ANTIOXIDANT POTENTIAL OF *CLERODENDRON VISCOSUM VENT.* ROOTS

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Summary

The free radical scavenging potential of the roots of *C. viscosum* was studied by using different antioxidant models of screening. The ethanolic extract at 1000 μg/ml showed maximum scavenging of the radical cation, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate)(ABTS) observed upto 98.92% followed by scavenging of nitric oxide radical (96.75%), ferric ion radical (94.43%), 1,1- diphenyl, 2-picryl hydrazyl (DPPH) (92.25%) and antilipid peroxidation potential (81.13%) The aqueous extract showed only moderate activity. The finding justifies the therapeutic application of the plant in the indigenous system of medicine, augmenting its therapeutic value.

Keywords: *Clerodendron viscosum*, Antioxidant, Free radicals.

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Introduction

It is increasingly being realized that a majority of the disease of today are due to the shift if the balance of the pro-oxidant and the antioxidant homeostatic phenomenon in the body. Pro-oxidant conditions dominate either due to the increased generation of the free radicals or due to the excessive oxidative stress of the depletion of the dietary antioxidant[1]. Free radicals have been implicated in causation of ailments such as cancer, inflammation, diabetes, liver cirrhosis, nephrotoxicity etc [2]. Together with other derivatives of oxygen they are inevitable by products of biological redox reaction [3]. Reactive oxygen species (ROS) such as superoxide anions ($O_2^{\cdot -}$), hydroxyl radical (.OH), ferric ion and nitric oxide (NO) inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation [4], and thus have been shown to augment collagen synthesis and fibrosis. The increased production of toxic oxygen derivatives is considered a universal feature of stress conditions. Plants and other organisms have evolved a wide range of mechanisms to contend with this problem, with a variety of antioxidant molecules and enzymes.

Clerodendrum viscosum Vent. (Verbanaceae) are gregarious tawny-villous shrub found through out India as undergrowth in forest up to 1800 meters and as a weed along the roadsides and waste lands [5]. The plant has been used traditionally as antiseptic, anti-inflammatory, anti pyretic, vermifuge, expectorant and in the treatment of snakebites, scorpion sting, leprosy and skin diseases [6, 7, 8]. Traditionally *Clerodendrum viscosum* has been also been used in the treatment of tumors [9]. A detailed review of literature afforded no information on the antioxidant potential of the plant. It was therefore worthwhile to investigate the antioxidant potential of *Clerodendron viscosum*.

Materials and Methods

All chemicals and solvents were of analytical grade and were obtained from Ranbaxy Fine Chemicals, Mumbai, India. 1,1- diphenyl, 2-picryl hydrazyl (DPPH), was obtained from sigma Chemicals, USA. The other chemicals used were 2,2-azinobis-(3-ethylbenzothiazoline-6-sulphonate)(ABTS), sodium nitroprusside, sulphanilamide,

potassium superoxide, o-phosphoric acid, napthyl ethylene diamine dihydrochloride, potassium chloride, ferric chloride, ferrous sulphate, thio barbituric acid, trichloro acetic acid (TCA), butyrated hydroxy toluene (BHT), nitroblue tetrazolium(NBT), dimethyl sulphoxide (DMSO), ethylene diamine tetra acetic acid (EDTA), ortho- phenanthroline and sodium hydroxide (NaOH).

The roots of *Clerodendron viscosum* were collected from the local areas of Udupi district, Karnataka, India during September 2004 and were authenticated by Prof. Gopalakrishna Bhat, Department of Botany, Poorna Prajna College, Udupi. The voucher specimen P515a has been submitted in herbarium of the college.

Preparation of Extracts

Total Aqueous Extracts: The dried powders (24#) 600 g were extracted with water by boiling on a water bath for 30 mints. The extracts were concentrated and dried under controlled temp of 60^{o}C.

Total Ethanolic extract: The shade dried powdered roots (500g) were exhaustively extracted with 95% ethanol using a soxhlet apparatus. The total Ethanolic extract was concentrated in vacuo to a syrupy consistency (yield 200g)

Preparation of rat brain homogenate [10] – Adult Wistar rats of either sex and of approximately the same age weighing about 200-250 g were used. The rats were fed with standard chow diet (Pranav Agro Industries Ltd., Sangali, and Maharashtra) and water *ad libitum*. They were housed in polypropylene cages maintained under standard conditions (12:12 hr L:D cycles; $25^{0} \pm 3^{0}$C; 35-60 % RH). The experimental protocols were subjected to the scrutinization of the Institutional Animal Ethics Committee and were cleared by the same (No: IAEC/ KMC/ 06/2004-2006). Randomly selected rats were fasted overnight. They were sacrificed by cervical dislocation, dissected and the whole brain except cerebellum was removed quickly. It was further processed to get 10% homogenate in 0.15 *M* KCl [11] using a Teflon homogeniser. The homogenate was filtered to get a clear solution and used as a source of polyunsaturated fatty acids for determining the extent of lipid peroxidation.

In vitro antilipid peroxidation assay- The extent of lipid peroxidation in rat brain homogenate was thiobarbituric acid reactive substances (TBARS). Different concentrations of the extracts (2-1000 µg/ml) were made up with ethanol and double distilled water. The ethanolic extract and aqueous extract were expressed in terms of dry weight (mg/ml) in ethanol and double distilled water. These samples were individually added to the brain homogenate (0.5 ml). This mixture was incubated with 0.15 M KCl (100 µl). Lipid peroxidation was initiated by adding 100 µl of 15 mM $FeSO_4$ solution. The reaction mixture was incubated at 37^0C for 30 minutes. An equal volume of TBA: TCA (1:1, 1ml) was of 1ml BHT. This final mixture was heated on a water bath for 20 min at 80^0C and cooled, centrifuged and absorbance read at 532nm [12] using a spectrophotometer (Shimadzu 160 IPC). The percentage inhibition of lipid peroxidation was calculated by comparing the results of the test with those of controls not treated with the extract as per the formula:

$$\text{Inhibition \%} = \frac{(\text{Control-Test})}{\text{Control}} \text{ X } 100$$

ABTS radical cation decolorization assay [13]– ABTS radical cation (ABTS) was produced by reacting ABTS solution (7 mM) with 2.45 mM ammonium per sulfate and the mixture was allowed to stand in dark at room temperature for 12 – 16 hr before use. For the study, different concentration (2 – 1000 µg/ ml) of the different extracts (0.5 ml) were added to 0.3 ml of ABTS solution and the final volume was made up with ethanol and double distilled water to make 1 ml. The absorbance was read at 745 nm [10] and the percentage inhibition calculated by using the same formula as given above.

DPPH radical scavenging activity **-** DPPH scavenging activity was measured by the spectrophotometer method [14]. To an ethanol solution of DPPH (200 µ M), 0.05 ml of ethanolic extract and aqueous extract were dissolved in ethanol and double distilled water were added at different concentration (2-1000 µg/ ml). An equal amount of ethanol and double distilled water was added to the control. After 20 minutes, the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated [12].

Scavenging of nitric oxide radical [15,16]- Nitric oxide was generated from sodium nitroprusside and measured by Griess's reaction as described previously[17,18]. Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentrations (2-1000 µg/ ml) of the extracts dissolved in phosphate buffer (0.025 M, pH 7.4) and the tubes were incubated at 25°C for 5 hr. control experiments without the compounds but with equivalent amounts of buffer were conducted in an identical manner. After 5 hr, 0.5 ml of incubation solution was removed and diluted with 0.5 ml of Griess's reagent (1% sulphanilamide, 2% O- Phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiment was repeated in triplicate.

Reduction of ferric ions by ortho- phenanthroline colour method [20]- Ortho substitute phenolic compounds were found more active than unsubstituted phenol. Hence, these compounds may exert pro- oxidant effect by interacting with iron. In the presence of scavenger, reduction of ferric ions will occur which is measured at 510 nm. The reaction mixture consisting of 1 ml ortho- phenanthroline (0.005g in 10 ml methanol), 2 ml ferric chloride 200 µM (3.24 mg in 100 ml distilled water) & 2 ml of various concentrations of the extracts (2 – 1000 µg/ml) were incubated at ambient temperature for 10 minutes. Then the absorbance of the same was measured at 510 nm. The experiment was performed in triplicate.

Results and Discussion

Several concentrations, ranging from 2-1000 µg/ml of the ethanolic extract and aqueous extract of roots *C. viscosum* were tested for their antioxidant activity in vitro models. It was observed that the test compounds scavenged free radicals in a concentration dependent manner up to the given concentration in all models (Table1 and Table2). The maximum percentage inhibition at 1 mg/ml concentration in all the models via, ABTS, DPPH, nitric oxide, lipid peroxidation and ferric ion of alcoholic extract, were 98.92, 92.25, 96.75, 81.13 and 94.43 % and aqueous extract were 71.05, 64.54, 69.47, 55.29 and 65.12 %.. Alcoholic extract showed better antioxidant activity than aqueous extract.

Oxidative stress has been implicated in the pathology of many diseases and conditions including diabetes, cardiovascular diseases, inflammatory conditions, cancer and ageing[2]. Antioxidant may offer resistance against the oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by many other mechanisms and thus prevent disease [21].

The peroxidation of membrane lipids initiated by oxygen radicals may lead to cell injury. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl-perferryl complex [22] or through OH radical by Fenton reaction [23] thereby initiating a cascade of oxidative reactions. The results obtained in the present studies may be attributed to several reasons viz, the inhibition of ferryl-perferryl complex formation; scavenging of OH or superoxide of OH or superoxide radical or by changing the ratio of Fe^{3+} / Fe^{2+}; reducing the rate of conversions of ferrous to ferric or by chelation of the iron itself [24]. The moderate activity of the extract may probably be due to the rapid and extensive degradation of the antioxidant principles in an *ex vivo* state thereby corroborating the finding that was observed in the study carried out in Australia with a group of human volunteers [25]. It is also known that the .OH radical which initiates lipid peroxidation has a very short lifetime (10^{-9} s at 37^0C) and hence very difficult to investigate by conventional method [26].

The ABTS assay is based on the inhibition of the absorbance of the radical cation $ABTS^+$, which has a characteristic long wavelength absorption spectrum [19]. The results obtained imply the activity of the extract either by inhibiting or scavenging properties of antioxidants towards $ABTS^{\cdot+}$ radicals have been reported earlier [13,27].

Nitric oxide is a free radical produced in mammalian cells, involved in the regulation of various physiological processes. However, excess production of NO is associated with several diseases [28,29]. In the present study, the nitrite produced by the incubation of solutions of sodium nitroprusside in standard phosphate buffer at 25^0C was reduced by the ethanolic and aqueous extract of *Clerodendron viscosum*. This may be due to the antioxidant principles in the extract, which complete with oxygen to react with nitric oxide [30] thereby inhibiting the generation of nitrite.

DPPH is a reactively stable free radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical $DPPH^{\cdot}$ From the present results, it may be postulated the *Clerodendron viscosum* reduces the radical to the corresponding hydrazine when it reacts with the hydrogen donors in the antioxidant principles [19]. DPPH radicals react with suitable reducing agents, the electrons become paired off and the solution loses color spectrophotometrically depending on the number of electrons taken up [31].

Reduction of ferric ions by ortho- phenanthroline, ortho- substituted phenolic compounds were found more active than unsubstituted phenol. Hence, these compounds may exert pro-oxidant effect by interacting with iron. In the presence of scavenger, reduction of ferric ions will occur which is to be measured [20].

Table 1: Effect of Alcoholic extract of Clerodendron viscosum on free radical scavenging activity using different models

S.No	Con μg/ml	% Scavenging				
		DPPH	ABTS	Nitric oxide	Ferric ion	Lipid peroxidation
1	2	3.52	1.31	10.08	56.04	18.18
2	4	8.9	3.60	12.36	55.83	29.67
3	8	10.41	3.71	18.78	57.21	34.14
4	16	27.10	7.65	24.31	58.14	39.23
5	32	38.01	13.69	54.28	64.80	47.74
6	64	66.40	18.54	73.31	74.22	59.51
7	128	90.33	40.82	89.96	81.77	65.78
8	256	92.21	65.69	93.20	92.32	79.25
9	512	92.21	98.88	95.21	94.43	79.61
10	1000	92.25	98.92	96.75	94.43	81.13

Table 2: Effect of Aqueous extract of *Clerodendron viscosum* on free radical scavenging activity using different models

S.No	Con μg/ml	% Scavenging				
		DPPH	ABTS	Nitric oxide	Ferric ion	Lipid peroxidation
1	2	4.48	2.47	11.69	41.12	11.35
2	4	1.15	7.35	14.29	40.32	14.16
3	8	2.17	10.61	16.73	41.17	18.95
4	16	2.28	17.02	21.15	42.65	22.76
5	32	7.30	18.21	31.65	44.11	27.11
6	64	9.35	20.07	36.19	46.29	28.20
7	128	19.68	22.84	48.20	58.10	45.72
8	256	37.15	49.19	54.65	64.36	51.53
9	512	64.15	70.47	69.09	65.25	53.66
10	1000	64.54	71.05	69.47	65.12	55.29

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