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INVITRO ANTIOXIDANT POTENTIAL OF HYDROMETHANOLIC EXTRACT OF LEAVES OF *CALOTROPIS GIGANTEA* (ASCLEPIADACEAE)

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ABSTRACT

Calotropis gigantea of the family Asclepiadaceae has been considered as an important shrub and is used as antifertility, anti-inflammatory, hepatoprotective, antimyocardial infraction, antidiarhoeal drugs etc. The present investigation aimed to evaluate the antioxidant potential of *Calotropis gigantea.* Antioxidant potential was determined by DPPH (1,1-diphenyl-2-picryl-hydrazyl) free radical scavenging activity using ascorbic acid as standard. The result showed that hydromethanolic extract of *Calotropis gigantea* possesses antioxidant property (IC_{50} 29.17µg/ml) compared to that of the standard ascorbic acid (IC_{50} 12.93µg/ml).

KEYWORDS: *Calotropis gigantea*, DPPH, IC_{50}.

INTRODUCTION

Calotropis gigantea belonging to family Asclepiadaceae, commonly known as Milkweed or Madar, is a erect shrub, widely distributed throughout the tropical and subtropical regions of Asia and Africa.[1,2] The bark of the plant is pale in nature. Leaves of the plant are elliptical to oblong-obovate & 10 to 20 centimeters long but subsessile and the stem are erect and milky. The flowers have five pointed petals with a small crown which holds the stamens. The sepal and petal clusters in flowers which may be white or lavender in color. The fruit is a follicle and when dry, seed are dispersed in air by wind. Different part of plant is used in traditional system of medicine,[3,4] Plant contains many phytochemicals including cardiac glycosides, b

sitosterol, madrine, saponins, alkaloids, tannins, trisaccharides and flavonols. Plant possess various pharmacological activities like antifertility, anti-inflammatory activity, hepatoprotective activity, antimyocardial infraction activity, antidiarhoeal activity, etc.[5]

Antioxidants are the compounds which protects the body from oxidative stress caused by free radical. A number of free radical scavenging antioxidants are present within the body.[6] Free radicals like reactive oxygen species (ROS) and other oxidants are the causative factors in the induction of many chronic and degenerative diseases.[7] Reactive oxygen species are (ROS) highly reactive molecules which are derived from oxygen metabolism. It includes superoxide radicals, hydroxyl radicals and hydrogen peroxide, etc.[8]

MATERIAL AND METHODS

Collection of plant material

The plant of *Calotropis gigantea* was collected from Bhopal M.P., India in the month of November 2013. The plant was acknowledged by Dr. Zia-ul Hasan, Department of Botany, Safia College of Science, Bhopal, Madhya Pradesh, India.

Preparation of Extract

Plant material was washed with water and then allowed to dry in shade for about 3 to 4 weeks. Dried plant materials were grinded by using the electronic grinder. The powder of the whole plants of *Calotropis gigantea* was extracted according to **(Harborne and Baxter., 1995).**[9] The dried plants sample was powered and filed into the soxhlet using petroleum ether and methanol respectively. Almost all the chlorophyll and lipid was deposited on the side of the flask and removed carefully. The extracts were stored in refrigerator till any further use.

ANTIOXIDANT ACTIVITY

DPPH free Radical Scavenging Assay

Principle: The scavenging reaction between (DPPH·) and antioxidant (H-A) can be written as:

$$\underset{\text{(Purple)}}{\text{(DPPH·) + (H-A)}} \longrightarrow \underset{\text{(Yellow)}}{\text{DPPH-H + (A)}}$$

❖ **Preparation of Standard Ascorbic acid solutions**

Various solutions of the ascorbic acid were prepared in 90% methanol to obtain different concentrations (1-100µg/ml). 200µM solution of DPPH (in methanol) was prepared and 1.5ml of this solution was added to 1.5ml of methanolic ascorbic acid solution of different concentrations and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517nm.

❖ **Preparation of Test solutions**

Various solutions of leaf extract were prepared in 90% methanol to obtain different concentrations (10-100µg/ml). 200µM solution of DPPH in methanol was prepared and 1.5ml of this solution was added to 1.5ml of methanolic extract solution of different concentrations and incubated for 30 min (at room temperature) in dark. After 30 minutes, the absorbance of each solution of ascorbic acid was taken against methanol (as blank) at 517nm.

❖ **Preparation of Control solution**

For control, 1.5ml of methanol was mixed with 200µM DPPH solution and incubated for 30 min at room temperature in dark. Absorbance of the control was taken after 30 min against methanol (as blank) at 517 nm.

The antioxidant activity of plant leaf extract and ascorbic acid were calculated by using the following formula in terms of % inhibition:

% Inhibition = [(Ac 515 nm- At 515 nm/ Ac 515 nm) x 100]

Where,

Ac = Absorbance of control

At = Absorbance of ascorbic acid/ hydro alcoholic leaf extract.

RESULTS AND DISCUSSION

DPPH is a purple colored stable free radical on reducing it becomes yellow-colored diphenyl-picryl hydrazine. DPPH radicals react with reducing agents thus electrons become paired-off and the solution loses colour stoichimetrically.[10]

The obtained results of the hydromethanolic extract of *Calotropis gigantea* plant are shown below. The scavenging activity of hydromethanolic extract of *Calotropis gigantea* leaves was found (IC_{50} 29.17µg/ml.). The scavenging effect was compared to that of the standard ascorbic acid with IC_{50} value 12.93µg/ml.

Table 1: % Inhibition data of DPPH free radical scavenging assay by ascorbic acid

S.No.	Conc. (µg/ml)	Absorbance (Control), Ac	Absorbance (Test), At	% Inhibition
1.	2	0.70	0.51	27.143
2.	4		0.485	30.714
3.	6		0.458	34.571
4.	8		0.424	39.429
5.	10		0.395	43.571
6.	12		0.362	48.286
7.	14		0.336	52
8.	16		0.302	56.857

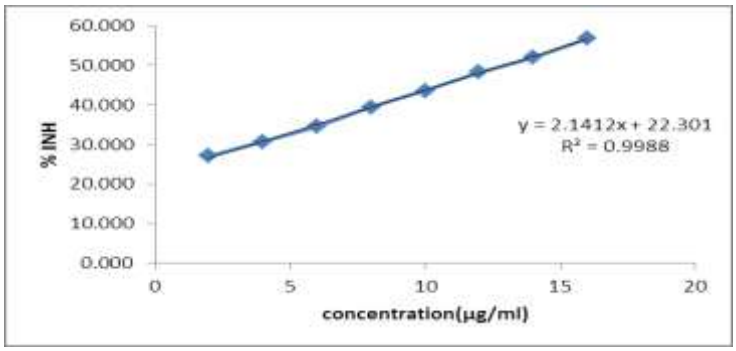

Fig 1: Standard curve of ascorbic acid Graph representing regression curve of ascorbic acid by DPPH assay method.

Table 2: % Inhibition data of DPPH free radical scavenging by hydromethanolic extract of leaves of *Calotropis gigantea*

S.No.	Conc.(µg/ml)	Absorbance (Control), Ac	Absorbance (Test), At	% Inhibition
1.	10	0.70	0.229	25.250
2.	20		0.171	37.340
3.	30		0.152	48.560
4.	40		0.114	66.230
5.	50		0.105	78.22

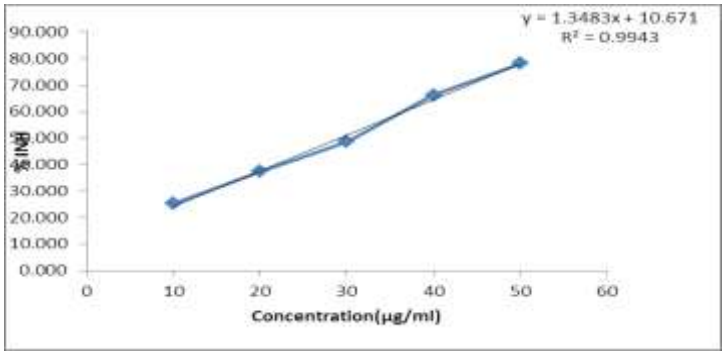

Fig 2: % DPPH inhibition curve of leaf extract. Graph representing regression curve of hydro-methanolic extract of *Calotropis gigantea* leaf extract by DPPH free radical scavenging assay method

Table 3: IC_{50} of ascorbic acid and hydromethanolic extract of *Calotropis gigantea*

S. No.	Sample	IC_{50}
1.	Ascorbic acid	12.93 µg/ml
2.	Leaf extract	29.17µg/ml

CONCLUSION

The present study was carried out to investigate the antioxidant property of hydromethanolic extract of the plant *Calotropis gigantea*. The scavenging activity of the plant hydromethanolic extract through DPPH radicals was investigated using ascorbic acid as standard. By performing the above work, it can be concluded that *Calotropis gigantea* hydromethanolic extracts possess anti-oxidant activity. The antioxidant property of the plant can be use for the treatment of various diseases.

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