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Antioxidant and Radical Scavenging Effect of *Clerodendrum inerme* (L.)

S. Gurudeeban, K. Satyavani, R. Shanmugapriya,
T. Ramanathan, G. Umamaheswari and K. Muthazagan

C.A.S. in Marine Biology, Annamalai University, Parangipettai 608502, Tamil Nadu, India

Abstract: *Clerodendrum inerme* (L.) is a medicinal plant traditionally used as an abortifacient and to treat constipation, oedema, bacterial infections, cancer and diabetes. Preliminary phytochemical screening of the plant showed the presence of large amounts of phenolics and flavonoids. Subsequent quantification showed the presence of 0.74% (*m/m*) phenolics (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 us to evaluate its antioxidant activity. In the present study, methanolic leaves extract of *Clerodendrum inerme* was screened to evaluate its highest antioxidant and free radical scavenging ability of the leaves extract was observed at a concentration of 2500 mg mL $^{-1}$.

Key words: *Clerodendrum inerme* · Methanolic extract · Antioxidants · Free radical scavenging

INTRODUCTION

C. inerme is an important medicinal plants reported to be used in the treatment of skin diseases, venereal infections, elephantiasis, asthma, topical burns [1] and for rheumatism [2]. *Clerodendrum inerme*, leaves commonly known as Seaside clerodendrum (or clerodendron), embrert, Indian privet, glorybower. It is also used as substitute of quinine [2]. A glycoside ester namely Verbascoside has been isolated from the root of this plant, which has analgesic and antimicrobial properties [3, 4]. 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 [5, 6]. 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 [7]. 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.* [8]. 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 nitroprusside obtained from Qualigens Fine Chemicals, Glaxo Smithkline Pharmaceutical Ltd., India, naphthylethylenediamine dihydrochloride, *N*-1-naphthylethylenediamine 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.

Corresponding Author: Dr. T. Ramanathan, C.A.S.in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India, Tel: +04144-243223

Plant Material: *Clerodendrum inerme* leaves were collected from the southeast coast of Parangipettai, (Tamilnadu) India. The specimen was certified by Botanical Survey of India (BSI) Coimbatore and by the Herbaria of C.AS.in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu and India. Mature leaves were separated manually from the aerial part of the plant. Then, the leaves was 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 2.78 g of dry extract.

Total Phenolic Content: The total phenolic content of *Clerodendrum inerme* leaves (CIL) extract was determined spectrometrically [9]. Folin-Ciocalteu's reagent, 1 mL previously diluted with 20 mL distilled water, was added to 1 mL of sample (250 mg mL^{-1}) and mixed thoroughly. To the mixture, 4 mL of sodium carbonate (75 g L^{-1}) 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 760 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 [10]. Methanolic leaves extracts or standard solution of catechin (500 mg mL^{-1}) was added to 10 mL volumetric flask containing 4 mL of water. To the above mixture, 0.3 mL of 5% $NaNO_2$ was added. 2 mL of 1 mol L^{-1} 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 Free Radical Scavenging Activity: The free-radical scavenging activity of CIL extract was measured by the decrease in absorbance of methanolic solution of DPPH [11]. A stock solution of DPPH (33 mg L^{-1}) was prepared in methanol and 5 mL of this stock solution was added to 1 mL of the CIL extract solution at different concentrations (250, 500, 1000, 1500, 2000 and 2500 mg mL^{-1}). After 30 min, absorbance was measured at 517 nm and compared with the standards, *i.e.*, ascorbic acid, BHA and a-tocopherol (10–50 mg mL^{-1}). Scavenging activity was expressed as the percentage inhibition.

Hydroxyl Radical Scavenging Activity: Methanolic extract at different concentrations was placed in a test tube and evaporated to dryness. One mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5 mL of 0.018% EDTA, 1 mL of DMSO -0.85%, *V/V*, in 0.1 mol L^{-1} phosphate buffer, pH 7.4 and 0.5 mL of 0.22% ascorbic acid were added to each tube [12]. The tubes were capped tightly and heated in a water bath at 80–90 °C for 15 min. The reaction was terminated by adding 1 mL of ice-cold TCA (17.5% *m/V*). Three ml of Nash reagent (75.0 g ammonium acetate, 3 mL glacial acetic acid and 2 mL acetyl acetone were mixed and water was added to a total volume of 1 L) was added to each tube; the tubes were left at room temperature for 15 min for colour development. The intensity of the yellow colour formed was measured at 412 nm against a blank of the reagent. Percentage inhibition was determined by comparing the results of the test and standard compounds.

Nitric Oxide Scavenging Activity: Nitric oxide scavenging activity was measured spectrophotometrically [13]. Sodium nitroprusside (5 mM L^{-1}) in phosphate buffered saline pH 7.4, was mixed with different concentrations of the extract (250–2500 mg mL^{-1}) prepared in methanol and incubated at 25 °C for 30 min. A control without the test compound, but with an equivalent amount of methanol, was taken. After 30 min, 1.5 mL of the incubated solution was removed and diluted with 1.5 mL of Griess reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% N-1-naphthylethylenediamine dihydrochloride). Absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with N-1-naphthylethylene diamine dihydrochloride was measured at 546 nm and the percentage scavenging activity was measured with reference to the standard.

Statistical Analysis: Values were represented as mean ± SD of three parallel measurements and data were analyzed using the t-test.

RESULTS AND DISCUSSION

From the results on the total phenolic content, it was found that there were 0.74% of gallic acid equivalents of phenolic compounds while the total flavonoid content

Table 1: Antioxidant profile of *Clerodendrum inerme* L. leave extract

Sample	Concentration	DPPH radical scvanging (%)		Hydroxyl radical scvanging (%)		Nitrite radical scvanging (%)	
CIL	2500	88.21±	2.1	71.4	3.2	61.3	3.5
Ascorbic acid	50	89.13±	1.1	-		86.4	3.3
BHA	50	83.11±	1.2	83.2	3.1	-	-
Tocopherol	50	67.15±	0.9	-	-	-	-

a Mean _ SD, $n = 3$.

was 0.13% of catechin equivalent of fresh mass of *C. colocynthis* leaves extract. The results of antioxidant and free radical scavenging activity are given in Table I. 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 CIL extract. The scavenging effect of CIL extract on the DPPH radical was 88.0 ± 2.7% ($p < 0.005$), at a concentration of 2500 mg mL^{-1} compared to the scavenging effects of ascorbic acid, BHA and a-tocopherol at 50 mg mL^{-1} of 89.5 ± 1.1, 83.2 ± 1.1 and 67.5 ± 0.8% ($p < 0.05$) respectively. Hydroxyl radicals are the major active oxygen species that cause lipid oxidation and enormous biological damage [14]. The percentage of hydroxyl radical scavenging increased with the increasing concentration of leaves extract. Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity [15]. The percentage inhibition of nitric oxide generation by CIL at 2500 mg mL^{-1} concentration was found to be 61.4 ± 3.8% ($p < 0.005$). On the other hand, ascorbic acid at 50 mg mL^{-1} concentration showed 86.0 ± 3.5% ($p < 0.05$) inhibition of nitric oxide.

CONCLUSIONS

Free radical scavenging effect of CIL increases with increasing concentration and maximum antioxidant activity was observed at 2500 mg mL^{-1}. Antioxidant activity may be due to phenolic compounds in CIL but further work should be done on the isolation and identification of other antioxidant components of *Clerodendrum inerme*.

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