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Invitro antioxidant evaluation of the various extracts of *Clerodendrum inerme* gaertn leaves and root

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ABSTRACT

Clerodendrum inerme Gaertn leaves and root was extracted with different solvents like Pet ether, Chloroform, ethyl acetate, ethanol and aqueous, using different protocols Soxhlet extraction followed by cold-extraction especially for aqueous. To evaluate the antioxidant potential of the extracts, two *invitro* methodology were employed, i.e., DPPH(2,2-diphenyl-1-picrylhydrazyl)and Phospho molybdenum assay. All extracts exhibited antioxidant activities particularly ethanolic root extract exhibited strong antioxidant DPPH radical scavenging activity with IC_{50}value 41.52 μg/mL and Phospho molybdenum assay with IC_{50} value 51.26 μg/mL respectively. The strongest antioxidant activity of ethanol extract of root could be due to the presence of flavonoids and phenols. The results showed that the ethanolic extracts, could be considered as natural antioxidants and may be useful for curing diseases arising from oxidative deterioration. Findings offer affirmation that the crude ethanol extract of *C. inerme* root is a potential wellspring of natural antioxidants and this legitimized its uses in old folklore claim.

Keywords*:* *Clerodendrum inerme Gaertn,* DPPH; Phospho molybdenum.

1. INTRODUCTION

Free radicals are any molecules which have one or more unpaired electron in the furthest shell[1]. These unpaired electrons are extremely unstable and can assault contiguous atoms, for example, lipids, proteins and sugars and instigate cell harm[2]. Physiological and biochemical procedures of living cells may bring about the era of free radicals and other responsive oxygen species as by-items. Free radicals can bring about oxidative harm to lipids, proteins and DNA, in the long run prompting numerous perpetual sicknesses, for example, tumor, diabetes, maturing and other degenerative malady in people [3]. The fundamental characteristic of an antioxidant agent is its capacity to trap free radicals which may oxidize nucleic acids, proteins, lipids or DNA. A few techniques were created as of late to measure the total antioxidant capacity (TAC) as a result of the trouble in measuring every antioxidant agent independently[4,5].

Antioxidant agents from characteristic sources assume a foremost part in helping endogenous cell reinforcements to kill oxidative stress. A few epidemiological, clinical and exploratory information recommend that plant based antioxidants agents have useful impacts in light of avoidance on incessant ailments[6,7].

Clerodendrum inerme Gaertn commonly grown along the seacoasts on both sides, in tidal forests, often planted in gardens and perhaps run wild inland. It is a strangling shrub, with corolla-tube usually 1-1.5 in. long and a dry brown drupe. It is called as Batraj in Hindi, Pisung in Telugu and Pinchil, Sangam in Tamil and Garden Quinine in English. The traditional uses of the plant was found to be useful in treatment of febrifuge, eczema, scabies, leucorrhoea, poisonous bites and glandular swellings[8,9]. Though the information on the physiological properties of the *Clerodendrum inerme* Gaertn was known, its antioxidant properties are not thoroughly worked out. The objectives of this study were to determine the antioxidant potential of leaves and roots of *Clerodendrum inerme* by DPPH and total antioxidant capacity (TAC) by Phosphomolybdenum assay.

2. MATERIALS AND METHODS

Plant material Leaves and Roots of *Clerodendrum inerme* Gaertn was collected from local areas of Chennai and was authenticated by Botanical Survey of India (BSI/SRC/5/23/2012-13/Tech-705). Plant material was shade dried and ground into uniform powder using milling machine to obtain a coarse powder and then passed through a 40 mesh [10]. The successive extraction procedures were carried out using soxhlet apparatus with Pet Ether, Chloroform, Ethyl acetate and Ethanol as a solvent followed by aqueous extract by cold maceration technique. Then the Leaves and root extract of *Clerodendrum inerme* Gaertn was concentrated to dryness under reduced pressure and used for the experiment.

2.1. *Invitro* antioxidant study (*DPPH Radical scavenging test).*

Radical scavenging activity of plant extracts against stable DPPH was determined by the slightly modified method of Brand-Williams et al 1995[11,12]. DPPH reacts with an antioxidant compound, which can donate hydrogen, and reduce DPPH. The antioxidant scavenging activity of serial concentrations of different extracts (20:100 µg/mL) on DPPH free radical will be performed according to the method of Chen, et al. [13]. 1.0 ml (0.25 mM) DPPH in methanol was added to 2.0 mL solution of the extracts and standard, and allowed to stand at room temperature in a dark chamber for 30 min. The change in colour from deep violet to light yellow was then measured at 514 nm on a spectrophotometer. The decrease in absorbance was then converted to percentage radical scavenging antioxidant activity using the formula. The non-reacted radical form of DPPH, absorbed in the visible range, and the spectroscopic method is based on the measurement of color intensity at 514 nm against blank solution.

The decrease in optical density of DPPH will be calculated in relation to control as follows:

% Inhibition = ((A control - A sample) / A control) x 100

Whereas Blank = Methanol (1.0 mL) plus sample solution (2.0 ml), negative control =DPPH solution (1.0 mL, 0.25 mm) plus Methanol (2.0 mL), Ascorbic acid was used as standard

2.2 Evaluation of Total antioxidant capacity by Phospho molybdenum method

The total antioxidant capacity of the ethanol extract was evaluated by the phospho molybdenum method. The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid ρH. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The absorbance of the reaction mixture was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Ethanol (0.3 ml) in the place of extract was used as the blank[14,15,].

3. RESULTS AND DISCUSSION

The DPPH radical searching test is a simple fast and sensitive strategy for the cancer antioxidant screening of plant extracts. Various strategies are accessible for the determination of free radical searching action however the measure utilizing the stable DPPH has gotten the greatest consideration attributable to its usability and its comfort. DPPH is one of a few stable and commercially available organic nitrogen radicals. A freshly prepared DPPH solution exhibits a deep violet color with absorption maximum at 514 nm. Upon reduction, the solution color fades to yellow and the reaction progress is conveniently monitored by a spectrophotometer. Thus, antioxidant molecules can quench DPPH free radicals either by electron donation or by providing hydrogen atoms resulting in a decrease in absorbance at 514 nm. This test is a commonly employed assay in antioxidant studies of specific compounds or extracts across a short time-scale [16,17].The main advantage of DPPH is that its reduction is easily measured spectrophotometrically and it gives reliable information on the ability of the tested compounds. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability.

Table 1, 2 and figure 1, 2, shows the amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} values of the extract and L-ascorbic acid. In the present study, all extracts were found to be effective scavengers against DPPH radical. The antioxidant activities of various extracts were compared with the reference standard antioxidant ascorbic acid. The results showed that the ethanolic extract of root and leaves showed better anti-oxidant property when compared to other extracts.

The result of total antioxidant activity (TAC) is shown in table 3, 4 and figure 3, 4. It is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid ρH. TAC of the phosphor molybdenum model evaluates both water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity). The results indicate a concentration dependent total antioxidant capacity. It means that the ethanol extract of *C.inerme* root will contain as much quantity of

antioxidants compounds as equivalents of ascorbic acid to effectively reduce the oxidant in the reaction matrix[14,18].The IC_{50} value was found to be 51.26 µg/mL.The various extracts were found to have different levels of antioxidant activity.

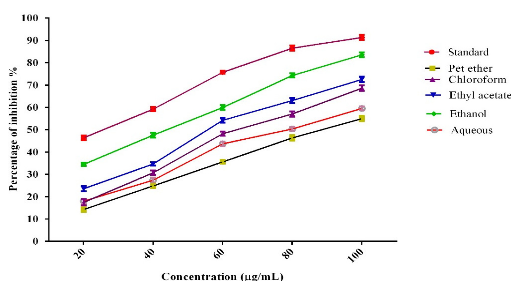

Figure - 1: Anti oxidant activity of various extract of *C.inerme* leaves by DPPH Method.

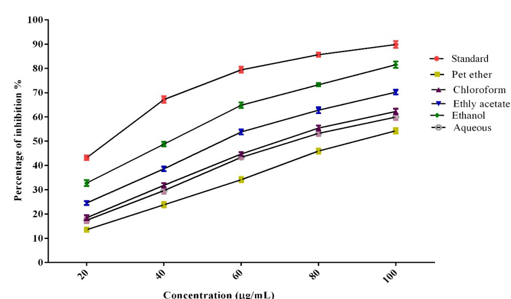

Figure - 2: Anti oxidant activity of various extract of *C.inerme* root by DPPH Method.

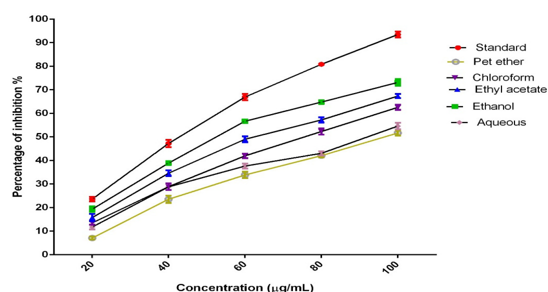

Figure - 3: Phosphomolybdenum assay of various extract of *C.inerme* leaves.

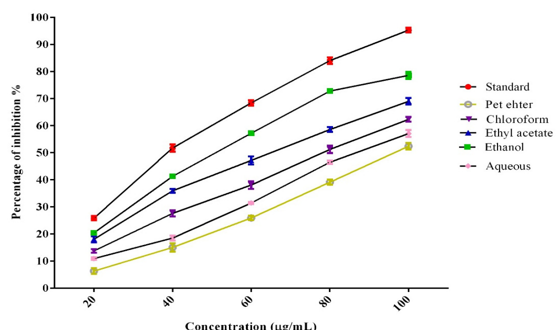

Figure - 3: phosphomolybdenum assay of various extract of *C.inerme* root.

The extracts demonstrated electron-donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products[19].

Table - 1: Anti-oxidant activity of various extract of *Clerodendrum inerme* Leaves by DPPH Method

Test	% Inhibition at various concentration in µg/mL					IC_{50} µg/mL
	20	40	60	80	100	
Standard	46.37±1.06	59.21±0.96	75.73±0.69	86.54±1.14	91.33±1.09	25.78
Pet Ether	14.26±1.02	24.82±1.90	35.61±0.75	46.33±1.21	54.98±1.15	88.66
Chloroform	17.48±1.35	30.69±1.03	48.22±0.91	57.05±1.17	68.59±1.22	66.11
Ethyl acetate	23.56±1.10	34.68±0.82	54.21±1.07	63.08±1.13	72.53±1.18	55.63
Ethanol	34.48±0.76	47.55±1.07	59.96±1.11	74.27±0.93	83.56±1.04	43.98
Aqueous	18.01±1.09	27.42±1.15	43.67±1.06	50.36±0.79	59.53±0.87	79.27

Data was shown as mean ± S.E.M. of triplicate experiment

Table - 2: Anti-oxidant activity of various extract of *Clerodendrum inerme* Root by DPPH Method

Test	% Inhibition at various concentration in µg/mL					IC_{50} µg/mL
	20	40	60	80	100	
Standard	43.20±0.94	67.18±1.33	79.42±1.23	85.64±0.86	89.86±1.42	25.51
Pet Ether	13.53±0.85	23.82±1.16	34.17±1.06	45.95±1.02	54.28±1.10	89.47
Chloroform	18.48±1.05	31.83±0.91	44.73±0.72	55.41±1.08	62.26±1.15	69.57
Ethyl acetate	24.56±0.84	38.64±0.97	53.82±1.04	62.78±1.20	70.21±0.95	54.85
Ethanol	32.77±1.22	48.80±0.93	64.86±1.11	73.28±0.64	81.53±1.31	41.52
Aqueous	17.44±1.18	29.66±1.25	43.38±0.68	53.24±1.07	59.93±1.26	73.31

Data was shown as mean±S.E.M. of triplicate experiment

Table - 3: Phosphomolybdenum assay of various extract of *Clerodendrum inerme* Leaves

Test	% Inhibition at various concentration in μg/mL					IC_{50} μg/mL
	20	40	60	80	100	
Standard	23.65±0.96	47.23±1.53	66.92±1.31	80.84±0.14	93.48±1.27	43.06
Pet Ether	7.10±0.63	23.56±1.52	33.84±1.38	42.08±0.74	51.67±1.05	96.55
Chloroform	13.54±0.81	28.89±1.42	41.97±0.94	52.33±1.32	62.58±1.11	75.28
Ethyl acetate	15.78±1.52	34.62±1.23	49.03±1.25	57.21±1.10	67.35±0.87	61.98
Ethanol	19.36±1.21	38.91±0.86	56.70±0.75	64.82±0.78	73.13±1.42	52.53
Aqueous	11.75±0.97	28.83±1.36	37.64±1.03	43.07±0.88	54.62±1.39	91.54

Data was shown as mean ± S.E.M. of triplicate experiment

Table - 4: Phosphomolybdenum assay of various extract of *Clerodendrum inerme root*

Test	% Inhibition at various concentration in μg/mL					IC_{50} μg/mL
	20	40	60	80	100	
Standard	25.87±0.78	51.76±1.34	68.43±1.98	84.05±1.20	95.31±0.83	38.34
Pet Ether	6.33±1.07	13.08±1.54	25.93±0.79	39.14±0.93	52.45±1.18	97.47
Chloroform	13.75±0.67	27.63±1.09	38.09±1.43	51.29±1.36	62.34±0.88	78.18
Ethyl acetate	18.06±1.14	35.98±0.72	47.17±1.50	58.61±0.85	69.03±1.19	64.91
Ethanol	20.43±0.64	41.32±0.55	57.21±0.25	72.83±0.42	78.59±1.34	51.26
Aqueous	10.94±0.48	18.53±0.91	31.46±0.35	46.51±0.69	57.12±1.28	86.54

Data was shown as mean ± S.E.M. of triplicate experiment

4. CONCLUSION

Natural antioxidant constituents of plant extracts will help to develop new medicationdrug molecules for antioxidant therapy[20]. The plants might be considered as decent wellsprings of natural antioxidant agents for therapeutic uses, for example, against aging and different ailments identified with radical systems[21].

This study has demonstrated that ethanolic extract of roots of *C. inerme* have cell reinforcement power and the capacity to rummage free radicals. The extract additionally demonstrated that the higher the grouping of extracts, the higher the ability to scavenge free radicals[22]. Further examination on the confinement and distinguishing proof of cancer prevention agent parts in the plant may prompt synthetic substances with potential for compound use. Since this examination is a preparatory study, a point by point investigation of the antioxidant mechanisms of particular segments is a flat out need, and is in advancement. In light of the above displayed results, different solvent extracts of *C. inerme* could be explored as a conceivable new wellspring of natural antioxidants in the Pharmaceutical industry.

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