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IN VITRO ANTIOXIDANT AND RADICAL SCAVENGING EFFECT OF *Clerodendrum inerme* (L)

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ABSTRACT

Clerodendrum inerme (L) is a medicinal plant traditionally used as an aborticide and to treat constipation, oedema, bacterial infections, cancer and diabetes. Preliminary phytochemical screening of the plant showed the presence of large amounts of phenolic compounds and flavonoids. Subsequent quantification showed the presence of 0. 4% (m ^m) phenolic (calculated as gallic acid) and 0.13% (m ^m) flavonoids calculated as catechin equivalents per 100 g of fresh mass. The presence of phenolic compounds prompted to carry out this work to evaluate its antioxidant activity. Methanolic leaves extract of *Clerodendrum inerme* was screened to evaluate its highest antioxidant and free radical scavenging ability of the leaves extract. These finding was observed at a concentration of 1400 mg ml

Keywords: *Clerodendrum inerme* - Methanolic extract - Antioxidants - Free radical Scavenging

INTRODUCTION

Clerodendrum inerme (L) is an important medicinal plant reported to be used in the treatment of skin diseases, venereal infections, elephantiasis, asthma, topical burns (Anonymous, 2001) and for rheumatism (Miller, 1996). *Clerodendrum inerme* (L). (Common name: Gloriybower; Tamil: Pinari) is a species of flowering plant in the family of Verbinaceae. This plant has simple leaves, white flowers. It is a tropical plant; it is mainly found in India. It is also used as substitute of quinine (Halliwell, 1999). A glycoside ester namely Verbascose has been isolated from the root of this plant, which has analgesic and antimicrobial properties (Fauvel, 1989 and Govindarajan, et al., 2003; Velavan,. 2015). The root of C. inerme is used as therapeutic agent and because of this the whole plant needs to be destroyed, which has disturbed its natural population leading to unavailability of good quality plant material for therapeutic use. In this scenario, there is an emerging need to systematically plan for cultivation and conservation of this plant. The present investigation describes a method which can be used for in situ and ex situ conservation of better- quality plant material of C. inerme using tissue culture technique. Reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide and hydroxyl, nitric oxide radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases (Kirtikar, et al., 1991 and Devasagayam, et al., 2004). Antioxidants act as a major defense against radical mediated toxicity by protecting the damages caused by free radicals. Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases, like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer, have appeared in the last three decades (Marinova, et al., 2005; Kalavathi and Sagayagiri, 2014). This has attracted a great deal of research interest in natural antioxidants. Flavonoids and phenolic compounds are widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free

radical scavenging abilities, anti-inflammatory, anticarcinogenic, etc. (Fauvel, et al., 1989; Kalavathi and Sagayagiri, 2016). The aim of the present investigation was to evaluate in vitro antioxidant and free radical scavenging activity of the *Clerodendrum inerme* leaves extract.

MATERIALS AND METHODS

Chemicals:

Chemicals used in this study were 1,1-diphenyl-2-picrylhydrazyl (DPPH) obtained from Sigma-Aldrich, India, NADH and sulfanilamide obtained from Himedia, Laboratories Pvt. Ltd., India, Folin-Ciocalteu reagent, potassium ferricyanide and sodium nitropruss ide obtained from Qualigens Fine Chemicals, Glaxo Smithkline Pharmaceutical Ltd., India, naphthylethylenediamine dihydrochloride, N-1- naphthylethylene diamine dihydrochloride, sodium nitrite, trichloroacetic acid, butylated hydroxy anisole (BHA), ascorbic acid, a-tocopheryl acetate, ethylenediamine tetraacetic acid, phosphoric acid, nitro blue tetrazolium, phenazine methosulfate, ferrous ammonium sulfate, DMSO are obtained from Sd Fine Chemicals Ltd, India. All reagents used in the study were of analytical grade.

Plant Material:

Clerodendrum inerme leaves were collected from Thanjavur, (Tamilnadu) India. The specimen was certified by Dr. S. John Britto, Director, The Rapinet Herbarium and Center for Molecular Systematic, St. Josephs College, Thiruchirappalli-602 002, India. Mature leaves were separated manually from the aerial part of the plant. Then, the leaves were dried and minced with a grinder into a powder in preparation for extraction.

Extraction: *Clerodendrum inerme* leaves (100 g) in powdered form were extracted with methanol using a Soxhlet assembly for 48 h, filtered and last traces of the solvent were evaporated under reduced pressure in a rotary evaporator. The yield was 2.8 g of dry extract.

Total Phenolic Content: The total phenolic content of *Clerodendrum inerme* leaves extract was determined spectrometrically (Rastogi, et al., 1998). Folin-Ciocalteu's reagent, 1 ml previously diluted with 20 ml distilled water, was added to 1 ml of sample (250 mg ml) and mixed thoroughly. To the mixture, 4 ml of sodium carbonate and 10 ml of water were added and mixed well. The mixture was allowed to stand for 2 h at room temperature. Contents were then centrifuged at 2000 g for 5 min and the absorbance of the supernatant was taken at 60 nm using a double beam spectrophotometer 2202 (Systronics, India). A standard curve was obtained using various concentrations of gallic acid. Results were expressed as percentage of gallic acid equivalents (GAE) per 100 g fresh mass.

Total Flavonoid Assay: Total flavonoid contents were measured with the aluminum chloride colorimetric assay (Sreejayan, et al., 1996). Methanolic leaves extracts or standard solution of catechin (500 mg ml ') was added to 10 ml volumetric flask containing 4 ml of water. To the above mixture, 0.3 mL of 5% NaNO, was added. 2 ml of 1 mol L ' NaOH was added and the total volume was made up to 10 ml with water. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 510 nm. Total flavonoid content of the leaves was expressed as percentage of catechin equivalent per 100 g fresh mass.

DPPH and Radical scavenging activity- (Braca, et al., 2002). The antioxidant activity of the C. inerme leaves extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH)- free radical activity by modified method (Braca, et al., 2002). The diluted working solutions of the test extracts were prepared in methanol. The samples were mixed with DPPH and solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm. Methanol with DPPH solution was used as blank.

Reducing power assay- (Benzie and Strain, 1999).Substances which have reduction potential react with potassium ferric cyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), (Velavan Sivanandham. 2011) which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.FRAP assay was performed according to the methods of Benzie and Strain (1999) with slight modification. A known volume of the plant sample was made up to 3 ml with phosphate buffer, 1% potassium ferric cyanide. It is cooled and 10% TCA, was added distilled water and ferric chloride. It is kept for 10 minutes in room temperature and the absorbance was read at 700 nm against standard ascorbic acid equivalent.

Statistical analysis (Harvey and Paige, 1998). Values were expressed as mean □ SD for six rats in each group and statistically significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Turkey's test for multiple comparisons. The results were statistically analyzed by SPSS version 16 was

used and p< 0.05 was considered to be significant.

RESULTS AND DISCUSSION

From the results on the total phenolic content, it was found that there were 0.4% of gallic acid equivalents of phenolic compounds while the total flavonoid content was 0.13% of catechin equivalent of fresh mass of *C. inerme* leaves extract. The results of antioxidant and free radical scavenging activity are given in Table 1. The free radical scavenging activity was evaluated by using various in vitro assays. DPPH radical was used as a substrate to evaluate the free radical scavenging activity of *Clerodendrum inerme* leaves extract. Free radical scavenging activities of ethanolic extract of *Clerodendrum inerme* leaves were studied. The ethanolic extract was screened for *in vitro* antioxidant activity by DPPH and Ferric reducing antioxidant power assay at different concentrations (20µg/ml to 80µg/ml). Throughout this studies *C. inerme* leaves extracts showed marked antioxidant activity. The half inhibition concentration (IC5o) of DPPH radical scavenging activity of ascorbic acid and *C. inerme* leaves extract were 2.68 µg/ml and 3.08µg/ml respectively.

The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants. DPPH radical scavenging activity of *Clerodendrum inerme* leaves extracts and standard as ascorbic acid are presented in Table-2. The half inhibition concentration (IC_{50}) of DPPH radical scavenging of activity of ascorbic acid and *C. inerme* leaves extract exhibited a significant dose dependent inhibition of *C. inerme* leaves extract were 2.68 µg/ml and 3.08µg/ml respectively, the DPPH activity (Fig -1). The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

Depicts the reductive effect of *Clerodendrum inerme* L. leaves extract similar to the antioxidant activity, the reducing power of *C. inerme* leaves extract and standard ascorbic acid increased with increasing dosage (Fig-2). All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Clerodendrum inerme* L. leaves extract consist of hydrophilic polyphenolic compounds that cause the greater reducing power. The reducing capacity of compounds may serve as a significant indicator of its potential antioxidant activity (Table-2).

CONCLUSION

In conclusion, free radical scavenging effect of *Clerodendrum inerme* increases with increasing concentration and maximum antioxidant activity was observed at 1400 mg mL ' Antioxidant activity may be due to phenolic compounds in *Clerodendrum inerme* but further work should be done on the isolation and identification of other antioxidant components of *Clerodendrum inerme* (L).

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Table 1: DPPH radical scavenging activity

S. No.	Standard – Ascorbic acid			Plant sample
	Volume (µl)	Concentration of plant extract (µg)	% of Inhibition	% of Inhibition
1.	10	280	12.21±0.8547	18.42±1.2894
2.	20	560	25.60±1.792	29.57±2.0699
3.	30	840	61.26±4.2882	44.32±3.1024
4.	40	1120	88.98±6.2286	68.47±4.7929
5.	50	1400	99.34±6.9538	82.32±5.7624
IC_{50} (mg/ml)			2.68	3.08

Values were expressed as mean ± Standard deviation for triplicates;
IC: Inhibitions concentration

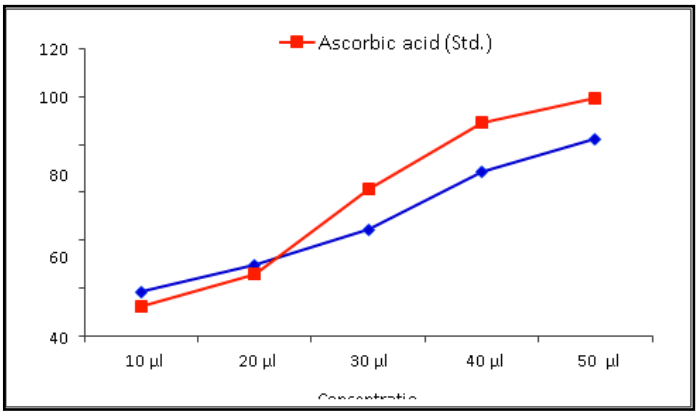
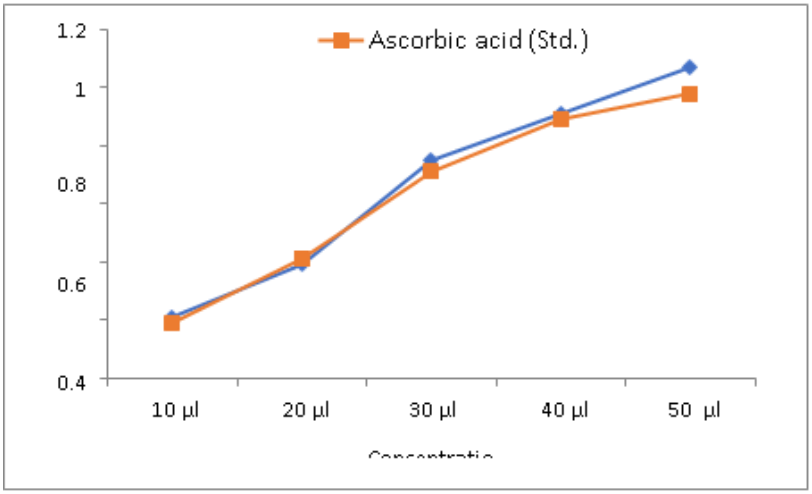


Figure 1: DPPH radical scavenging activity

Table - 2: Reducing power assay of *Clerodendrum inerme* L. leaves extract

S. No.	Standard – Ascorbic acid			Plant sample
	Volume (µl)	Concentration of plant extract (µg)	% of Inhibition	% of Inhibition
1	10	280	0.19±0.0133	0.21±0.0147
2	20	560	0.41±0.0287	0.39±0.0273
3	30	840	0.71±0.0497	0.75±0.0525
4	40	1120	0.89±0.0623	0.91±0.0637
5	50	1400	0.98±0.0686	1.07±0.0749

Fig- 2: Reducing power assay of *Clerodendrum inerme* L. leaves extract



Values are expressed as mean ± SD for triplicates

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