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Free Radical Scavenging and *In-vitro* Antioxidant Effects of Ethanol Extract of the Medicinal Herb *Chromolaena odorata* Linn.

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Research Article

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ABSTRACT

The ethanol extract of the leaf of *Chromolaena odorata* (Linn) was assessed for free-radical-scavenging and antioxidant potentials. Ability of the extract to scavenge reactive intermediates (superoxide ion $O_2^{\cdot-}$, hydrogen peroxide H_2O_2, nitric oxide $NO^{\cdot}$, hydroxyl radical $OH^{\cdot}$) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, were used to assess its free radical scavenging potentials. Antioxidant potential was studied by assessing *in-vitro* inhibition of lipid peroxidation in both the brain (Neuro-protective potentials) and liver homogenates of Fenton-oxidant stressed rabbits. Inhibition of protein oxidation was assessed *in-vitro* by loss of protein thiol (P-SH), while assessment of the reducing power of the extract was further used to assess antioxidant capacity. Results obtained showed the ability of the extract to scavenge free radicals and reactive intermediates in a dose-response manner. The plant also had good antioxidant capacity. The secondary plant metabolites found earlier in the extract may explain reasons for the bio-efficacy of the plant. These findings are of great importance in view of the availability of the plant and its observed possible diverse applications in medicine and nutrition.

Keywords: Chromolaena odorata; hepatoprotection; antioxidation; reactive species scavenging;

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1. INTRODUCTION

Chromolaena odorata (L.) R. KING & H. ROBINSON (formerly *Eupatorium odoratum* L.), a perennial belonging to the *Asteraceae* (=Compositae) family, is a diffuse, scrambling shrub that is mainly a weed of plantation crops and pastures of Southern Asia and Western Africa. This common plant called Siam weed is known among the Igbos of the South-Eastern Nigeria as: 'Elizabeth', 'Independence leaf', 'Enugu plantation weed', or 'Awolowo weed'.

Results of a number of studies showed that the extract of the leaves of *C. odorata* has diverse biological activities ranging from inhibition of the growth of some bacteria (Alisi and Onyeze, 2009; Irobi, 1997) to enhancement of haemostasis and blood coagulation (Triaratana et al., 1991), anti-inflammatory, astringent, diuretic, and hepatotropic activities (Iwu, 1993) among others.

Several classes of flavonoids have been isolated from *C. odorata* extracts. The phenolic acids present in ethanol extract of *C. odorata* are protocatechuic, *p*-hydroxybenzoic, *p*-coumaric, ferulic and vanillic acids (Phan et al., 2001). The complex mixtures of lipophilic flavonoid aglycones present in *C. odorata* included flavanones, flavonols, flavones and chalcones. These phenolic compounds are major, powerful antioxidants to protect cultured skin cells against oxidative damage (Phan et al., 2001). Three flavanones and one flavone were isolated and proved to be responsible for blood coagulation. The extract also contains high concentrations of amino acids (Thang et al., 2001).

The potential antioxidant effect of this plant is of great importance because, there exists convincing evidence that oxidative stress triggered by reactive oxygen species (ROS) play an important role in the etiology and/or progression of a number of human diseases. The medical significance of oxidative stress has become increasingly recognized to the point that it is now considered to be implicated in virtually every disease process. Free radicals are known to be the major cause of various chronic and degenerative diseases, including aging, coronary heart disease, inflammation, stroke, diabetes mellitus and cancer (Cheng *et al*., 2003; Slater, 1984). Reactive oxygen species (ROS) which include $^{\cdot}O_2^-$ (superoxide anion), $^{\cdot}OH$ (hydroxyl radical), H_2O_2 (hydrogen peroxide) and $^{1/2}O_2$ (singlet oxygen) can cause cellular injuries and initiate peroxidation of polyunsaturated fatty acids in biological membranes (Compori, 1985; Halliwell and Gutteridge 1997). The tissue injury caused by ROS may also include DNA damage (Halliwell & Gutteridge, 1984), protein damage (Bartold *et al*; 1984), and oxidation of important enzymes (Varani et al., 1985) in the human body. These events could consequently lead to the emergence of various free radical- related diseases.

Recently, natural foods and food-derived antioxidants such as vitamins and phenolic phytochemicals have received growing attention, because they are known to function as chemo-preventive agents against oxidative damage. The beneficial health effects of fruits and vegetables have been attributed, in part, to antioxidant flavonoids present in them (Riboli and Norat, 2003; Bosetti et al., 2005; Graziani, 2005). Crude extracts of fruits, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest because they retard oxidative degradation of lipids (Rice – Evans et al., 1995; Hung and Yen 2002; Lee et al., 2003) by different mechanisms (Soobrattee et al., 2005).

Scanty information is available on the possible potential of *C. odorata* extract in free radical scavenging (Alisi and Onyeze, 2008). It is our intention in this work to evaluate the

antioxidant and free radical scavenging effects of *C. odorata* extract of the medicinal herb *Chromolaena odorata*-Linn.

2. MATERIAL AND METHODS

2.1 Collection and Preparation of Plant Samples

Fresh aerial parts of *Chromolaena odorata* were collected from Egbu and Ihiagwa in Owerri, Imo State in and authenticated by a plant taxonomist, at the Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. Voucher specimen has been retained at the authors' laboratory.

The leaves were shed, dried at 30^0C and then reduced to a coarse powder in a mill (Kenwood BL357). A 500g portion was extracted with 2 litre ethanol by shaking for 48hrs. Soluble extract was recovered by distillation under reduced pressure at 49^oC in a Buchi rotavapour (Switzerland). The extract was then dried to solid form in vacuum desiccator (CNS Simax), and stored in a freezer (4.0 ^{0}C) until needed.

2.2 Inhibition of Superoxide Radical Production

The effect of *C. odorata* on the superoxide radical production was evaluated using the nitroblue tetrazolium (NBT) reduction method of McCord and Fridovich (1969). In the EDTA-NACN-riboflavin-NBT system, superoxide anion derived from the dissolved oxygen by the coupling reaction reduces NBT. The decrease in the absorbance with antioxidants thus indicates the consumption of the generated superoxide anion in the reaction mixture. Briefly, the reaction mixture consisted of EDTA (6µM) + 3µg NACN, riboflavin (2µM), NBT (50µM), *C. odorata* extract (0 - 1000 µg/ml) and phosphate buffer (67mM, pH 7.8) in a final volume of 3ml. The tubes were uniformly illuminated with an incandescent lamp for 15 minutes and the absorbance was measured at 530nm before and after illumination. Superoxide radical-scavenging ability (SORSA) was calculated thus:

$$\text{SORSA (\%)} = 100 - \frac{\text{Absorbance of medium containing } \textit{C. odorata} \text{ X } 100}{\text{Absorbance of control medium}}$$

Where: absorbance of medium = absorbance after illumination – absorbance before illumination.

2.3 Scavenging of Hydrogen Peroxide Radical

The method as described by Wettashinge and Shaidi (2000) was used. Briefly, *C. odorata* ethanol extracts were dissolved in 3.4ml of 0.1M phosphate buffer (pH 7.4) and mixed with 600µl of a 43mM solution of hydrogen peroxide (prepared with the same buffer). (+)Catechin was used as the reference antioxidant. Concentration of extract and standard were graduated between 0 and 300µg/ml. The absorbance values of the reaction mixture were recorded after 30 minutes at 230nm wavelength against a reagent blank. The concentration (mM) of hydrogen peroxide in the medium was determined from its calibration curve ($y = 0.0072x$, $r^2=0.9978$). Percentage scavenging was calculated.

$$\%\text{Inhibition} = 100 - \frac{Test}{Control} \times 100 \quad \text{.....Eqn (1)}$$

2.4 Scavenging of Nitric Oxide Radical

Based on the principle that the compound sodium nitroprusside (SNP) is known to decompose in aqueous solution at physiological pH (7.2) producing $NO^{\cdot}$. Under aerobic conditions, $NO^{\cdot}$ reacts with oxygen to produce stable products: nitrate and nitrite, the quantities of nitrate and nitrite can be determined using Griess reagent. The scavenging effect of *C. odorata* extract on nitric oxide was measured according to the method of Marcocci et al. (1994) with little modification (Alisi and Onyeze, 2008). Briefly 4ml of extract solution at different concentrations were added (in the test tubes) to 1ml of sodium nitroprusside (SNP) solution (25mM) and the tubes incubated at 29°C for 2 hours. A 2ml aliquot of the incubation solution was diluted with 1.2ml Griess Reagent (1% sulfanilamide in 5% H_3PO_4 and 0.1% naphthylethylenediamine-dihydrochloride). The absorbance of the chromophore that formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine dihydrochloride was immediately read at 550nm and the concentration determined from a standard curve ($y = -0.4289x^2 + 1.1534x + 0.0093$, $r^2 = 0.9968$) of sodium nitrite salt treated in the same way with Griess reagent. Inhibition of nitrite formation by extract or the standard plant antioxidant (Quercetin) were calculated relative to the control (eqn. 1).

2.5 Scavenging of Hydroxyl Radicals

Free radical dependent 2-deoxyribose degradation was studied using the Fenton oxidant reaction mixture of Fe^{+3}/ascorbic acid and H_2O_2 as described by Halliwell *et al*. (1987). Hydroxyl radical scavenging ability of *C. odorata* extract was measured by studying the competition between deoxyribose and the test compounds for hydroxyl radicals generated from the Fe^{3+} / ascorbate / EDTA / H_2O_2 system. The reaction mixture contained: Deoxyribose(2.8mM), $Fecl_3$ (0.1mM), EDTA(0.1mM), H_2O_2 (1mM), ascorbic acid(0.1mM) KH_2PO_4–KOH buffer (20mM, pH 7.4) and the extract (0-3000µg/ml) in a final volume of 1.0ml. After incubation for 1 hr at 37°C, the deoxyribose degradation was measured as thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al*. (1979), as modified by Liu et al., (1990). Briefly, 1.5ml of 20% acetic acid (pH 3.5), 1.5ml of 0.8% thiobarbituric acid (TBA) and 0.2ml of 8.1% sodium dodecyl sulphate (SDS) prepared in distilled water and the mixture incubated at 100°C for 1 hr, cooled and 2ml of trichloroacetic acid added. The mixture was vortexed vigorously and centrifuged at 3000g for 10 minutes and the absorbance of the supernatant read at 532nm wavelength (λmax). Concentration of TBARS was determined using its molar extinction. Hydroxyl radical scavenging of extract was calculated relative to control (eqn. 1).

2.6 Scavenging of 1,1-diphenyl-2-picrylhydrazyl (dpph) Radical

The scavenging activity of extract for the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured as described by Velazquez *et al* (2003). Radical scavenging activity of *C. odorata* extract for the radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) was measured on the principle that antioxidants reduce the DPPH radical to a yellow-coloured compound (diphenylpicrylhydrazin) and the extent of the reaction will depend on the hydrogen donating

ability of the antioxidant (Bondent et al., 1997). Extracts were dissolved in methanol at concentrations of 2.65–170 mg/ml, and 0.75 ml of each sample was mixed with 1.5 ml of DPPH (0.02 mg/ml) in methanol, with methanol serving as the blank sample. The mixtures were left for 15 min at room temperature and its absorbance then measured at 517 nm. Catechin (0–50 mg/l) was used as positive controls. The radical-scavenging activity was calculated as follows:

$$\% \text{ DPPH radical Scavenging} = \frac{\text{Blank absorbance - Sample absorbance}}{\text{Blank absorbance}} \times 100$$

2.7 Evaluation of Possible Hepatoprotective Effect

The possible hepatoprotective effect of the extract was assessed using the possible inhibition of lipid peroxidation in rabbit liver homogenates. Fe^{2+}-ascorbic acid mixture is well known to stimulate lipid peroxidation in rat liver *in vivo*, and microsomes and mitochondria of rat liver *in vitro.* Antioxidants inhibit the metal-catalysed initiation of peroxidation. Reaction mixtures containing rabbit liver homogenate in Tris HCl, ferrous ammonium sulphate, ascorbic acid, and different concentrations of extract were incubated and the resulting thiobarbituric acid reacting substances (TBARS) measured by the method of Ohkawa *et al*., (1979) as modified by Liu et al., (1990) as previously described. Briefly, 1,5ml of 20% acetic acid (pH 3.5), 1.5ml of 0.8% thiobarbituric acid (TBA), 0.2ml of 8.1% sodium dodecyl sulphate (SDS) and the incubation mixtures were heated at 100^{o}C for one hour, cooled and 2ml of TCA added. The mixtures were vortexed vigorously and centrifuged at 3000g for 10minutes and its absorbance read at 532nm wavelength (λmax). Concentration of each thiobarbituric acid reactive substances (TBARS) tube was determined using the molar extinction coefficient of 1.56 x $10^{5}M^{-1}cm^{-1}$ (Raja et al., 2007).

2.8 Evaluation of Possible Neuroprotective Potential

Neuroprotective potential of the extract was measured by the ability of *C. odorata* extract to inhibit lipid peroxidation in rabbit brain. This was studied by incubating rabbit brain homogenate treated with hydrogen peroxide (10 µM) and different concentrations of *C. odorata* extract (0–800 µg/ml) Muralikrishnan et al., (2008). Whole rabbit brain was homogenized in phosphate buffered saline (10% w/v). Rabbit brain homogenate (200µl), *C. odorata* extract from 0 to 800µg/ml, Hydrogen peroxide (10µM), were incubated for one hour. Thiobarbituric acid reactive substances were measured according to the schemes of Liu et al. (1990) as previously described.

2.9 Measurement of the Effect of *C. odorata* Ethanol Extract on Protein Oxidation

The effect of *Chromolaena odorata* extract on protein oxidation was investigated according to the modified method of Wang *et al* (2006). This method measures loss of protein thiol (P-SH) groups. Bovine serum albumin (BSA) 1mg/ml was incubated at 25^{0}C in solution with 2.5mM H_2O_2, 1.0mM $FeCl_2$, 1.0mM Ascorbate and 3.0mM EDTA in the presence of the plant extract. After incubation for 45 minutes, protein was precipitated with 10% TCA, centrifuged at 5000rpm for 10 minutes and the supernatant decanted. Protein pellets were dissolved in 1ml of 50mM potassium phosphate buffer of pH 7.5. Ellmann's reagent (5,5-Dithiobis -2-nitrobenzoic acid) was used to determine protein sulphydryl group (PSH) at 412nm

wavelength. Percentage inhibition of protein oxidation was calculated relative to control (without the extract).

2.10 Reducing Power Determination

The reducing power of the prepared *Chromolaena odorata* extract was determined according to method of Oyaizu (1986). This method is used to investigate the Fe^{3+}/Fe^{2+} transformation in the presence of the test compound. Different amounts (0 - 5.0mg) of the plant extract or standard (Butylated hydroxyl toluene) were dissolved in a buffered medium to give final concentrations of 0-1000µg/ml in a reaction mixture mixed thoroughly with 2.5ml of 0.2ml phosphate buffer at pH 6.6 and 2.5ml of 1% $K_3Fe(CN)_6$. Each mixture was incubated at 50^0C for 20 minutes before adding 2.5ml of 1% TCA and centrifuging at 3000rpm for 5 minutes. Thereafter, 2.5ml of the upper layer of the solution was mixed with 2.5ml of distilled water and 0.5ml of 0.1% $FeCl_3$. The absorbance was read at 700nm. Reducing power was determined from the plot of optical density against concentration of extract. Reducing power (RP 0.5_{AU}) was taken as the concentration of extract able to give 0.5 absorbance reading.

2.11 Data Analysis

The inhibition data (means values from triplicate determinations) were fitted into kinetic equations (2-6) which are LogNormCum, logistic dose response, decay 1+1 kinetic sigmoid abcd, and sigmoid abc models respectively. The parameters were estimated by iterative minimization of least squares using Levenberg-marquardt algorithm (Table curve 2D SYSTAT USA) Marquardt (1964).

2.11.1 Equations

$$\%\text{Inhibition} = 100 - \frac{Test}{Control} \times 100 \qquad \text{.....eqn 1}$$

$$y = a + 0.5\text{berfc}\{-\ln(x/c)/(2^{0.5}d)\} \qquad \text{.....eqn 2}$$

$$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d} \qquad \text{.....eqn 3}$$

$$y = a + b\exp(-cx) + d\exp(-ex). \qquad \text{..... eqn 4}$$

$$y = a + \frac{b}{1 + \text{Exp}\left(-\frac{(x - c)}{d}\right)} \qquad \text{.....eqn 5}$$

$$y = \frac{a}{1 + \exp\left(-\frac{x - b}{c}\right)} \qquad \text{.....eqn 6}$$

3. RESULTS

Result obtained showed that the ethanol extract of *C. odorata* caused a dose-dependent scavenging of superoxide radicals (Figure 1a). The consumption followed a LogNormCum

(eqn. 2), where a = 2.2910672, b=130.6974, c=362.5704 d=1.9312477. R^2-Value = 0.9857. Threshold inhibitory concentrations are shown in Table 1. The percentage hydrogen peroxide scavenging activity of the ethanol extracts of *C. odorata* increased with increase in extract concentration. Scavenging of hydrogen peroxide followed a logistic dose response (eqn 2). Threshold % Hydrogen peroxide scavenging ability of *C. odorata* (PSC) is shown in Table 1. Decomposition of sodium nitroprusside with formation of nitrite (Figure 1c) mimicked decay 1 + 1 (abcde) kinetic model (eqn 4). Nitrite concentration was logistically diminished with increase in extract concentration. % Inhibition of nitrite formation followed a logistic dose response model, (eqn. 3). Threshold inhibitory concentrations are shown in Table 1. Results obtained in the present study (Figure 1d) showed that increase in extract concentration caused a decrease in the thiobabituric acid reactive substances formation. Percentage Hydroxyl radical scavenging activity followed a logistic dose response (eqn.3). Threshold % Hydroxyl radical scavenging activities of *C. odorata* are shown in Table 1.

The DPPH radical is a stable organic free radical with an absorption maximum band around 515-528nm. It is a useful reagent for evaluation of antioxidant activity and free radical scavenging ability of compounds (Sanchez-Moreno, 2002). In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-coloured compound, diphenylpicrylhydrazin, and the extent of the reaction depended on the hydrogen donating ability of the antioxidant (Bondent et al., 1997). The result of the DPPH-radical scavenging by ethanol extract of *C. odorata* (Figure 1e) showed that percentage reduction of DPPH radical to a yellow-coloured compound by ethanol extract of *C. odorata* had a sigmoid relationship with its graded concentrations. Scavenging of the radicals by the extract and standard increased in a dose-dependent manner that mimicked sigmoid model (abcd, and abc) (Eqn 5 & 6). Percentage reduction of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical followed a sigmoid (abc) model - equation (6), Threshold inhibitory concentrations are shown in Table 1.

Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generate a number of degradation products. Malondialdehyde, one of the products of lipid peroxidation has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress (Janero, 1990). Fe^{2+}-ascorbic acid mixture used in this study is well known to stimulate lipid peroxidation in rat liver *in* vivo, and microsomes and mitochondria of rat liver *in vitro.* The ethanol extract of *C. odorata* diminished lipid peroxidation of the rabbit liver homogenate in a dose dependent fashion. This was evident in the malondialdehyde concentrations which were reduced as the extract concentration increased (Table 2). The results (Table 2) showed that the ethanol extract of *C. odorata* compared with quecetin as a good inhibitor of the lipid peroxidation process in liver homogenates.

Lipid peroxidation in biological systems has long been thought to be a toxicological phenomenon that can lead various pathological consequences (Hochstein and Atallah, 1988). The results obtained from the studies showed that the ethanol extracts of *C. odorata* inhibited the formation of the thiobarbituric acid reactive substances (TBARS) (Table 2). Percentage inhibition of % H_2O_2-induced Lipid peroxidation in Rabbit brain homogenates are as shown in Table 2. The pattern of inhibition was a logistic dose response. Inhibition of % H_2O_2-induced Lipid peroxidation in Rabbit brain homogenates are shown in Table 1. Ethanol extract of *C. odorata* suppressed lipid peroxidation by extending the lag phase and reducing the propagation rate in a similar fashion with that of known antioxidants.

Table 1. Threshold inhibitory concentrations, scavenging ability of Ethanol extract of *C. odorata* on some free radicals and reactive intermediates

Free radicals and reactive intermediates	Threshold Inhibitory concentration/Scavenging power of Ethanol extract of *C. odorata* (µg/ml)				
	IC_{20}	IC_{50}	IC_{70}	IC_{80}	IC_{100}
Superoxide radical ($^{\cdot}O_2^-$) scavenging	57.66 ± 4.03	222.38 ± 14.66	*	686.92 ± 15.56	1639 ± 48.10
Hydrogen peroxide (H_2O_2) scavenging	6.86 ± 0.46	17.66 ± 1.23	*	43.90 ± 3.20	241.85 ± 8.90
Nitric oxide radical ($NO^{\cdot}$) scavenging	0.602 ± 0.04	9.860 ± 0.68	*	123.55 ± 8.10	3041.60 ± 106.90
Hydroxyl radical ($^{\cdot}OH$) scavenging	33.70 ± 2.30	151.48 ± 9.61	*	559.76 ± 39.20	2188.00 ± 153.16
2,2-Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging power	41.84 ± 2.93	124.08 ± 8.50	220.59 ± 15.11	299.35 ± 20.95	657.46 ± 46.01

*Data are means ± standard deviations of triplicate determinations. * = Not determined*

Table 2. Threshold inhibitory concentrations of ethanol extract of *C. odorata* on oxidative breakdown of some macromolecules

Parameters	Threshold Inhibitory concentration of Ethanol extract of *C. odorata* (µg/ml) on oxidation of macromolecules				
	IC_{20}	IC_{50}	IC_{70}	IC_{80}	IC_{100}
Lipid peroxidation in liver homogenates	57.66 ± 4.03	222.38 ± 14.66	*	686.92 ± 15.56	1639 ± 48.10
Lipid peroxidation in Brain homogenates	50.22 ± 0.46	177.97 ± 1.23	*	867.90 ± 3.20	3717.74 ± 8.90
Protein oxidation	17.85 ± 0.04	120.13 ± 0.68	*	381.60 ± 8.10	7077.2 ± 106.90

*Data are means ± standard deviations of triplicate determinations. * = not determined*

The effect of *C. odorata* ethanol extract on Bovine serum albumin (BSA) oxidation induced by Fe^{2+}-ascorbate-H_2O_2 system was used to assess the extracts ability to inhibit protein oxidation. Free radicals generated by the Fenton oxidant mixture caused oxidation of Protein-SH groups.

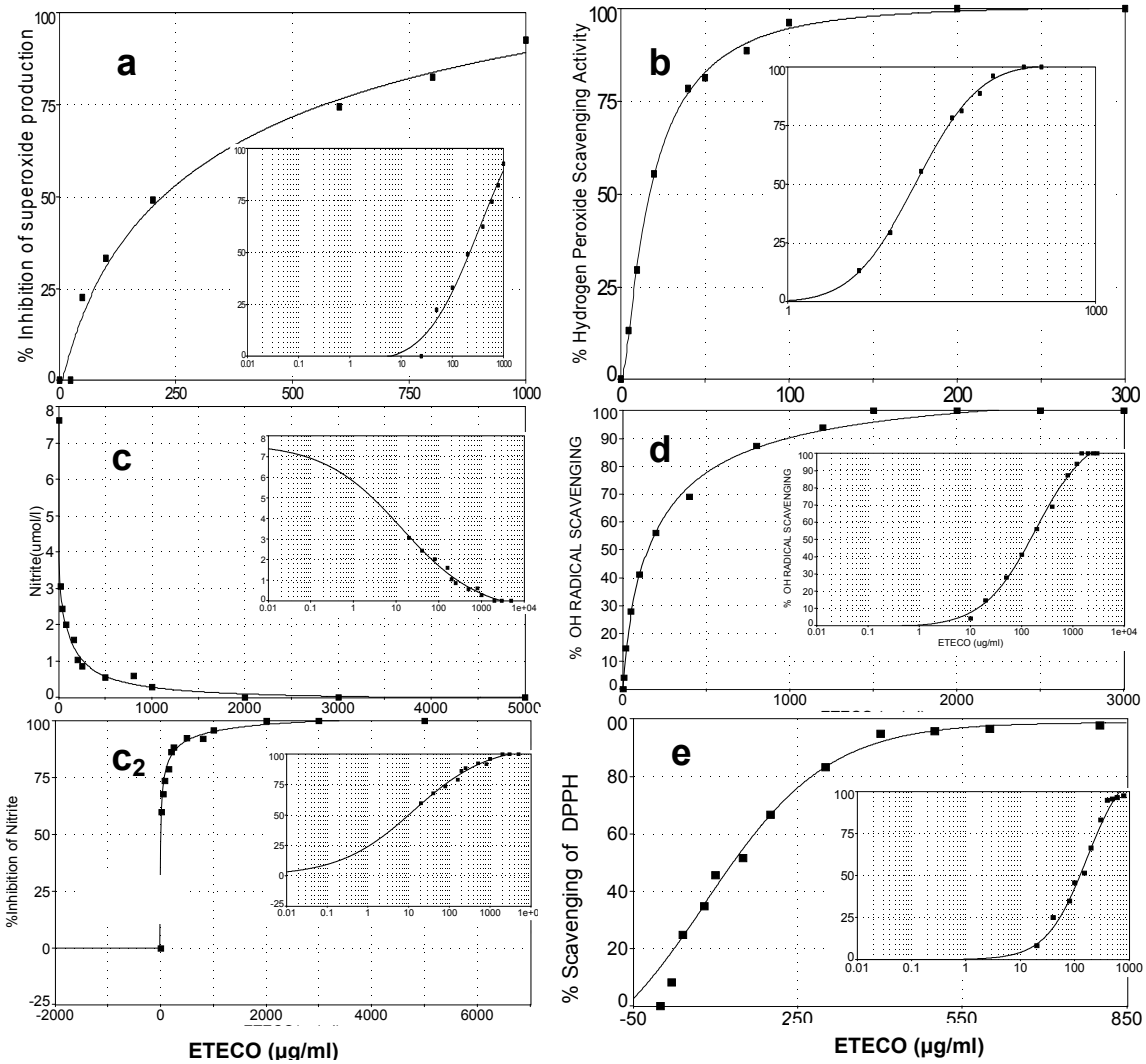

Fig. 1. Effect of graded concentrations of ethanol extract of *C. odorata* on scavenging of (a) superoxide radicals, (b) hydrogen peroxide, (c) nitric oxide radicals, (d) hydroxyl radicals, and (e) DPPH radicals. Insets are logarithm scale of the x-axis.

The oxidative protein damages, provoked by free radicals, have been demonstrated to play a significant role in aging and several pathological events (Stadtman and Levin, 2000). Radical mediated damages to proteins might be initiated by electron leakage, metal-ion dependent reactions, and autoxidation of lipids and sugars (Dean et al., 1997). The extract inhibited protein oxidation in a dose dependent fashion (table 2). Percentage inhibition of

protein oxidation followed a logNormCum (a,b,c,d), model - equation (2). Inhibitory concentrations of protein oxidation are shown in Table 2.

The reducing capacity of compound may serve as a significant indicator of its potential antioxidant activity (Hsu et al., 2006). We investigated the Fe^{3+}/Fe^{2+} transformation in the presence of the test compound (*C. odorata*). Increased absorbance of the reaction mixture correlates with greater reducing power. Reducing power of the ethanol extract of *C. odorata* was (RP 0.5_{AU} = 142.59 µg/ml) (Figure 2a,b) and compared with standard. RP 0.5_{AU} is the amount of extract able to give 0.5 absorbance unit. Reducing power was dose dependent and obeyed a sigmoid association (eqn. 6) (R^2 = 0.9963). Eqn. 6 on logarithmic scale gave a good linearization of reducing power.

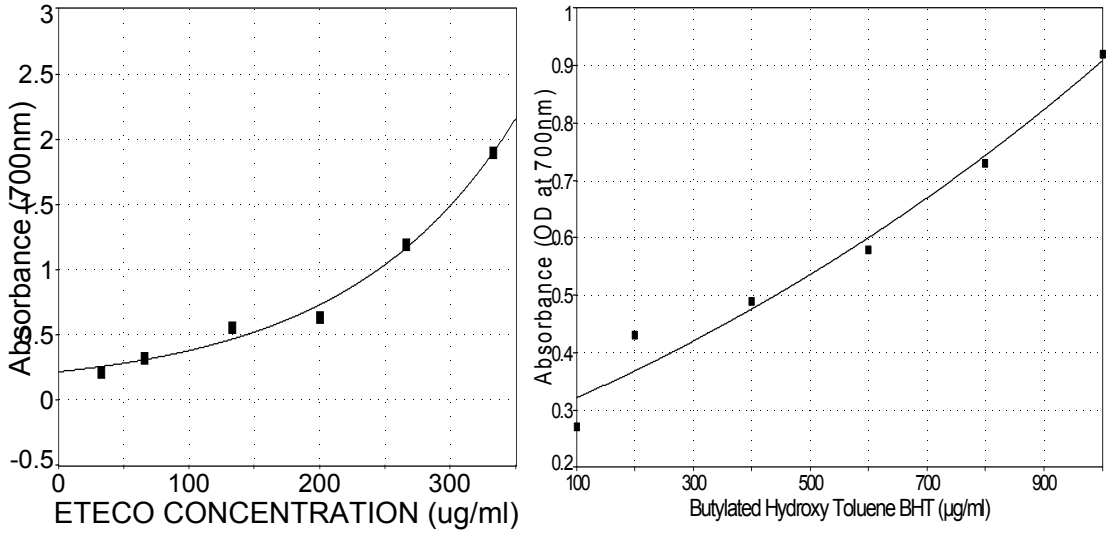

Fig. 2. Reducing power of ethanol extract of *C. odorata* (RP 0.5_{AU} is the concentration of extract able to give 0.5 absorbance reading

4. DISCUSSION

Under oxidative stress conditions, superoxide radical ($O_2^{\bullet -}$) acts as an oxidant of [4Fe–4S] cluster-containing enzymes and facilitates •OH production from H_2O_2 by making Fe^{2+} available for the Fenton reaction (Leonard et al., 2004). Scavenging of superoxide radicals by *C. odorata* extract (Figure 1a) is considered an early step in the intervention strategy against oxidative stress (Valko et al., 2005). Fluxes of superoxide radicals have been found to inactivate and nitrate the human superoxide dismutase enzyme thereby reducing antioxidant status (Demicheli et al., 2007). Endothelial dysfunction has been observed in abnormal glucose metabolism (Esper et al., 2008). This dysfunction has been associated with increased superoxide anion production leading to various vascular complications. *C. odorata* may bring about reduction in vascular complication via scavenging of superoxide.

Results obtained in the present study showed that ethanol extract of *C. odorata* scavenged hydrogen peroxide (Figure 1b). We had shown that the extract is rich in phenolic compounds (Alisi and Oyeze 2008). Scavenging of H_2O_2 by *C. odorata* may be attributed to their phenolic content which could donate electrons to H_2O_2, thus neutralizing it to water. The neutralization

of H_2O_2 by ethanol extract of *C. odorata* will prevent its participation in the Haber-weiss reaction. Neutralization of H_2O_2 by *C. odorata* extract is a valuable intervention in the process leading to modification of proteins, lipid peroxidation, strand damages in DNA, and indeed modification of all molecules in the living cell.

Result of the study (Figure 1c), showed that the ethanol extract of *C. odorata* scavenged nitric oxide *in vitro*. Nitric oxide scavenging effect of *C. odorata* extracts could be due to diverse phytochemicals including the phenolic compounds in the plant. Phenolic compounds have been implicated in antioxidant metabolism (Chung et al., 1998) and the nitric oxide scavenging activity of flavonoids and phenolic compounds are known (Crozier et al., 2000; Jagethia et al., 2004). Emerging trends in antioxidant research point to the fact that other secondary plant metabolites, vitamins and mineral elements may contribute to the synergy that translates to higher medicinal value. Both the accumulation of oxidative stress markers during and the impaired respiration after reoxygenation can be prevented by blocking NO production. Scavenging of nitric oxide radical may be one antioxidant mechanism of *C. odorata* extract.

Increase in *C. odorata* extract concentration caused a corresponding decrease in the thiobabituric acid reactive substances concentration. •OH is one of the most damaging free radicals in the body and can be important mediator of damage to cell structures, nucleic acids, lipids and proteins (Valko et al., 2007). The •OH scavenging capacity of the extracts of *C. odorata* was high enough to consider it as one possessing antioxidative potentials. The hydroxyl radical scavenging activity of *C. odorata* may be partly due to the presence of *p*-hydroxy benzoic (*p*-HBA). *p*-HBA has been found to be an important natural antioxidant. It is well established as an *in vitro* effective hydroxyl radical scavenger and has Trolox equivalent antioxidant activity (TEAC) (Rice-Evans et al., 1997).

The extract had a DPPH radical scavenging ability which is less than that found in catechin. Catechin was three fold as strong as ethanol extract of *C. odorata* in DPPH radical scavenging. It is a useful tool for evaluation of antioxidant activity and free radical scavenging ability of compounds (Sanchez-Moreno, 2002).

The ethanol extract of *C. odorata* inhibited lipid peroxidation process in the rabbit liver and brain homogenates in a dose dependent fashion (Table 2). The extracts were effective against oxidative attack on lipids. It suppressed lipid peroxidation by extending the lag phase and reducing the propagation rate, thus reflecting typical characteristic of a chain breaking antioxidant, similar to that of known antioxidant, butylated hydroxyl toluene (BHT). It is known that metal-catalysed generation of ROS results in an attack not only on DNA and proteins, but also on other cellular components involving polyunsaturated fatty acid residues of phospholipids, which are extremely sensitive to oxidation (Siems et al., 1995). Inhibition of lipid peroxidation in rabbit brain homogenate is analogous to neuro-protection (Muralikrishnan et al., 2008). The brain is particularly vulnerable to oxidative damage because of its high oxygen utilization, its high content of oxidisable polyunsaturated fatty acids and the presence of redox-active metals (Cu, Fe). Naturally occurring antioxidants have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress (Shahidi, 2000). Polyphenols cross the blood–brain barrier and can exert their antioxidant and iron chelating properties in the brain. Polyphenols such as (-)-epigallocatechin-3-gallate prevents N-methyl- 4-phenyl-1, 2,3,6-tetrahydropyridine (MPTP)-induced dopaminergic neurodegeneration (Levites et al., 2001). Protection of the polyunsaturated fatty acids in the rabbit brain by *C. odorata* is a

measure of neuro-protection and can be exploited in the management of neurodegenerative disease.

The effect of *C. odorata* ethanol extract on Bovine serum albumin (BSA) oxidation induced by Fe^{2+}-ascorbate-H_2O_2 system was used as a good assessment of its ability to inhibit protein oxidation. Free radicals generated by the Fenton oxidant mixture caused oxidation of Protein-thiol groups. Results of the present study (Table 2) indicated that the ethanol extract of *C. odorata* inhibited oxidation of the protein (–SH) moiety in a dose response fashion. This implied that the extract may have acted by blocking electron leakage, metal-ion dependent reactions, and autoxidation. The -SH moiety of cysteine is highly prone to oxidative attack by several mechanisms, leading to the formation of disulfide bonds and thiyl radicals (Shacter, 2000). At the experimental concentrations (0 - 2000µg/ml), the extract could not achieve complete inhibition of protein (–SH) oxidation. Inhibition of protein-SH oxidation by the plant extract showed that the ethanol extract of *C. odorata* was capable of protecting the body from glutathione depletion.

C. odorata have a good reducing power (RP 0.5_{AU} = 142.59µg/ml) (Figure 2). Most antioxidants and pharmacologically therapeutic agents used in the treatment of oxidative stress related diseases have been shown to have strong reducing power (Amin and Razieh, 2007). Based on these results, it might be concluded that *C. odorata* ethanol extract is an electron donor capable of neutralizing free radicals. Reducing power provides effectiveness in the conversion of free radicals to more stable products and thus brings about the termination of free radical initiated chain reaction.

5. CONCLUSION

C. odorata exerts some of its antioxidant effects by scavenging of superoxide ion $O_2^{\cdot-}$, hydrogen peroxide H_2O_2, nitric oxide $NO^{\cdot}$, and hydroxyl radical OH. It exerts reducing power and protects biological macromolecules against oxidative attack. Based on these results, it might be concluded that *C. odorata* ethanol extract is an electron donor capable of neutralizing free radicals.The total phenolic compounds, flavonoids, and other secondary plant metabolites found in the extract are thought to be acting as proton sink and synergistically bring about the antioxidant and free radical scavenging potentials observed. This explains reasons for the bio-efficacy of the plant thus justifying its ethno-medical usage. These findings are of great importance in view of the availability of the plant and its observed possible diverse applications in medicine and nutrition.

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