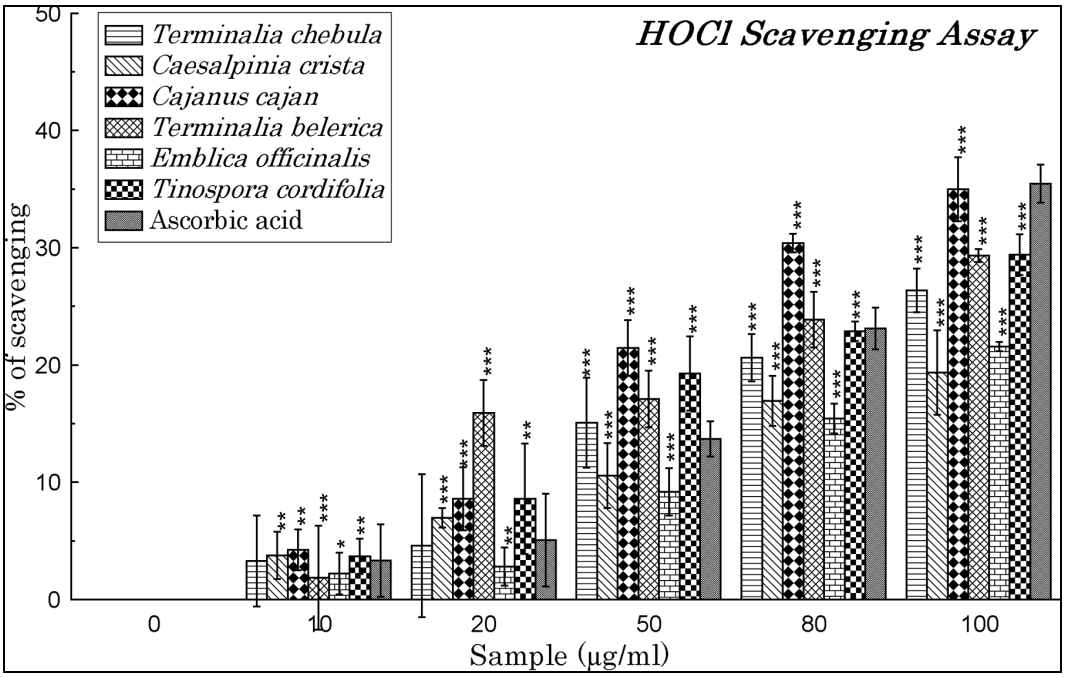


***Figure 7***

*Correlation between the total phenolic or flavonoid contents with the antioxidant activity*

As shown in Figure 8, the total phenolic content of the six plants are correlated to their respective antioxidant activity, with the correlation coefficients for all approaching the value $R = 1$, which proved that the phenolic contents of these plants are reciprocated to their respective antioxidant activity. The correlation coefficients for the plants are: *T. chebula* ($R = 0.8812$); *T. belerica* ($R = 0.9519$); *E. officinalis* ($R = 0.7469$); *C. crista* ($R = 0.9154$); *C. cajan* ($R = 0.7426$) and *T. cordifolia* ($R = 0.8264$). Similar observations are found for the correlation of antioxidant activities of the plants with their flavonoid contents (Figure 9), with the values of the coefficients for the plants as: *T. chebula* ($R = 0.7206$); *T. belerica* ($R = 0.8636$); *E. officinalis* ($R = 0.7453$); *C. crista* ($R = 0.8165$); *C. cajan* ($R = 0.7280$) and *T. cordifolia* ($R = 0.8126$). To sum up, the results showed that the total phenolic and flavonoid contents of the different plant parts are thoroughly correlated to their individual antioxidant activities.

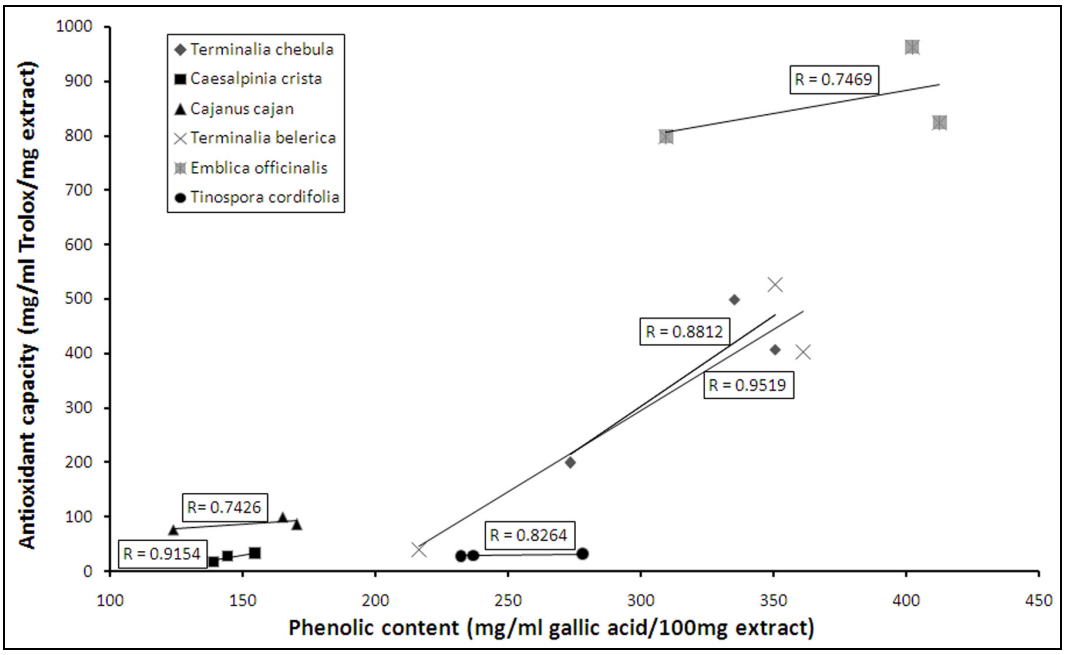


***Figure 8***

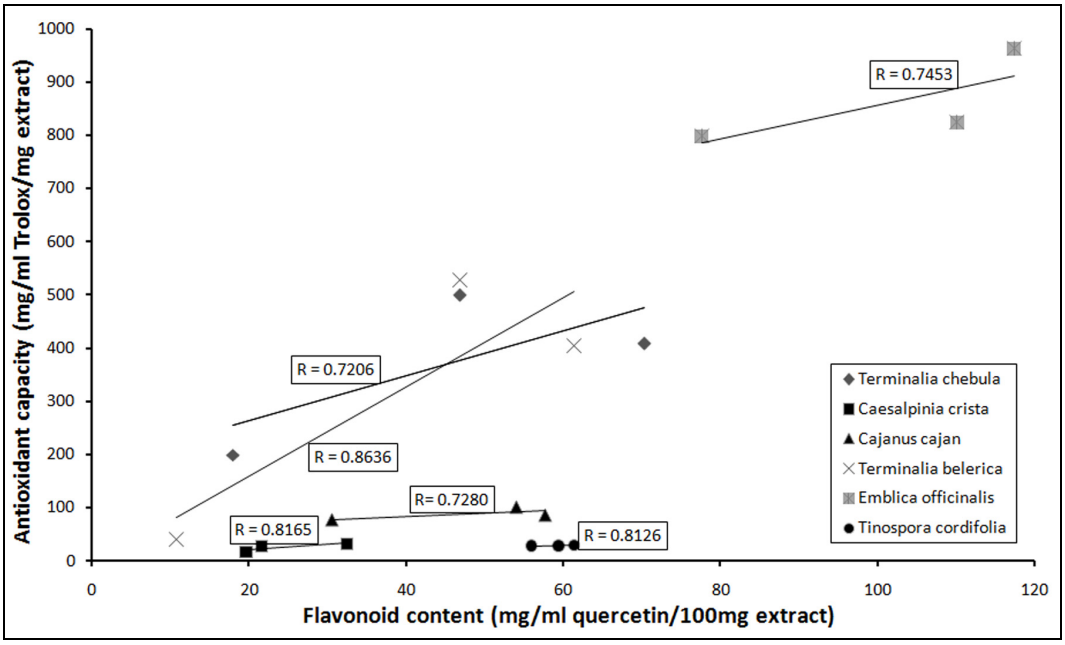


***Figure 9***

**Table 1: Comparison of the antioxidant capacities, free radical scavenging activities of 60% ethanolic extracts of *Terminalia chebula*, *Caesalpinia crista, Cajanus cajan, Terminalia belerica*, *Emblica officinalis* and *Tinospora cordifolia***

| Name of Assay | 60% ethanolic crudes of | | | | | | Standard | Values of Standard compounds |
|---|---|---|---|---|---|---|---|---|
| | ***Terminalia chebula*** | ***Caesalpinia crista*** | ***Cajanus cajan*** | ***Terminalia belerica*** | ***Emblica officinalis*** | ***Tinospora cordifolia*** | | |
| **TEAC Values (Antioxidant activity)** | 1.499 ± 0.005 | 0.152 ± 0.002 | 0.611 ± 0.003 | 1.49 ± 0.004 | 4.6 ± 0.05 | 0.359 ± 0.0035 | -- | -- |
| **† Phenolic content** | 0.202 ± 0.008 | 0.0801 ± 0.001 | 0.083 ± 0.001 | 0.18 ± 0.002 | 0.193 ± 0.004 | 0.0822 ± 0.0 | -- | -- |
| **‡ Flavonoid content** | 0.108 ± 0.006 | 0.0473 ± 0.001 | 0.081 ± 0.001 | 0.087 ± 0.005 | 0.168 ± 0.003 | 0.0841 ± 0.008 | -- | -- |
| **Ø $IC_{50}$ values for free radical scavenging capacity of:** | | | | | | | | |
| **Superoxide ($O_2^{\cdot-}$)** | 14.03 ± 0.261*** | 139.4 ± 17.23*** | 49.99 ± 4.02** | 20.23 ± 0.31*** | 27.03 ± 4.02*** | 293.21 ± 23.09*** | Quercetin | 42.06 ± 1.35 |
| **Nitric oxide ($NO^{\cdot}$)** | 38.72 ± 10.37*** | 52.21 ± 7.28*** | 57.89 ± 15.67** | 47.38 ± 6.62*** | 66.55 ± 20.67* | 199.33 ± 27.47*** | Curcumin | 90.82 ± 4.75 |
| **Peroxynitrite ($ONOO^-$)** | 1056.60 ± 84.49*** | 4229.17 ± 522.79*** | 3145.22 ± 203.89*** | 1108.81 ± 26.38*** | 817.96 ± 15.82$^{NS}$ | 5590.89 ± 580.20*** | Gallic acid | 876.25 ± 56.96 |
| **Singlet oxygen ($^1O_2$)** | 95.0 ± 10.38*** | 425.24 ± 48.27*** | 841.86 ± 636.98* | 139.43 ± 6.69*** | 45.91 ± 9.06$^{NS}$ | 132.7 ± 14.08*** | Lipoic acid | 46.16 ± 1.16 |
| **Hypochlorous acid (HOCl)** | 295.03 ± 28.79** | 410.48 ± 81.2** | 187.42 ± 19.15*** | 235.5 ± 24.54*** | 415.62 ± 26.11*** | 241.69 ± 14.64** | Ascorbic acid | 235.96 ± 5.75 |

**† Phenolic content (mg/ml Gallic acid equivalent per 100 mg plant extract)**
**‡ Flavonoid content (mg/ml Quercetin equivalent per 100 mg plant extract)**
**Ø $IC_{50}$ Values are in µg/ml.**
*** p< 0.05; ** p< 0.01;*** p< 0.001; NS = Non significant**

## Conclusions

From our results, it is observed that most of the plant materials shown quite reasonable activities in their hydroalcoholic crude extract. The plant extracts showed their efficacies in antioxidative studies through ROS scavenging. It may be drawn from our detailed study that ROS generation is an important step for the formation of cancer, and it may be for sure that all these plants, especially *Terminalia chebula*, *Terminalia belerica* and *Emblica officinalis* will be more than useful in therapeutic point of view through their antioxidant activities. In addition, the other three plants, viz., *Caesalpinia crista*, *Cajanus cajan* and *Tinospora cordifolia*, which may not be as effective as the previous three, can play important role in amelioration of oxidative stress, while incorporated as dietary supplement.

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## Figure Legends

**Figure 1 - Total antioxidant activity**

Effects of the plant extracts and reference compound trolox on ABTS radical cation decolorization assay. The percentage of inhibition is plotted against concentration of sample. The value represented as mean ± S.D. (n = 6). **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs. 0 mg/ml for each individual plant extract.

**Figure 2 – Reducing power**

The reductive ability of the plant extracts and standard Ascorbic acid. The absorbance ($A_{700}$) was plotted against concentration of sample. Each value represents mean ± S.D. (n=6). **p* < 0.05 and ****p* < 0.001 vs. 0 mg/ml for each individual plant extract.

**Figure 3 - Superoxide radical scavenging activity**

Scavenging effects of the plant extracts and the standard quercetin on superoxide radical. The data represent the percentage of superoxide radical inhibition. Each value represents mean ± S.D. (n=6). ***p* < 0.01 and ****p* < 0.001 vs. 0 µg/ml for each individual plant extract.

**Figure 4 - Nitric oxide radical scavenging activity**

Nitric oxide radical scavenging activities of the plant extracts and standard curcumin. The data represent the % of nitric oxide inhibition. Each value represents mean ± S.D. (n=6). **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs. 0 mg/ml for each individual plant extract.

**Figure 5 – Peroxynitrite anion radical scavenging activity**

Peroxynitrite radical scavenging activities of the plant extracts and standard gallic acid. The data represent the % of peroxynitrite radical inhibition. Each value represents mean ± S.D. (n=6). **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs. 0 mg/ml for each individual plant extract.

**Figure 6 – Singlet oxygen scavenging activity**

Effects of the plant extracts and standard lipoic acid on the scavenging of singlet oxygen. Each value represents mean ± S.D. (n=6). **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs. 0 mg/ml for each individual plant extract.

**Figure 7 - Hypochlorous acid scavenging activity**

Hypochlorous acid scavenging activities of the plant extracts and standard ascorbic acid. Each value represents mean ± S.D. (n=6). **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs. 0 mg/ml for each individual plant extract.

**Figure 8 - Correlation of antioxidant activity with phenolic contents**

The relationship between total phenolic content in individual plant extract and their antioxidant capacity. The correlation analyses were described as linear correlation coefficient (R). The differences were considered statistically significant if $p < 0.05$.

**Figure 9 - Correlation of antioxidant activity with flavonoid contents**

The relationship between total flavonoid content in individual plant extract and their antioxidant capacity. The correlation analyses were described as linear correlation coefficient (R). The differences were considered statistically significant if $p < 0.05$.