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ANTIOXIDANT EFFECTS OF LEAVES OF *CLERODENDRUM INFORTUNATUM* (LINN.)

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ABSTRACT

Over the past decade, herbal and ayurvedic drugs have become a subject of world importance, with both medicinal and economical implications. A regular and widespread use of herbs throughout the world has increased serious concerns over their quality, safety and efficacy. Thus, a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. In the present study we examined the antioxidant effects of ethanolic extract of leaves of *Clerodendrum infortunatum* Linn at various concentrations in the DPPH radical scavenging assay, FRAP assay (Ferric Reducing Antioxidant Power) and the Hydrogen peroxide radical scavenging assay. The results of the present study revealed that the plant extract has significant antioxidant activity and are encouraging for further assessment to elucidate the mechanism of action and to identify the bioactive compounds implicated in the antioxidant effect and the membrane-stability.

INTRODUCTION: Oxidative stress is believed to be a primary factor in various diseases as well as in the normal process of aging [1, 2]. Free radicals and reactive oxygen species (ROS) are well known inducers of cellular and tissue pathogenesis leading to several human diseases such as cancer, inflammatory disorders, atherosclerosis and cardiovascular diseases. Cardiovascular diseases are the most common cause of death in the industrialized countries. The beneficial effects of phytochemicals are associated with a multitude of biological activities, including antioxidant and free radical scavenging properties[3].

Clerodendrum infortunatum Linn. (Verbanaceae: Bhat in Hindi, Ghentu in Bengali, Bhania in Oriya) is a terrestrial shrub having square, blackish stem and simple, opposite, decussate, petiole, exstipulate, coriacious, hairy leaves with a disagreeable odour[4, 5]. The plant is common throughout the plains of India. Various parts of the plant have been used by tribes in colic, scorpion string, snake bite, tumour and certain skin diseases[6, 7] also used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy[10- 12]. Fresh juice of the leaves has been used as vermifuge and in treatment of malaria[7, 8]. *Clerodendrum infortunatum* leaves on preliminary chemical analysis are found to contain saponin, clerodin (a bitter diterpene) [7-9] and some enzymes. Leaves also contain a fixed oil which consists of Glycerides of Lenoleic, oleic, stearic and lignoceric acid[9]. Previous phytochemical investigation of the plant revealed the presence of alkyl sterols and 2, - (3, 4-dehydroxyphenyl) ethanol 1- O- α- 2 rhamnopyranosyl- (1→3) - β- D- (4- O- caffeoyl) glycopyranoside (acteoside) in this plant[13, 14].

MATERIAL AND METHODS:

Plant Material and Extraction: The fresh leaves of *Clerodendrum infortunatum* of were collected in the months of July-August from the local market of Amaravati, Maharashtra state, India, and authenticated by the authority of the botany department, VMV, Amaravati. A voucher specimen was submitted at Institute's herbarium department for future reference. Dried leaves were ground to coarse powder. Powder was first defatted with pet. Ether and then extracted with ethanol, which is further evaporated to dryness to obtain alcoholic extract aqueous extract was obtained by maceration for 24 hours.

ANTIOXIDANT ACTIVITY:

RSA assay (DPPH Radical Scavenging Activity): In DPPH radical scavenging activity, test sample solution (200 µl) was added to 4 ml of 100 mM/l ethanolic DPPH, then the mixture was incubated for 10 minutes at room temperature and the absorbance at 517 nm was measured. The difference in absorbance between a test sample and a control (ethanol) was considered as activity. The activity was shown as IC50 value (50% of inhibitory concentration in mg/ml). Ascorbic acid was used as standard substance. All

values are shown as the mean of three measurements [15, 16].

Table 1: Antioxidant activity of *Clerodendrun infortunatum* Linn. (DPPH radical scavenging activity)

Concentration (mg/ml)	Ethanolic extract	Vitamin C	Aqueous extract
	% inhibition	% inhibition	% inhibition
0.020	21	25	12
0.040	38	44	20
0.060	55	60	31
0.080	68	73	40
0.10	80	88	48

FRAP assay (Ferric reducing antioxidant power): In ferric reducing antioxidant power assay, one ml of test sample was mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide. The reaction mixtures were incubated in a temperature-controlled water bath at 50 0C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionised water and 200 µl of 0.1% FeCl3. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm. The reducing power was expressed as an increase in A700 after blank subtraction[17, 18].

Table 2: Antioxidant activity of *Clerodendrun infortunatum* Linn. (FRAP Assay)

Concentration (mcg/ml)	Ethanolic extract	Vitamin C	Aqueous extract
	Absorbance	Absorbance	Absorbance
20	0.153	0.185	0.11
40	0.285	0.344	0.20
60	0.421	0.483	0.284
80	0.543	0.673	0.415
100	0.701	0.853	0.528

Ferric Thiocyanate (FTC) Method: A mixture containing 4 mg of the sample in 4 ml of 99.5% ethanol (final concentration 0.02%). 4.1ml of 2.52% linoleic acid in 99% ethanol, 8 ml of 0.05M phosphate buffer (pH 7.0) and 3.9 ml of water was placed in a vial with screw cap and then placed in an incubator at 400 C in the dark. To 0.1 ml of this mixture 9.7 ml of 75% ethanol (v/v) and 0.1 ml of 30% ammonium thiocyanate were added. Precisely 3 minutes later the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% hydrochloric acid was added to reaction mixture; (the absorbance of red color indicated the antioxidant activity) was measured at 500 nm for every 24 hours until the absorbance of the control reached maximum. The control and the standard were subjected to the same procedures as the sample except that for the control, only the solvent was used, and for the standard 4mg of the sample was replaced by 4 mg of Vitamin E and C. [19].

Table 3: **Antioxidant Activity of *Clerodendrun infortunatum Linn*. (FTC method)**

Extract	Absorbance								
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
Control	0	0.30	0.32	0.40	0.44	0.451	0.462	0.468	0.475
Vitamin C	0	0.035	0.047	0.052	0.0612	0.0702	0.0758	0.0801	0.0847
Ethanolic extract	0	0.021	0.035	0.044	0.0526	0.0615	0.0708	0.0754	0.0794
Aqueous extract	0	0.0125	0.0182	0.0298	0.0356	0.0448	0.0562	0.0624	0.0692

Thiobarbituric Acid (TBA) Method: TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100 0C), melonaldehyde binds TBA to form a red complex that can be measure at 532 nm. 2 ml of 20% trichloroacetic acid and 2 ml of 0.67% TBA solutions were added to 2 ml of the mixtures containing the sample prepared in the FTC method. This mixture was kept in water bath (100 0C) for 10 minutes and after cooling to room temperature, was centrifuged at 3000 rpm for 20 minutes. Antioxidant activity was based on the absorbance of the supernatant at 532 nm on the final day of the assay [24]. The percentage of antioxidant activity was calculated by following formulae for both FTC and TBA;

Percentage Scavenging

$$= \frac{\text{Absorbance of control- Absorbance of Sample}}{\text{Absorbance of control}} \times 100$$

RESULTS: In DPPH radical scavenging assay, *Clerodendrum infortunatum* leaves extract at various concentrations (0.020-0.10 mg/ml) and ascorbic acid (0.020-0.10 mg/ml) showed the significant inhibitory activity. In reducing power assay, a linear increase in reducing power was observed over the concentration range 20 – 100 µg/ml sample, equivalent to 20 – 100 µg/ml ascorbic acid. In FTC method, the total antioxidant activities elicited by the extracts were shown in table 3 in terms of absorbance at 500 nm. The percentage of antioxidant activity was shown in table 3 at concentration of 0.02%. In TBA method, the control produced highest absorbance value (0.512) followed by extracts, aqueous extracts (0.0736) and ethanolic extract (0.084) which is shown in table 4. The results indicated that extract consist of hydrophilic polyphenolic compounds that cause the greater reducing power. Result shows that reducing power of the ethanolic extract of leaves as a function of sample concentration in hydrogen peroxide scavenging assay, the inhibitive effect of extract was found to be moderate when compared to other assays.

Table 4: Antioxidant activity of *Clerodendrun infortunatum* Linn. (TBA method on 9th day)

Extract	Absorbance
Control	0.512
Vitamin C	0.0896
Ethanolic extract	0.084
Aqueous extract	0.0736

DISCUSSION: DPPH (1, 1- diphenyl- 2- picryl-hydrazil) is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The model of scavenging the stable DPPH radical is widely used for relatively rapid evaluation of antioxidant activities Compared to other methods[20]. The reduction capability of the DPPH radical is determined by its absorbance decrease at 517 nm, as induced by natural antioxidants[3]. The antioxidant activity has been attributed to various mechanisms, among which are the prevention of chain initiation, the binding of transition metal ion catalysts, decomposition of peroxides, the prevention of continued hydrogen abstraction, the reductive capacity and radical scavenging[21]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[22].

CONCLUSION: The results of the present study shows that the ethanolic extract of the leaves of *Clerodendrum infortunatum* Linn possess antioxidant activity through the DPPH free radical scavenging activity, reducing power assay and scavenging of hydrogen peroxide. The preliminary phytochemical investigation indicates the presence of flavonoids in the plant. Polyphenols like flavonoids and tannins are the well known natural antioxidants[24]. So, the antioxidant potential of the plant may be attributed to the presence of flavonoids. The separation and identification of flavonoids present in the leaves can help researchers find new molecules which can be used as natural antioxidants. Further studies are currently infact underway to isolate and characterize the active constituents responsible for its antioxidant activity.

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