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Total phenolic content, flavonoid content and in vitro antioxidant activities of Dendrophthoe falcata (L.f.) Ettingsh

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Abstract: The total phenolic content(TPC) and total flavonoid content(TFC) of chloroform(CEDF), methanol(MEDF) and hydroalcoholic(HEDF) extracts of an Indian ayurvedic plant *Dendrophthoe falcata* (L.f) Ettingsh were measured using Folin–Ciocalteau and aluminum chloride colorimetric methods, respectively. The *in vitro* antioxidant activities were also investigated by using *in vitro* antioxidant models including 2,2´-azinobis–(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$), 1,1-diphenyl-2-picrylhydrazyl(DPPH), nitric oxide(NO), hydroxyl radical(OH^-), super oxide(SO) and reducing power (Fe^{3+} to Fe^{2+} transformation) assay. The TPC were 21.78 ± 3.145, 38.66 ± 1.862 and 46.43 ± 2.55 mg gallic acid equivalents (GAE)/g extract, while TFC were 9.74 ± 1.324, 21.59 ± 1.09 and 33.42 ± 2.083 mg quercetin equivalents (QRT)/g extract sample for CEDF, MEDF and HEDF, respectively. Analysis of the free radical scavenging activities of the fractions revealed a concentration-dependent antiradical activity resulting from reduction of $ABTS^+$, DPPH, NO, OH^- and SO radicals to non-radical form. The scavenging activity of ascorbic acid, a known antioxidant used as positive control, was however higher and scavenging potential was in the order: ascorbic acid > HEDF > MEDF > CEDF. The reducing power of ascorbic acid, CEDF, MEDF and HEDF increased gradually with increasing concentration. The order of the reduction potential was ascorbic acid > HEDF > MEDF > CEDF. These results obtained in the present study indicate that *D. falcata* extracts can be a potential source of natural antioxidant with strong antiradical capacity.

Key words: *Dendrophthoe falcata*, total phenolics content, total flavonoid content, antiradical activity, reducing power.

INTRODUCTION

Oxidative stress depicts the existence of products called free radicals and reactive oxygen species (ROS), which are formed under normal physiological conditions but become deleterious when not being eliminated by the endogenous systems. In fact, oxidative stress results from an imbalance between the generation of reactive oxygen species and endogenous antioxidant systems. ROS are major sources of primary catalysts that initiate oxidation *in vivo* and *in vitro* and create oxidative stress which results in numerous diseases and disorders [1, 2] such as

cancer[3], cardiovascular disease[4], Alzheimer's disease[5], parkinsons disease[6], ulcerative colitis[7], arthrerosclerosis[8]. Oxygen derived free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are cytotoxic and give rise to tissue injuries[9]. In addition, oxidative stress causes inadvertent enzyme activation and oxidative damage to cellular system [10].

Cells are equipped with different kinds of mechanisms to fight against ROS and to maintain the redox homeostasis of cell. For example, antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) play important roles in scavenging the free radicals and preventing cell injury[11]. Molecules such as ascorbic acid and α-tocopherol inhibit lipid peroxydation in cell. When the mechanism of antioxidant protection becomes unbalanced in human body, antioxidant supplement may be used to help reduce oxidative damage.

Medicinal plants are an important source of antioxidants[12]. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases such as cancer, heart diseases and stroke[13]. The secondary metabolites like phenolics and flavonoids from plants have been reported to be potent free radical scavengers. They are found in all parts of plants such as leaves, fruits, seeds, roots and bark[14]. There are many synthetic antioxidants in use. It is reported, however, they have several side effects[15], such as risk of liver damage and carcinogenesis in laboratory animals[16, 17, 18]. There is therefore a need for more effective, less toxic and cost effective antioxidants. Medicinal plants appear to have these desired comparative advantages, hence the growinginterest in natural antioxidants from plants.

Dendrophthoe falcata (L.f) Ettingsh, commonly known as 'Banda' (Hindi) is an evergreen hemiparasitic shrub with bark smooth grey, leaves opposite unequal, thick 1.6 - 25.4 cm long, flowers single, orange-red or scarlet softly pubescent, berries soft ovoid-oblong, 1.3cm diameter and indigenous to India, Srilanka, Thailand, Indo-china, Australia[19]. The aerial parts are used in wounds, menstrual troubles, asthma, psychic disorders, pulmonary tuberculosis, consumption and mania by the tribal of India[20, 21]. Leaf paste is used in skin diseases and abortion[22]. Its paste is applied on boils, setting dislocated bones and extracting pus[23]. Traditional physicians of Korku, a tribe inhabiting the forest areas of Melghat region of Amravati district, Maharastra state of India, use *D. falcata* as antifertility agent in women[24]. In addition to its medicinal value, the fruit of *D. falcata* tastes sweet and is consumed as a food[25]. The plant has been scientifically proved to have antilithiatic, diuretic, cytotoxic, immunomodulatory, wound healing and chemopreventive potentials[26, 27, 28, 29]. Recently our research group reported the effects of *Dendrophthoe falcata* in lowering the incidence of breast tumor growth in the experimental female wistar rats[30, 31].The plant is also proved to have antifertility efficacy in wistar female rats[32]. The merit of the traditional use of *D. falcata* has also been reported by the isolation and identification of several possible active chemical constituents such as β-sitostirol, stigmasterol[33], kaemferol, quercetin–3-O-ramnoside, rutin, quercetin, myrecitin and their glycosides[34], (+)-catechin, leucocyanidine, gallic acid, chebulinic acid[35] and some pentacyclic triterpenes, kaempferol-3-O-α-l-rhamnopyranoside and quercetin-3-O-α-l-rhamnopyranoside, etc. [25]. It is evident from the above literature that *D. falcata* is therapeutically useful in oxidative stress induced diseases.

The aim of the present work was to evaluate the total phenolics content, flavonoid content of chloroform, methanol and hydroalcoholic extracts of *D. falcata*, and to evaluate its *in vitro* antioxidant potential. Because different antioxidant compounds may act *in vivo* through different mechanisms, no single method can fully evaluate the total antioxidant capacity. For this reason, several complimentary test systems, including 2,2′-azinobis – (3-ethylbenzothiazoline-6-sulfonic acid), 1,1-diphenyl-2-picrylhydrazyl, nitric oxide, hydroxyl radical, super oxide and reducing power assay methods were used in this study.

MATERIALS AND METHODS

Plant material and extract preparation: Fresh aerial parts of *D. falcata*, growing on the host plant *Azadirachta indica* were collected in the month of March from the thick forest areas of Similipal biosphere reserve, Mayurbhanj district of Orissa, India. *Dendrophthoe falcata*(L.f)Ettingsh (Loranthaceae) was authenticated from Botanical Survey of India, Hawrah, West Bengal, India (CNH/I-I/32/2010/Tech.II/237 - 2). One set of the herbarium has been preserved in our laboratory for future reference. The aerial parts were air-dried, pulverized to a coarse powder in a mechanical grinder, passed through a 40 mesh sieve and extracted with petroleum ether (60º - 80 ºC) and further with chloroform (CEDF), methanol (MEDF) and ethanol-water (8:2) (HEDF), separately. The extracts were decanted, filtered with Whatman No. 1 filter paper. The entire extraction process was repeated twice. The filtrates were individually pooled and solvents were removed from the filtrates under reduced pressure (Rotavapor RII, Buchi Labortechnik AG, Switzerland). Finally, *D. falcata* extracts were cooled in a dessicator for 30 min before the yield of each

extract was calculated. Extracts from the aerial parts of *Dendrophthoe falcata* were kept at 4^0C for further use.

Phytochemical screening:
An attempt was also made to observe the presence and absence of different phytochemical constituents in CEDF/MEDF/ HEDF. The tests for flavonoids, sterols and tannins were carried out by using the methods previously described by Tona *et al*. (1998) [36]. Two milligrams of each extract were separately dissolved in 2 ml of the adequate solvent. The detection of major chemical groups was carried out by thin-layer chromatography (TLC) on silica gel 60 F_{254} from Merck (Dramstadt, Germany) (layer thickness, 0.25mm) as follows: for flavonoids, TLC was developed in n-Butanol/acetic acid/water 4:1:5, then spots were visualized with 1% $AlCl_3$ solution in methanol under ultraviolet (UV) 366 nm. Terpenes and steroids were detected with Libermann-Burchard as a reagent using n-hexane/CH_2Cl_2 1:9 as a mobile phase. Ranges of colours are produced after heating sprayed plates for 10 min at 100^0C. Tannins were detected with $FeCl_3$. Each class of tannins gave a specific coloration. And alkaloids (Dragendorff's test), glycosides (Molisch's reagent), fixed oil (spot test), proteins (Ninhydrine test), carbohydrates (Fehling's solution test) and polysaccharides (Iodine test) were detected according to standard methods[37, 38].

Total Phenolic content (Folin-Ciocalteau assay):
Total phenolic contents of CEDF/MEDF/ HEDF extracts were determined using Folin–Ciocalteu assay[39]. Briefly, 0.1 ml of extracts solutions were mixed with 2.5 ml of 10-fold diluted Folin–Ciocalteau reagent, and 2.0 ml of 7.5% sodium carbonate (Na_2CO_3). After incubation at 40 ºC for 30 min, the absorbance of the reaction mixtures was measured at 760 nm by using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan). Three replicates were made for each test sample. Gallic acid was used as a standard and TPC of the extracts were expressed in milligram gallic acid equivalents (mg GAE/g extract).

Total flavonoid content:
Total flavonoid content was determined by the aluminium calorimetric method[40], using quercetin as a standard. Briefly, the test samples were individually dissolved in DMSO. Then, the sample solution (150 µl) was mixed with 150 µl of 2% $AlCl_3$. After 10 min of incubation at ambient temperature, the absorbance of the supernatant was measured at 435 nm by using a spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan). Three replicates were made for each test sample. The total flavonoid content was expressed as quercetin equivalents in miligram per gram extract (mg QRT/g extract).

***In vitro* antioxidant assays**
ABTS assay: The antioxidant potential was measured by 2,2´-azinobis – (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay that measures the relative ability of antioxidant substances to scavenge the $ABTS^+$ cation radical generated in the aqueous phase. 3.5 ml reaction mixture contained 0.17 mM ABTS, 25 – 250 µg/ml CEDF/MEDF/ HEDF/ ascorbic acid and phosphate buffer (pH 7.4). The method used was based on Miller and Rice-Evans (1996) [41] modified by Lister and Wilson (2001) [42]. The absorbance at 734 nm was measured using UV–vis spectrophotometer. The antioxidant capacities of samples were measured against the standard.

Determination of DPPH radical scavenging activity: The free radical scavenging activity was evaluated by the DPPH assay described by Blois (1958) [43]. In its radical form, DPPH absorbs at 517nm, but upon reduction by an antioxidant or a radical species, the absorption decreases. Briefly, 1ml of 0.25mM solution of DPPH in methanol was added to 1ml of CEDF/MEDF/ HEDF solution in methanol (25 – 250 µg/ml). After 20min, the absorbance was measured at 517nm. Ascorbic acid was used as a positive control. The percentage DPPH decolorisation of the sample was calculated by the equation,

% of DPPH scavenging =
$[(A_{control} - A_{extarct})/ A_{control}] \times 100$

Where A is the absorbance

Nitric oxide radical scavenging (NO) assay: The nitric oxide radical inhibition activity was measured by the method of Garratt (1964) [44] using Griess reagent. Briefly, sodium nitroprusside (10mM) in phosphate buffered saline was mixed with different concentrations of CEDF/MEDF/ HEDF and ascorbic acid dissolved in methanol and incubated at room temperature for 150 min followed by addition of 0.5 ml of Griess reagent (1% sulfanilamide, 2 % H_3PO_4 and 0.1% *N*-(1-naphthyl)ethylenediamine dihydrochloride). The absorbance of the chromophore formed was read at 546 nm.

Hydrohyl radical scavenging (OH) assay: Hydroxyl radical scavenging activity was determined by the method of Halliwell *et al.* (1987) [45] based on the ability to compete with deoxyribose for hydroxyl radicals. Hydroxyl radicals produced by the reduction of H_2O_2 by iron, in presence of ascorbic acid degrade

deoxyribose to form products, which on heating with 2-thiobarbituric acid (TBA) form a pink colored chromogen. Briefly, the reaction mixture, of a final volume of 1.0ml, containing 0.4 ml of 20mM sodium phosphate buffer (pH 7.4) , 0.1 ml of 25 – 250 µg/ml of CEDF/MEDF/ HEDF, 0.1 ml of 60 mM deoxyribose, 0.1 ml of 10 mM H_2O_2, 0.1 ml of 1 mM ferric chloride, 0.1 ml of 1 mM EDTA and 0.1 ml of 2 mM ascorbic acid, was incubated at 37º C for 1h. The reaction was terminated by the addition of 1 ml of 17 mM TBA and 1 ml of 17 mM trichloroacetic acid(TCA). The mixture was boiled for 15 min, cooled in ice, and the absorbance measured at 532 nm. Ascorbic acid was used as a positive control. Distilled water in place of test extracts or ascorbic acid was used as control and the sample solution without deoxyribose as sample blank.

Superoxide radical scavenging (SO) assay:
The super oxide anion scavenging activity was determined by the method of Nishimiki *et al.* (1972) [46]. SO anion derived from dissolved oxygen by a phenazine methosulfate(PMS)/NADH coupling reaction reduces nitroblue tetrazolium (NBT), which forms a violet colored complex. A decrease in color after addition of the antioxidant is a measure of its superoxide scavenging activity. To the reaction mixture containing phosphate buffer (100 mM, pH 7.4), NBT (1mM) solution, NADH (1mM) and CEDF/MEDF/ HEDF (25 – 250 µg/ml) in methanol, 1ml of 1mM PMS was added. After incubation at 25ºC for 5 min, the absorbance was measured at 560 nm against a blank. Ascorbic acid was used as a positive control.

Ferric reducing antioxidant power (FRAP) assay:
The reductive potential was determined according to the method of Oyaizu (1986) [47] based on the chemical reaction of Fe^{3+} to Fe^{2+}. To 100 – 500 µg/ml CEDF/MEDF/ HEDF and ascorbic acid standard in 1 ml of methanol, 2.5 ml each of phosphate buffer (0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (1% w/v) was added and the mixture incubated at 50 ºC for 20 min, followed by addition of 2.5 ml of TCA (10% w/v). The mixture was centrifuged for 10 min at 1000g, the upper layer (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% w/v), and the absorbance was measured at 700 nm.

Statistical analysis:
The data are expressed as Mean ± Standard Deviation (S.D.) from triplicate determination. Linear regression analysis was used to calculate IC_{50} values whenever needed. Data were analysed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test with equal sample size. The difference was considered significant when p value < 0.05.

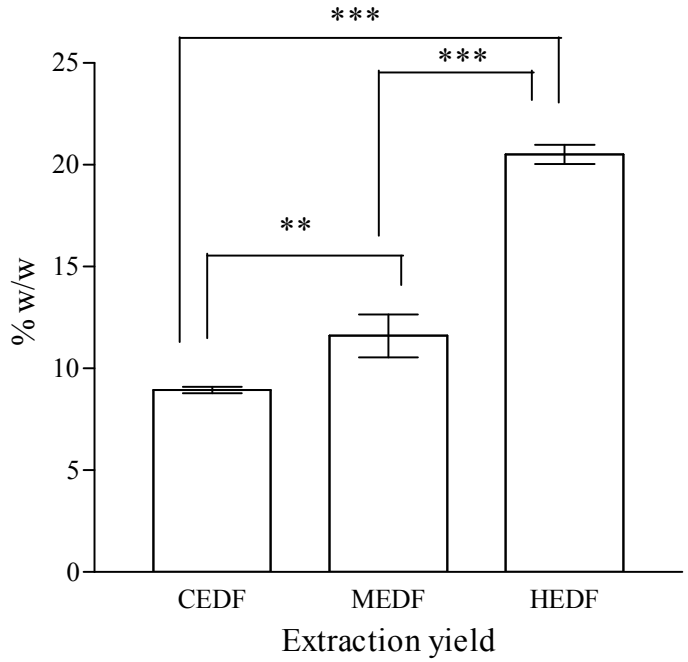


Fig.1: Extraction yield of *D. falcata* extracts (n=3);
Data are represented as mean ± S.D.; $^{**}p<0.01$; $^{***}p<0.001$.

Table 1: Total phenolics content and total flavonoid content of *D. falcata* extracts (n=3)

Sl. No.	Samples (extracts)	Total Phenolic content (mg GAE/g extract)	Total flavonoid content (mg QRT/g extract)
1	CEDF	21.78 ± 3.145	9.74 ± 1.324
2	MEDF	38.66 ± 1.862 a**	21.59 ± 1.09 a***
3	HEDF	46.43 ± 2.55 b***, c***	33.42 ± 2.083 b***, c***

Values are expressed as mean ± standard deviation (S.D.) from triplicate determination;
a, b: MEDF and HEDF compared to CEDF; c: HEDF compared to MEDF; **:p<0.01; ***p<0.001

RESULTS

Extraction yield: Fig. 1 represents the yield of *D. falcata* extracts. The yield of the extracts varied from 8.94 ± 0.164 % to 20.493 ± 0.469 %. Among all the tested extracts the highest yield was obtained from HEDF (p<0.001). The yield of different extracts from the areal parts of *D. falcata* is presented in the following order: HEDF > MEDF > CEDF (p<0.01). Low extraction yield of the CEDF is probably due to the low solubility of the major components of the areal parts in chloroform.

Phytochemical screening: The results of the preliminary phytochemical screening of the HEDF revealed the presence of steroids, terpenes, glycosides, tannins, proteins, flavonoids, carbohydrates and polysaccharides. CEDF showed the positive results for steroids, terpenes, flavonoid and MEDF revealed the presence of steroids, tannins, terpenes, glycosides and flavonoids.

Total phenolic content and total flavonoid content: The content of phenolics compounds in the different extracts were determined through a linear gallic acid standard curve ($y = 0.009 x + 0.044$, $r^2 = 0.9970$). The total phenolic content of the extracts varied form 21.78 ± 3.145 to 46.43 ± 2.55 mg GAE /g extract. The highest content of total phenolics compounds was detected in HEDF (46.43± 2.55 mg GAE/g extract) whereas the lowest content was measured in CEDF (21.78 ± 3.145 mg GAE/g extract) (p<0.001). Total phenolics content of the extracts is arranged in the following ascending order: CEDF < MEDF < HEDF (p<0.01) (Table 1). In this study, the total flavonoid content (TFC) of the extracts was evaluated by aluminum colorimetric assay. Quercetin (QRT) was used as a standard and the total flavonoid content of *D. falcata* extracts were expressed in miligram of quercetin equivalents per gram of extract (mg QRT/g extract) ($y=0.007 x + 0.131$, $r^2 = 0.9980$). The TFC of the extracts were varied from 9.74 ± 1.324 to 33.42 ± 2.083 mg QRT/g extract. The data presented in Table 1 indicates that the highest flavonoid content of 33.42 ± 2.083 mg QRT/g extract was observed in the HEDF and the lowest content was observed in CEDF (9.74 ± 1.324 mg QRT/g extract) (p<0.001). TFC of the extracts is arranged in the following sequence: HEDF > MEDF > CEDF (p<0.01).

***In vitro* antioxidant assay:** Analysis of the free radical scavenging activities of the extracts revealed a concentration-dependent antiradical activity resulting from reduction of $ABTS^+$ (fig. 2), DPPH (fig. 3), NO (fig. 4), OH^- (fig. 5) and SO (fig. 6) radicals to non-radical form. The scavenging activity of ascorbic acid, a known antioxidant used as positive control, was however higher and scavenging potential was in the order: ascorbic acid > HEDF > MEDF > CEDF. Fig. 7 presents the reduction potential of CEDF, MEDF and HEDF. The reducing power of ascorbic acid, CEDF, MEDF and HEDF increased gradually with increasing concentration. The order of the reduction potential was ascorbic acid > HEDF > MEDF > CEDF.

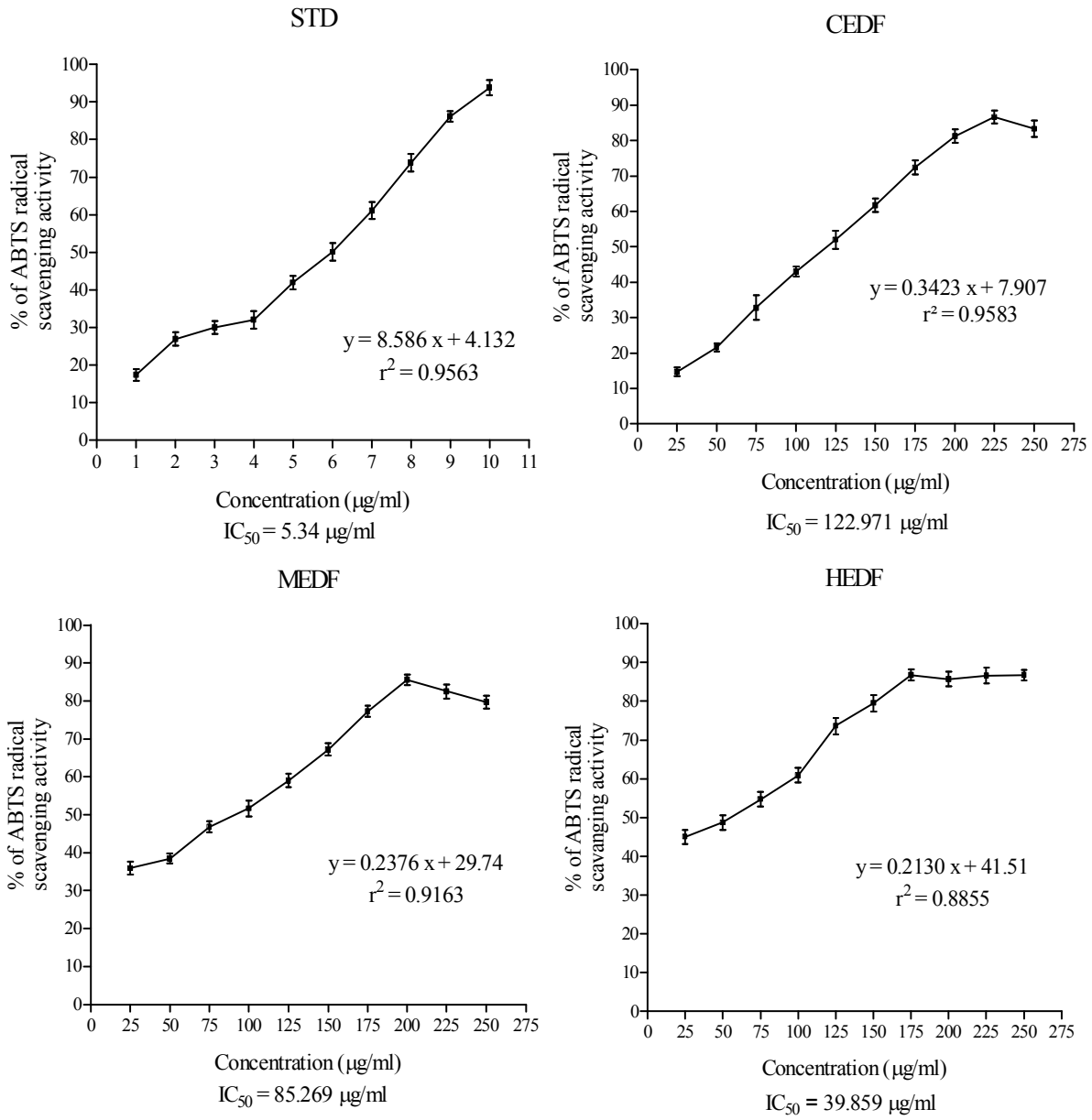


Fig. 2: Inhibition of ABTS radical by CEDF, MEDF and HEDF; Data are represented as mean ± S.D. of two independent experiments each.

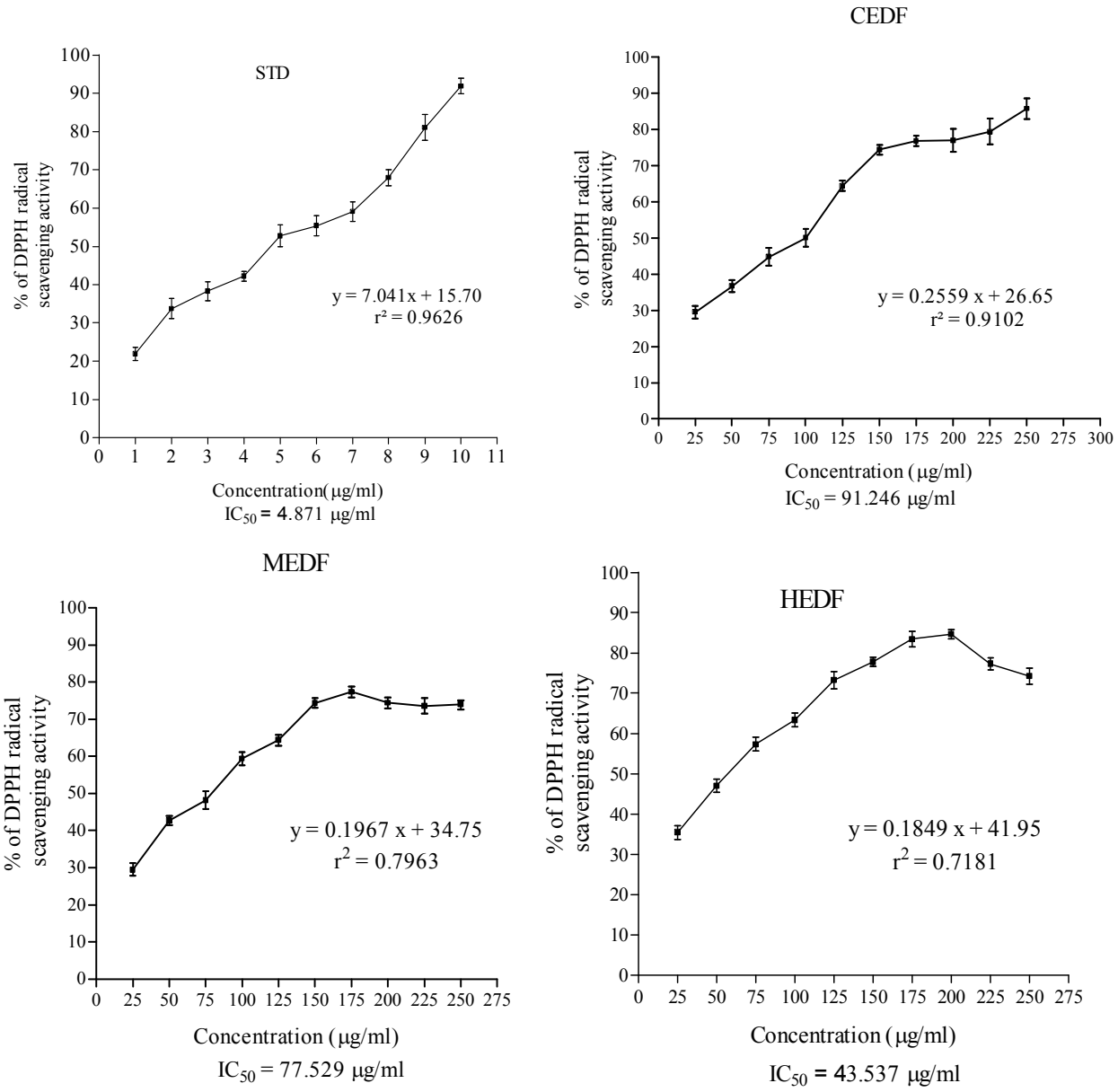


Fig. 3: Inhibition of DPPH radical by CEDF, MEDF and HEDF; Data are represented as mean ± S.D. of two independent experiments each.

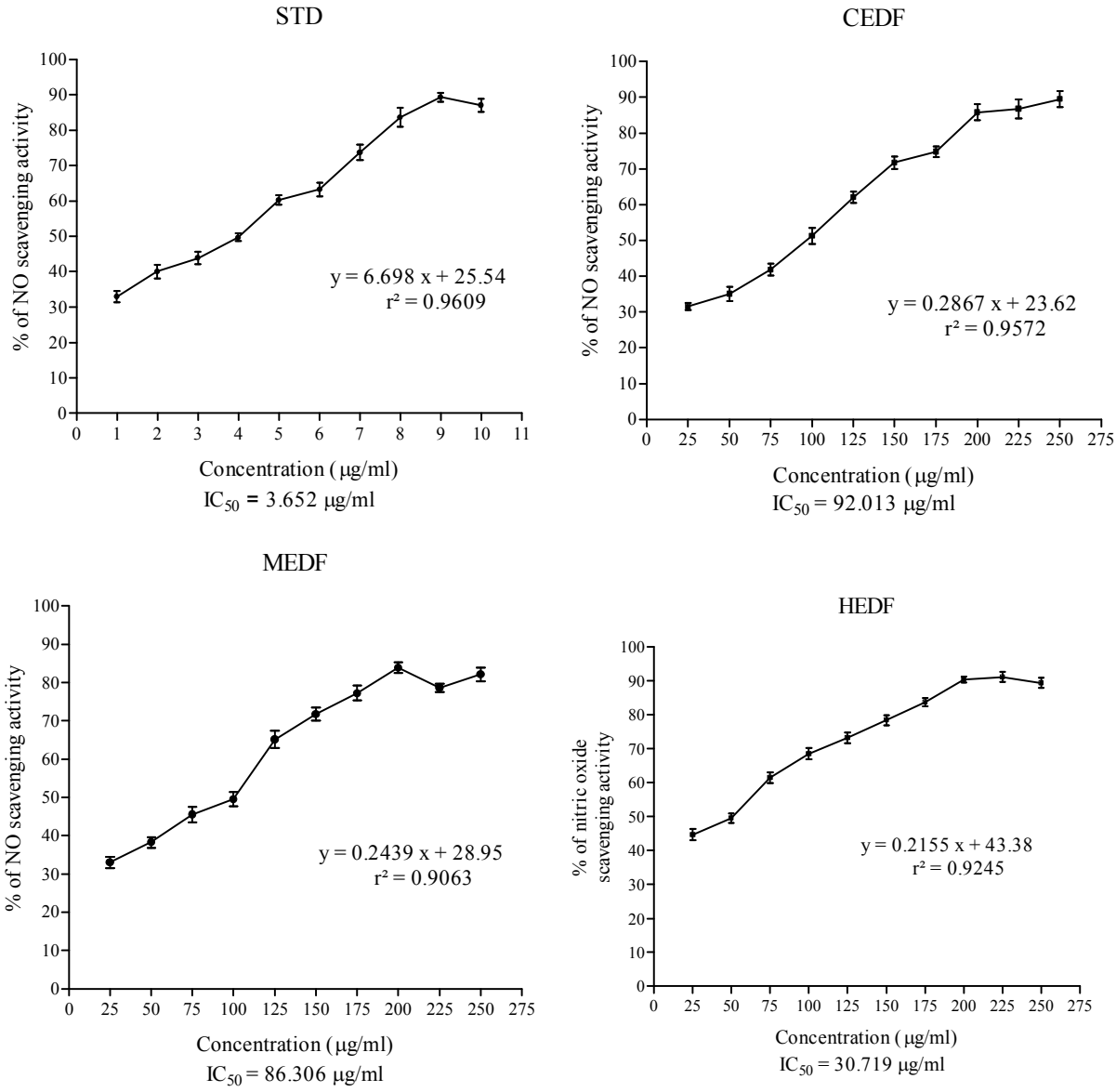


Fig. 4: Inhibition of nitric oxide radical by CEDF, MEDF and HEDF; Data are represented as mean ± S.D. of two independent experiments each.

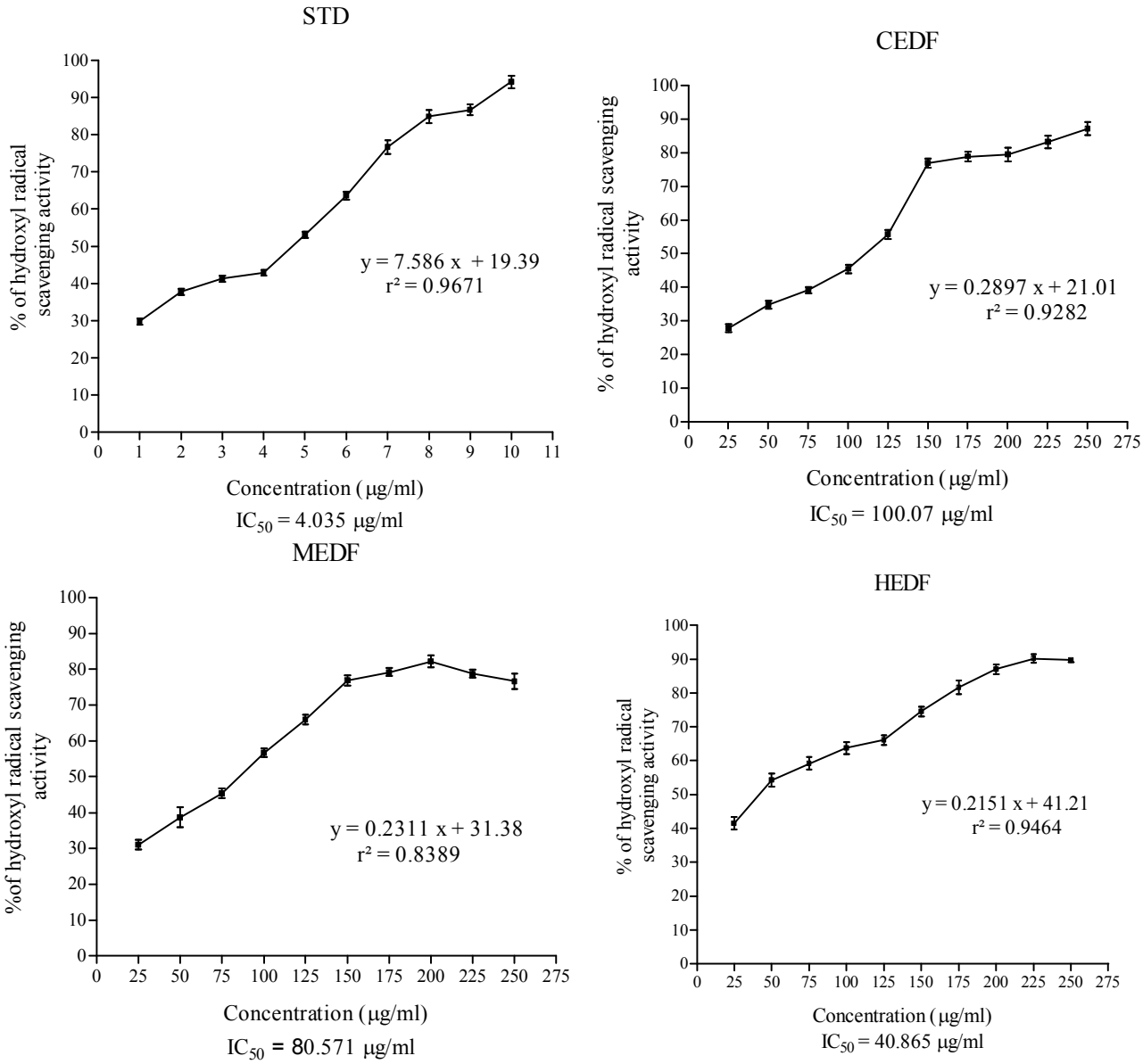
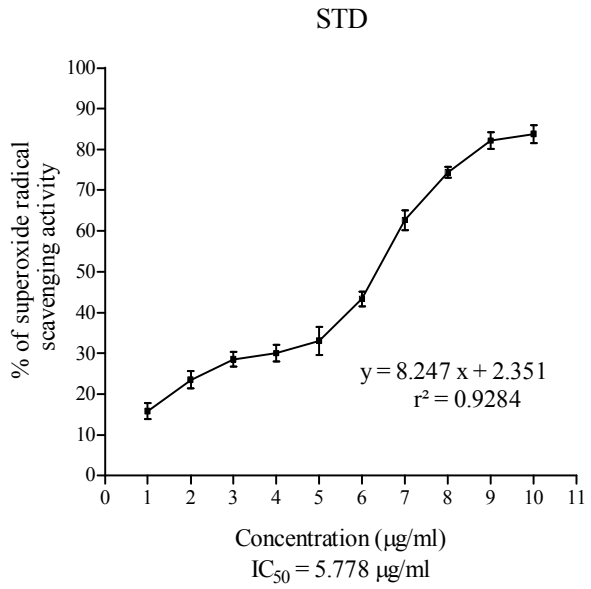
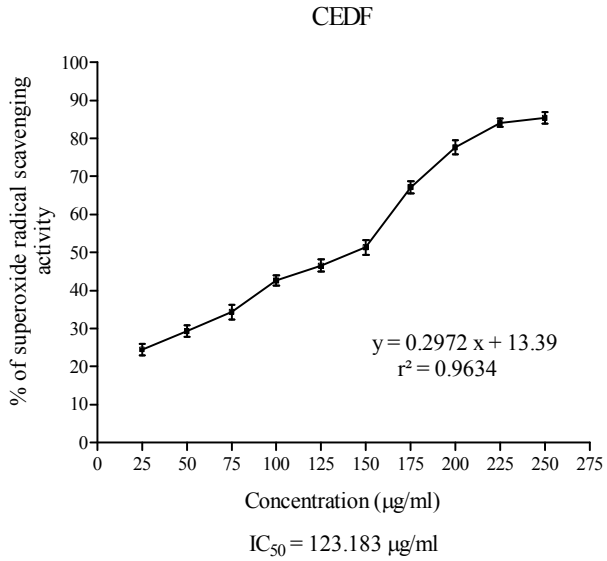
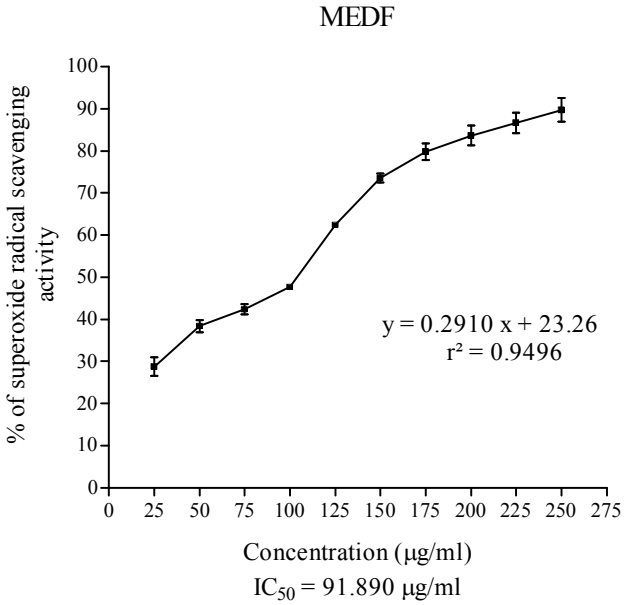


Fig. 5: Inhibition of hydroxyl radical by CEDF, MEDF and HEDF; Data are represented as mean ± S.D. of two independent experiments each.


STD
% of superoxide radical scavenging activity
y = 8.247 x + 2.351
r² = 0.9284
Concentration (µg/ml)
IC50 = 5.778 µg/ml


CEDF
% of superoxide radical scavenging activity
y = 0.2972 x + 13.39
r² = 0.9634
Concentration (µg/ml)
IC50 = 123.183 µg/ml


MEDF
% of superoxide radical scavenging activity
y = 0.2910 x + 23.26
r² = 0.9496
Concentration (µg/ml)
IC50 = 91.890 µg/ml

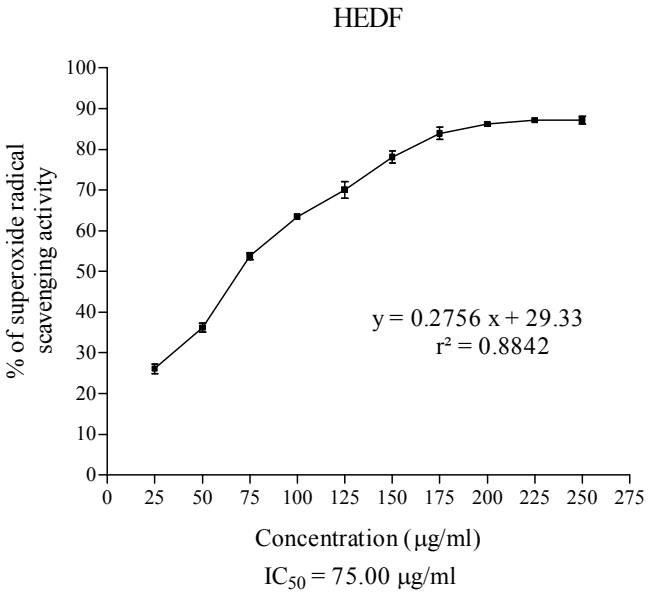

HEDF
% of superoxide radical scavenging activity
y = 0.2756 x + 29.33
r² = 0.8842
Concentration (µg/ml)
IC50 = 75.00 µg/ml

Fig. 6: Inhibition of super oxide radical by CEDF, MEDF and HEDF; Data are represented as mean ± S.D. of two independent experiments each.

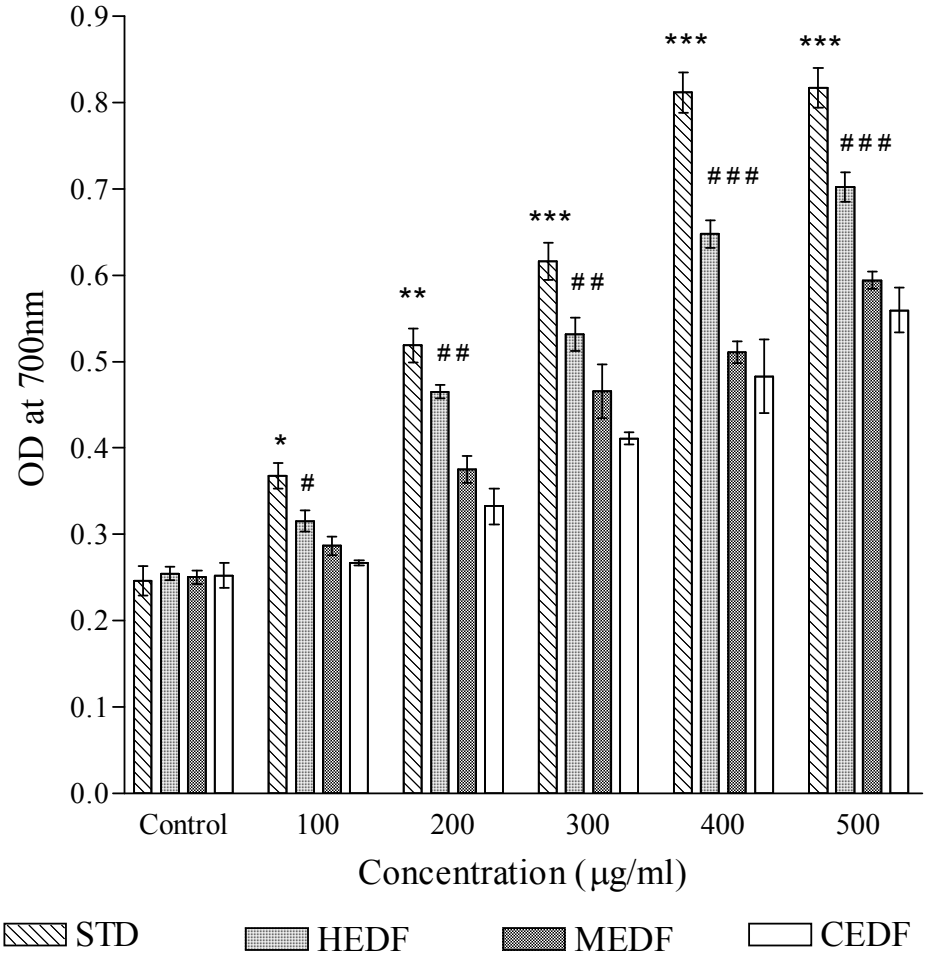


Fig. 7: The reduction potential of CEDF, MEDF and HEDF (mean ± S.D.; n = 6);

*: significantly different from HEDF, MEDF and CEDF ($p<0.05$); **: significantly different from HEDF, MEDF and CEDF ($p<0.01$); ***: significantly different from HEDF, MEDF and CEDF ($p<0.001$); #: HEDF significantly different form MEDF, CEDF ($p<0.05$); ##: HEDF significantly different form MEDF, CEDF ($p<0.01$); ###: HEDF significantly different form MEDF, CEDF ($p<0.001$).

DISCUSSION

These results suggest that the higher levels of antioxidant activity were due to the presence of phenolic components. The same relationship was also observed between phenolics and antioxidant activity in roseship extracts[48]. Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups[49]. The phenolic compounds may contribute directly to antioxidative action[50]. It is known that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when ingested up to 1 g daily from a diet rich in fruits and vegetables[51]. Phenolic compounds from plants are known to be good natural antioxidants. However, the activity of synthetic antioxidants was often observed to be higher than that of natural antioxidants[52]. Phenolic compounds, at certain concentrations, markedly slowed down the rate of conjugated diene formation. The interests of phenolics are increasing in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food[53]. The 2, 2′ -azinobis (3-ethyl-

benzothiazoline 6-sulfonate) (ABTS) formed from the reaction ABTS-e → $ABTS^+$ reacts quickly with ethanol/hydrogen donors to form colorless ABTS. The reaction is pH – independent. A decrease of the $ABTS^+$ concentration is linearly dependent on the antioxidant concentration. All extracts at tested doses (25 – 250 µg/ml) revealed good scavenging activity for $ABTS^+$ in a dose dependent manner, but the activity was higher in case of HEDF (IC_{50} = 39.859 µg/ml) (Fig. 2). The DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals was initiated by the lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [54]. The reduction capability of DPPH was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Positive DPPH test suggests that the samples were free radical scavengers. The scavenging effect of HEDF and ascorbic acid on DPPH radical was compared. On the DPPH radical, HEDF had significant scavenging effects with increasing concentration in the range of 25–250 µg/ml and when compared with that of ascorbic acid, the scavenging effect of HEDF was lower. The IC_{50} values were found to be 43.537, 77.529 and 91.246 µg/ml for HEDF, MEDF and CEDF, respectively (Fig.3). A higher DPPH radical-scavenging activity is associated with a lower IC_{50} value. Nitric oxide plays an important role in various types of inflammatory processes in the body. In the present study the crude extracts of the *D. falcata* aerial parts (CEDF, MEDF and HEDF) were checked for its inhibitory effect on nitric oxide production. Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by the extracts. The HEDF at varied concentrations showed remarkable inhibitory effect of nitric oxide radical- scavenging activity compared to CEDF and MEDF (Fig. 4). Results showed the percentage of inhibition in a dose dependent manner for all the extracts tested. The concentration of HEDF needed for 50% inhibition (IC_{50}) was found to be 30.719 µg/ml, whereas 86.306 and 92.013 µg/ml was needed for MEDF and CEDF, respectively. 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[55]. This radical has the capacity to join nucleotides in DNA and cause strand breakage which contributes to carcinogenesis, mutagenesis and cytotoxicity. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity [56]. The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins[38]. The effect of the extracts from *D. falcata* on the inhibition of free radical-mediated deoxyribose damage were assessed by means of the Iron (II)-dependent DNA damage assay. The Fentone reaction generates hydroxyl radicals (OH) which degrade DNA deoxyribose, using Fe^{2+} salts as an important catalytic component. Oxygen radicals may attack DNA either at the sugar or the base, giving rise to a large number of products. HEDF, MEDF and CEDF were also capable of reducing DNA damage at all concentrations used. Ascorbic acid was highly effective in inhibiting the oxidative DNA damage. As shown in Fig. 5, the extracts displayed potential inhibitory effect of hydroxyl radical-scavenging activity. All results showed hydroxyl radical scavenging activity in dose dependent manner. IC_{50} values were found to be 40.865, 80.571 and 100.07 µg/ml for HEDF, MEDF and CEDF, respectively (Fig. 5). 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 the chain reaction. Ascorbic acid was used as reference standard. Hagerman *et al.* (1998) [57] have also explained that high molecular weight and the proximity of many aromatic rings and hydroxyl groups are more important for the free radical-scavenging activity by phenolics than their specific functional groups. Super oxide is a reactive oxygen species, which can cause damage to the cells and DNA leading to various diseases. It was therefore proposed to measure the comparative interceptive ability of the antioxidant extracts to scavenge the super oxide radical. Several *in vitro* methods are available for generation of super oxide radicals [58]. In our study super oxide radicals were generated by auto-oxidation of hydroxylamine in presence of NBT (Nitro blue tetrazolium). The reduction of NBT in presence of antioxidants was measured. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. As shown in Fig. 6, the all extracts at varied concentrations had superoxide scavenging activity. IC_{50} values of CEDF, MEDF, HEDF and ascorbic acid were found to be 123.183, 91.890 and 75.00µg/ml, respectively. All of the extracts had a scavenging activity on the superoxide radicals in a dose dependent manner (25–250 µg/ml in the reaction mixture). Nonetheless, when compared to ascorbic acid, the superoxide scavenging activity of the extract was found to be low. Generally, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom[50]. The results of the ferric reducing assay indicated that HEDF had stronger

reducing power than CEDF and MEDF (Fig. 7). This could be due to the presence of more reactive concentration of bioactive constituents and mixture of other constituents in HEDF than other two extracts.

In the present study, there exists a positive correlation between the total phenolics content and the antioxidant activity which is in accordance with the earlier findings[59]. We found higher *in vitro* antioxidant activity in HEDF with higher polyphenols. The higher radical scavenging efficacy of HEDF may be due to retention of antioxidant phytochemicals in this extract. A strong evidence supports these findings that HEDF enhance the antioxidant effects *in vivo* rodent models of carcinogenesis[31].

Moreover, these results suggest that *Dendrophthoe falcata* may offer effective protection from free radicals and support that *D. falcata*, is a promising source of natural antioxidant. However, further work is required on the isolation and identification of the antioxidant components present in it since it is also a precondition for a more extensive understanding of the mechanisms involved in the antioxidant capacity.

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