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Research Article

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Evaluation of *in vitro* antioxidant activity of plant *Chrozophora plicata*

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ABSTRACT

Free radical induced oxidative stress is involved in the pathogenesis of various diseases and disorders. Antioxidants are the free radical scavengers which protect human body against the oxidative stress. The present study was carried out to evaluate the *in vitro* antioxidant properties of 70% ethanol extract of *Chrozophora plicata* plant (Leaves) by estimation of reducing power and scavenging activity of Superoxide anion, Hydroxyl radical and Nitric oxide anion. The Ethanolic extract of leaves had shown concentration dependent reducing power. This exhibited higher reducing power at 100µg concentration of extract as similar to standard drug (sodium metabisulphite 25µg). Similarly, Ethanolic extract of *Chrozophora plicata* also have shown concentration dependant superoxide scavenging activity, hydroxyl radical scavenging activity and Nitric oxide radical scavenging activities at 100µg but to a lesser extent than standard drug (sodium metabisulphite 25µg). From the preliminary studies, it was found that the leaves of *Chrozophora plicata* plant contain triterpenoids and related compounds (sterols, alcohols and hydrocarbons), phenolic compounds (flavonoids, lignans, coumarins, tannins, phenanthrenes, quinones, phenolic acids, etc.) that are possessing antioxidant properties.

Key words: *Chrozophora plicata*, Antioxidant activity, Ethanolic extract.

INTRODUCTION

Herbalism is becoming main stream as up-to-date analysis and research show their value in the treatment and prevention of disease. In present global scenario herbal medicines are gaining prominence, because they are economic, easily available & relatively free from side effects. World Health Organization (WHO) has defined herbal medicines as finished labeled medicinal product that contain active ingredients, aerial or underground parts of the plant or other plant material or combinations.[1] The post GATT (Generalized agreement on tariffs and trade) era and new international patenting pattern offers many challenges in the field of herbal medicines. The important role of antioxidant in maintaining the integrity of living organisms is gaining ever increasing recognition. Antioxidants are beneficial components that neutralize free radicals before they can attack cells.

From the phytochemical studies, it was found that the leaves of *Chrozophora plicata* contain triterpenoids and flavonoids that are possessing antioxidant properties. Since there are no reports on isolation of active antioxidant principles of *Chrozophora plicata*, the present study was planned to exploit the *in vitro* antioxidant activity of herbal plant named *Chrozophora plicata*, Family: *Euphorbiaceae*.

MATERIALS AND METHOD

Preparation of Extraction:

The Leaves of *chrozophora plicata* plant were collected in the month of May, 2012 from the garden of tirupathi in chitoor district, Andhra Pradesh. The plant material was taxonomically identified by the field botanist, Department of Botany, Sri Venkateswara University, and a specimen was deposited in their herbarium against issue of Vocher No.1674. The leaves were shade dried separately at room temperature and pulverized. The powder obtained was subjected to successive soxhlet extraction with 70% ethanol solvent which was used for *in vitro* antioxidant studies, after subjecting it to preliminary qualitative phytochemical studies.

The extract were concentrated under reduced pressure and stored in a desicator until further use and the percentage yield of corresponding extracts were calculated.

The following *in vitro* models were carried out to evaluate antioxidant activity.

1. Reducing power :

The reducing power of 70% Ethanolic extract of *Chrozophora plicata* leaves were determined according to the method of Oyaizu (Oyaizu, 1986)[2].

Procedure:

Different doses of 70% Ethanolic extract of *Chrozophora plicata* leaves were mixed in 1 mL of distilled water so as to get 20µg, 40µg, 60µg, 80µg and 100µg concentration. This was mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5mL, 1%). The mixture was incubated at 50°C for 20 minutes. A portion (2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was

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then centrifuged at 3000 rpm for 10 minutes. The upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700nm.

Increased absorbance (Abs.) of the reaction mixture indicates increase in reducing power. The %increase in reducing power was calculated by using following formula:

$$\% \text{ increase in reducing power} = \frac{\text{Abs. of Control} - \text{Abs. Test}}{\text{Abs. of Control}} \times 100$$

2. Superoxide anion scavenging activity :

Oxygen is essential for the survival of aerobic cells, but from the earlier days it has been known that, it is toxic to cells when supplied at concentration higher than the normal air. The biochemical mechanisms responsible for oxygen toxicity include lipid peroxidation and the generation of $H_2O_2^+$, the superoxide radical ($O_2\bullet$). This superoxide radical can inhibit or propagate the process of lipid peroxidation. Measurement of superoxide anion scavenging activity of 70% Ethanolic extract of *Chrozophora plicata* leaves done by using the method explained by Nishimiki (Nishimiki et al., 1972)[3] and modified by Ilhami et al.

Procedure:

About 1 mL of nitroblue tetrazolium (NBT) solution (156µM NBT in 100 mM phosphate buffer, pH 7.4), 1 ml Nicotinamide adenine dinucleotide (NADH) solution (468µM in 100 mM phosphate buffer, pH 7.4) and 0.1 mL of sample solution of 70% Ethanolic extract of *Chrozophora plicata* leaves were mixed in water to get 20µg, 40µg, 60µg, 80µg & 100µg concentrations and in the same manner standard solution was prepared by taking sodium metabisulphite. The reaction starts by adding 100µL of Phenazine methosulphate (PMS) solution (60µM PMS in 100 mM phosphate buffer, pH 7.4) to the mixture. The reaction mixture was incubated at 25°C for 5 minutes, and the absorbance at 560 nm was measured against blank.

Decreased absorbance (Abs.) of the reaction mixture indicated increased superoxide anion scavenging activity. Capability to scavenge the superoxide radical was calculated as % inhibition.

$$\% \text{ inhibition} = \frac{\text{Abs. Control} - \text{Abs. Test}}{\text{Abs. Control}} \times 100$$

3. Hydroxyl radical scavenging activity:

In biochemical systems, superoxide radical and H_2O_2 react together to form the hydroxyl radical (OH•), this can attack and destroy almost all the cells and tissues in the biologocal systems[4]. Phenylhydrazine when added to erythrocyte hosts cause peroxidation of endogeneous lipids and alteration of membrane fluidity. This peroxidation damage to erythrocytes is probably initiated by active oxygen species like $O_2\bullet$, OH• and H_2O_2 which are generated in solution from auto-oxidation of phenylhydrazine. This forms the basis of the experiment.

Procedure:

Hydroxyl radical generated by phenylhydrazine has been measured by using 2-deoxyribose degradation, Assay of Halliwell and Gutteridge[5]. 50mM phosphate buffer (pH 7.4), 1 mM deoxyribose and 0.2 mM Phenyl hydrazine hydrochloride were prepared. 0.4ml of 70% Ethanolic extract of *Chrozophora plicata* leaves of different concentrations (20-100µg) and 0.4 ml of standard were taken. To this add 0.6ml of 1mM Deoxyribose to both standard and test extracts to make the solutions to 1 ml. 0.6 mL phosphate buffer was added to both extract and standard to make reaction solutions 1.6mL. After 10 min incubation, 0.4mL of 0.2 mM phenyl hydrazine was added to both Std. and 70% extract. Incubation was terminated after 1 hr and 4 hrs and 1 mL each of 2.8% Trichloro acetic acid (TCA) and 1% (w/v) thiobarbituric acid were added to the reaction mixture (test extract and std) and heated for 10 mins in a boiling water bath. The tubes were cooled and absorbance was measured at 532 nm.

Decreased absorbance of the reaction mixture indicated increased hydroxyl radical scavenging activity. Capability to scavenge the hydroxyl radical was calculated as % inhibition.

4. Nitric oxide radical scavenging activity[6].

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes. Excess concentration of NO is associated with several diseases. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions, which act as free radicals. This forms the basis of this experiment.

Procedure:

Nitric oxide (NO) radical were generated from sodium nitroprusside solution at physiological pH. Sodium nitroprusside (1mL of 10mM) were mixed with 1mL of 70% Ethanolic extract of *Chrozophora plicata* leaves having different concentration (20-100 µg/mL) in phosphate buffer (pH 7.4) and also mixed with standard (sod.metabisulphite) The mixture was incubated at 25°C for 150 min. To 1 mL of the incubated solutions of test extract and standard, 1mL of Griess's reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% naphthyl ethylene diamine dihydrochloride) was added. Absorbance was measured at 546 nm.

Decreased absorbance (Abs.) of the reaction mixture indicated increased nitric oxide radical scavenging activity. Capability to scavenge the nitric oxide radical was calculated as % inhibition.

RESULTS AND DISCUSSIONS

Phytochemical Screening:

The results of the preliminary Phytochemical screening of 70% Ethanolic extract of *Chrozophora plicata* leaves have been presented below in Table No. 1.

Table No.1Phytochemical screening Ethanolic extract of *Chrozophora plicata* leaves

Types of Phytochemical constituents	70 %Ethanolic Extract
Alkaloids	–
Carbohydrates	++
Flavonoids	+++
Glycosides	++
Tannins	+++
Protein	+
Steroids	++
Saponins	++

– *absent* ++ *more clarity*
\+ *indicates presence* +++ *better response*

***In vitro*, Antioxidant activity**

Reducing power activity of 70% Ethanolic extract of *Chrozophora plicata*

It was observed that 70% Ethanolic extract of *Chrozophora plicata* had concentration dependent increase in the reducing property, where as sodium metabisulphate (std. 25µg) had 89.83 % reducing property compared to Control (C).However, 100µg of 70% ethanolic extract of *Chrozophora plicata* had shown comparable reducing power i.e. 82.03%. The results were graphically depicted in Fig No. 1.

Fig No. 1
Reducing power activity of 70% ethanolic extract of leaves of Chrozophora plicata

Superoxide anion, Hydroxyl radical and Nitric oxide anion scavenging activity

It was observed that 70% Ethanolic extract of *Chrozophora Plicata* had concentration dependent inhibition of superoxide anion, hydroxyl radical and nitric oxide anion, Where as 25µg of sodium metabisulphate (std.) had inhibition of 92.75 % superoxide anion, 63.31 % (1 hrs incubation), 52.65 % (4 hrs incubation) of hydroxyl anion, and 64.59 % nitric oxide radical compared to control (C). However all the test extracts even at 100µg have shown lesser inhibition than standard. The results are graphically depicted in Fig. No.2, 3, 4 & 5.

Fig. No. 2
Superoxide anion scavenging activity of 70 % ethanolic extract of leaves of Chrozophora Plicata

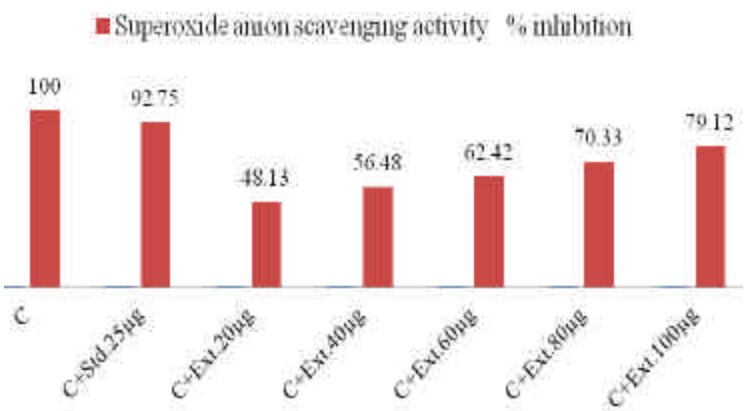


Fig.No. 3
Hydroxyl ion radical scavenging activity of 70% ethanolic extract of leaves of Chrozophora plicata(After 1 hr.)

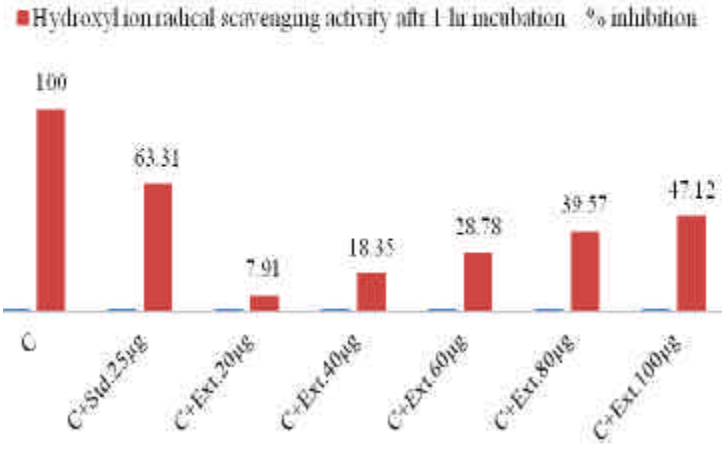


Fig.No. 4
Hydroxyl ion radical scavenging activity of 70% ethanolic extract of leaves of Chrozophora Plicata (After 4 hrs.)

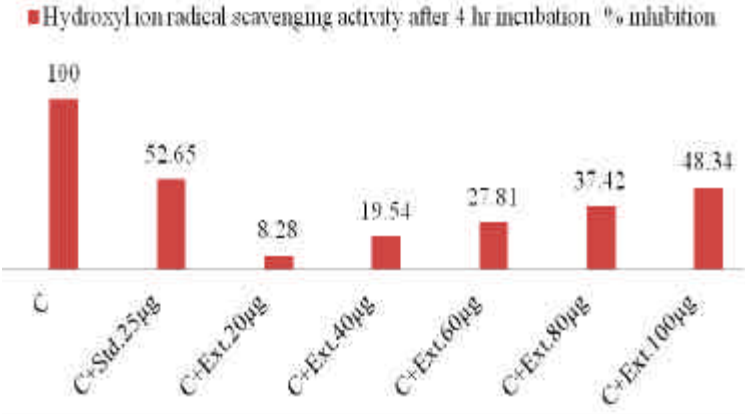
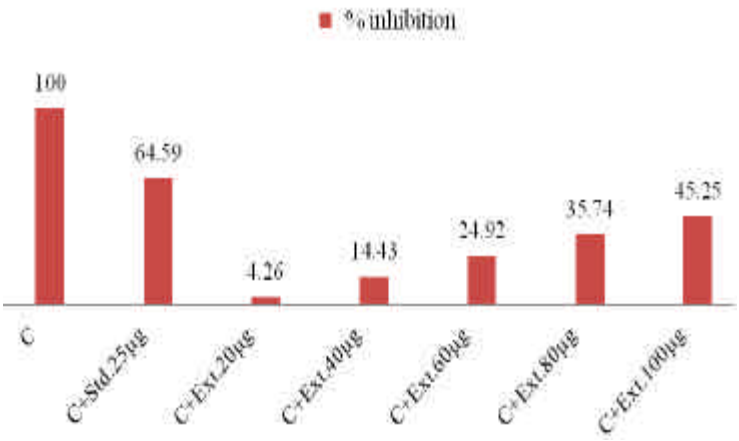


Fig.No. 5
Nitric oxide radical scavenging activity of 70% ethanolic extract of leaves of Chrozophora Plicata



CONCLUSIONS

The studies on *in vitro* models of antioxidants activity has indicated that the 70% Ethanolic extract of *Chrozophora Plicata* possess antioxidant activity and may have organ protective potential**.**

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