

## PHYTOCHEMISTRY AND EVALUATION OF ANTIOXIDANT ACTIVITY OF WHOLE PLANT OF *CALOTROPIS GIGANTEA* LINN

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### ABSTRACT

*Calotropis gigantea* Linn. belongs family Asclepiadaceae and contains flavonoids, tannins, reducing sugars cardiac glycoside alkaloids. In the present study, ethanolic extract of *Calotropis gigantea* was investigated for its antioxidant activity. Antioxidant activity was determined *in vitro* by reducing power, DPPH and nitric oxide method. Hydroalcoholic extract of *Calotropis gigantea* shown significant antioxidant activity. *Calotropis gigantea* (Asclepiadaceae) commonly known as arka and wild growing tropical plant, which possesses number of medicinal properties. It is reported to contain cardiac glycosides,  $\beta$ -sitosterol, madrine, saponins, alkaloids, tannins, trisecharoides and flavonols. The plant has been used for various disease conditions, including leprosy, ulcers, tumours and piles. Various pharmacological activities reported like antifertility, anti-inflammatory activity, hepatoprotective activity, antimyocardial infraction activity and antidiarrhoeal activity.

**KEYWORDS:-** *Calotropis gigantea*, Phytochemistry, Maceration, Total Phenolic Contents, Reducing Power Method, DPPH, Nitric Oxide Method, IC<sub>50</sub>

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## INTRODUCTION

*Calotropis gigantea* (Asclepiadaceae) is distributed throughout India. It is popularly known as arka in Hindi. India being a tropical country is blessed with best natural resources and ancient knowledge for its judicious utilization. However, in order to make these remedies acceptable to modern medicine, there is a need to scientifically evaluate them to identify the active principles and understand the pharmacological action.<sup>1</sup>

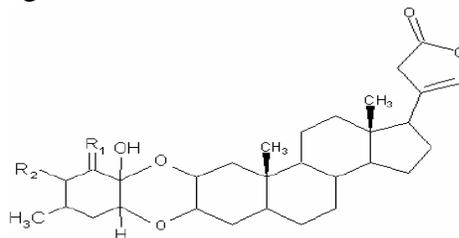
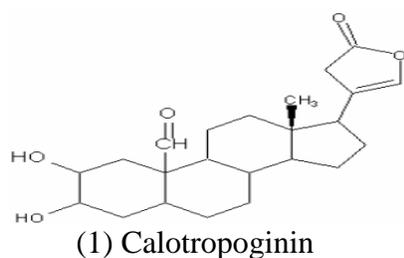
Humankind first utilized material found in environment on an empirical basis to cure various ailments. Natural products from plants and animals traditionally have provided the pharmaceutical industry with one of its important sources of lead compounds in search of new drugs and medicines. The search for new pharmacologically active agents from natural resources such as plants, animals and microbes led to discovery of many clinically useful drugs.<sup>2</sup>

India holds a pride of place largely because of its other used and economic values. The fibres extracted from the bark of the stem is white, silky, strong, flexible, durable and used in making ropes for cots, gunny bags, fishing nets, and bow strings. The wood is used as cheap fuel and latex is used in tanning industries<sup>3</sup>. The latex is used as wound healing agent by different traditional healers, it is also used as an abortifacient in folk medicines.<sup>4,5</sup>

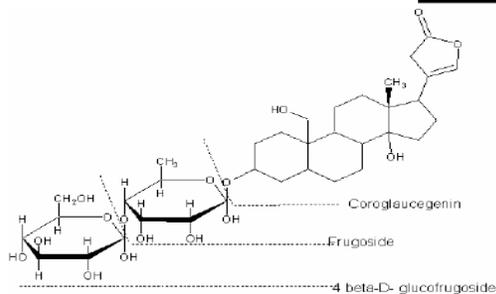
Phytochemically the plants have been investigated for cardenolides from the latex and leaves,<sup>5</sup> triterpenoids<sup>6</sup>, anthocyanins from flowers<sup>7</sup> and hydrocarbons<sup>8</sup>. The leaves and latex of *Calotropis gigantea* were found to have cardiac glycosides, various glycosides were isolated and<sup>9</sup>. An active principle 'mudarine' was isolated from leaves of *C. gigantea*. Beside this, a yellow bitter acid and resin were also found. The cardiac glycosides were identified as Calotropogenin (1), Calotropin (2), Uscharin (3) and Calotoxin (4), Calactin (5).<sup>7,10</sup> Three cardenolide glycosides Coroglaucigenin (6), frugoside (7), and 4-O-befa-D-glucopyranosylfrugoside (8), were obtained as the cytotoxic principles of "akond mul" (roots of *Calotropis gigantea* Linn.). The cytotoxicity of these compounds against various cell lines of human and mouse origin was tested. They showed similar cell line selectivity to those of cardiac glycosides such as digoxin and ouabain. They are toxic to cell lines of human origin, but not to those from mouse at two micrograms/ml<sup>11</sup>. According to Pal *et. al*,<sup>12</sup> studies on isolation, crystallization, and properties from *C. gigantea* confirm the structure of calotropins DI and DII.

Two new oxypregnane-oligoglycosides named Calotroposides A (9) and (10) have been isolated from root of *C. gigantea*, an Indonesian medicinal plant, and their chemical structures have been elucidated by chemical and spectroscopic methods as 12-O-benzoyllineolon 3-O-beta-D-cymaropyranosyl(1-4)-beta-D-oleandropyranosyl (1-4)-beta-D-oleandropyranosyl (1-4)-beta-D-cymaropyranosyl (1-4)-beta-D-cymaropyranoside and 12-O-benzoyl deacetyl metaplexigenin 3-O-beta-D-cymaropyranosyl(1-4)-beta-D-oleandropyranosyl (1-4)-beta-D-oleandropyranosyl (1-4)-beta-D-cymaropyranosyl (1-4)-beta-D-cymaropyranoside, respectively<sup>13</sup>. Besides isolation and characterization of isorhamnetin-3-O-rutinoside (11), isorhamnetin-3-O-glucopyranoside (12) and taraxasteryl acetate, a new flavonol trisaccharide was isolated from the aerial parts of *C. gigantea*, and its structure was established as isorhamnetin-3-O-(2-O-beta-D-galactopyranosyl-6-O-alpha-L-rhamnopyranosyl)-beta-D-glucopyranoside (13) by a combination of fast atom bombardment mass spectroscopy, <sup>1</sup>H and <sup>13</sup>C NMR spectra and some chemical degradations.<sup>14</sup>

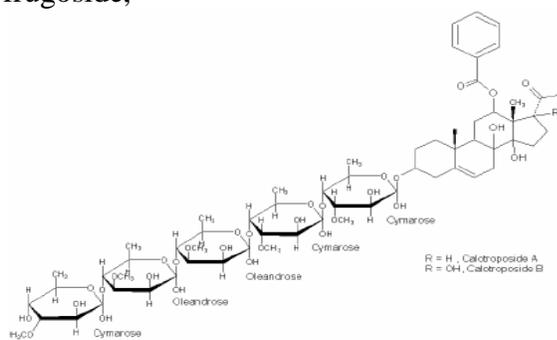
Giganticine, a novel non-protein amino acid, has been isolated from a methanol extract of the root bark of *Calotropis gigantea* and its structure established by spectroscopic methods. It exhibited a significant anti-feedant activity against nymphs of the desert locust *Schistocerca gregaria*.<sup>15</sup> Two proteinase containing carbohydrate, called calotropain-FI and calotropain-FII, were purified from *Calotropis gigantea* latex by CM-Sephadex C-50 chromatography. Both calotropain-FI and FII were found to be homogeneous by rechromatography<sup>16</sup>.



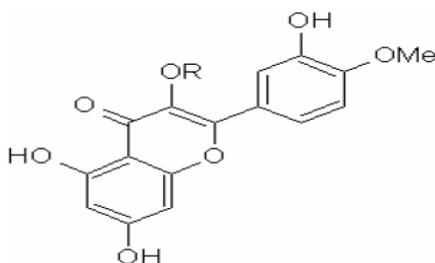
Structure No.	Nomenclature	R <sub>1</sub>	R <sub>2</sub>
2	Calotropin	$\alpha$ -OH, $\beta$ -H	H
3	Ucharin	$\left\{ \begin{array}{l} S-CH_2 \\ N-CH_2 \end{array} \right.$	H
4	Calotoxin	$\gamma$ -H, $\gamma$ -OH	H
5	Calactin	$\alpha$ -H, $\beta$ -OH	H



(6) coroglaucegenin, (7) Frugoside,  
(8) 4 beta-D-glucofrugoside,



(9) Clotroposide A, (10) Calotroposide B,



Structure No.	Nomenclature	R
11	Isorhamnetin -3-O-rutinoside	Glu-ORha O Gal
12	Isorhamnetin 3-O-glucoside	Glucose
13	Isorhamnetin rhamnoglucoside	Glucose-O-Rhamnose

## MATERIALS AND METHODS

Plant material of *Calotropis gigantea* Linn. were collected in the month of September-October from the campus of Barkatullah University Bhopal. Plants were identified and authenticated in the Department of Pharmacy, Barkatullah University, Bhopal (M.P.). The plant parts were washed properly and dried in shade. Dried plant material was subjected to reduction to coarse powder using hand grinder.

### Preparation of Extract

Approximately 200g of powdered crude drugs were extracted with hydro-alcoholic solvent (70:30) by double maceration process. The macerates were filtered with muslin cloth and concentrated using rotary evaporator to avoid thermal degradation. Phytochemical tests reveals the presence of carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, amino-acids and tannins.

### Total Phenolic Contents

The Folin-Ciocalteu reagent assay was used to determine the total phenolics content. The sample (0.2 ml) of different conc. (50-500 µg/ml) was mixed with 2.5 ml of Folin-Ciocalteu's phenol reagent and allowed to react for 5 min. Then, 2 ml of 7.5% Na<sub>2</sub>CO<sub>3</sub> solution was added, and the final volume was made up to 10 ml with deionized water. After 1 h of reaction at room temperature, the absorbance at 760 nm was determined. Gallic acid was used as standard for the calibration curve.

$$\frac{\text{Concentration of gallic acid (Cg)}}{\text{Concentration of sample (Cs)}} = \frac{\text{Absorbance of gallic acid (Ag)}}{\text{Absorbance of sample (As)}}$$

### In – Vitro Evaluation of Antioxidant Activity by Reducing Power Method

Extracts of different conc. in 1 ml distilled water were mixed with phosphate buffer (2.5 ml, 2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%); the mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (TCA, 10%) was added to the mixture which was then centrifuged at 1500g for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl<sub>3</sub> (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.<sup>17</sup>

### In – Vitro Evaluation of Antioxidant Activity by Nitric Oxide Method

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions which were measured by the Griess reaction. Sodium nitroprusside (10 mM) in 0.5M- phosphate buffered saline (PBS) was mixed with 3.0 mL. of different concentrations (20-

100µg/ml) of the drug dissolved in the suitable solvent systems and incubated at 25 oC for 150 minutes. The samples from the above were reacted with 0.5 mL of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 5% H<sub>3</sub>PO<sub>4</sub>) was added. The absorbance of the chromophore formed was measured at 546 nm. The same reaction mixture without the ethanolic extracts of plants but with equivalent amount of 0.5M phosphate buffer served as control. The percentage inhibition of nitric oxide generated was measured by comparing the absorbance values of control and test. Curcumin was used as a reference compound.

The nitric oxide radicals scavenging activity was calculated according to the equation:

$$\% \text{ Inhibition} = ((A_0 - A_1) / A_0 \times 100)$$

Where A<sub>0</sub> was the absorbance of the control (blank, without extract) and A<sub>1</sub> was the absorbance in the presence of the extract.<sup>18</sup>

### ***In – Vitro* Evaluation of Antioxidant Activity by DPPH Method**

Different concentrations of ethanolic extract of crude drug (25-250 µg/ml) in methanol shall be mixed with 400 mM methanolic solution of 1,1-Diphenyl-2-Picrylhydrazine(DPPH) at a ratio of 1:3. The mixture shall be left in the dark at room temperature for 90 min. Absorbance shall be measured by a Jasco V-530 Germany double beam spectrophotometer with matched quartz cells with corresponding 1 cm path length. The capacity of scavenging DPPH radical shall be then calculated (Table1)

## **RESULTS AND DISCUSSION**

The Total Phenolic content of *C. gigantea* was found to be 72.28 mg gallic acid equivalent per gm of extract. *In – Vitro* antioxidant activity by Reducing Power Method was also carried out and the results shows that the Reducing power of *Calotropis gigantea* = 56.34 mg Ascorbic acid equivalent per gm of extract as shown in Figure 1. For more appropriate work *In – Vitro* antioxidant activity was also done by Nitric Oxide and DPPH Method and the results are shown in Table No.1

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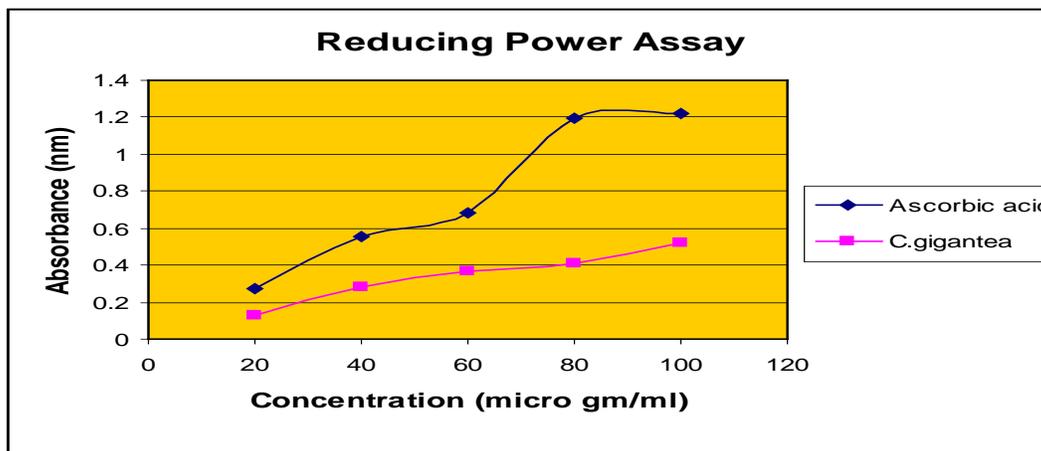


Figure 1: Reducing power of hydro alcoholic extract of *C.gigantea*

Table 1: Antioxidant activity of hydro alcoholic extract of *C.gigantea*.

Drug	Inhibitory concentration ( IC <sub>50</sub> µg/ml)	
	Nitric Oxide	DPPH
<i>C.gigantea</i>	86.56	54.29
Curcumin	21.14	--
Ascorbic acid	--	18.21

\*Curcumin used as a standard for Nitric oxide Method and Ascorbic acid used as a Standard for DPPH Method

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