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Phytochemical screening and antioxidant activity of *Calotropis gigantia* Linn. flowers in polar and non-polar solvents

Narwade Keshav[1], Marathe Vishal[2]
[1] Research Scholar, Department of Botany, Mahatma Gandhi Mahavidhyalaya, Ahemedpur, Latur, Maharashtra, India
[2] Assistant Professor, Department of Botany, NES Science College, Nanded, Maharashtra, India

Abstract
The plant *Calotropis gigantia* Linn. Belongs to the family Asclepiadaceae and is commonly known as 'Ark', which has aesthetic and religious value in Indian culture. In Ayurveda, various part of plants was used for traditional treatment of various diseases as well curative agent. The current information and knowledge regarding *Calotropis gigantia* Linn. flower world scientist and researcher have received more attention in concern with an ethnopharmacological view in recent years. The present research work focused on *Calotropis gigantia* Linn. Flower. Phytochemical analysis was done by standard method exposed a diverse group of phytochemicals such as alkaloids, flavonoids, terpenes, carbohydrates, saponins. The extract of *Calotropis gigantia* Linn. Flower in polar (Methanol) and non-polar (Hexane) solvent was evaluated for DPPH and OH^- radical scavenging activity.

Keywords: phytochemicals, *Calotropis gigantia* Linn, polar and non-polar solvent, DPPH

Introduction
The plant *Calotropis gigantia* Linn. Commonly called giant milkweed, is a wild xerophytic weed commonly found in the sub-tropical region of India. Genera *Calotropis* was a Greek-derived word that means beautiful coronal scales is a small shrub that grows in xeric condition. The studied plant belongs to the family Asclepiadaceae, characterized by the presence of taproot, stem, and leaf with soft tomentum, flower having lobed calyx, campanulate corollas, and coronal scales. Pollinia is the special character of this flower [1]. The flower of Calotropis gigantia invites worldwide researchers for pharmacological activities such as anti-inflammation and anti-ulcer [2]. The leaves extract of *C. gigantia* were screened for antibacterial, phytochemicals by using water, methanol, ethanol, chloroform, n-hexane, and ethyl acetate solvents. The extract of leaves was found to be most effective with MIC ranging from 0.25 to1.0 mg/ml against *Bacillus cereus* and *Salmonella typhi* in ethyl acetate [3]. Malformation in mango fruit and reduction in harvesting yield caused by fungal pathogen *Fusarium mangiferae*. Fungal mycelium growth inhibited by flower extract of *C. gigantia* in hexane, methanol, and water-methanol (70/30 v/v) with 5000 ppm and 10000 ppm concentration shows (2±0.9a &2±0.2a) (4±2.4b &5±0.) (5b 3± &2b 4±1.5b) (4). The dried powdered leaves of *C. gigantia* extraction in methanol, chloroform, n-hexane, ethyl acetate, and butanol extract found 3 new compounds *viz*., lignan, 90-methoxypinoresinol, and two new glycosylated 5-hydroxymethylfurfural, calofurfuralside A and calofurfuralside B have been isolated from the active fractions, CHCl3 (IC50, 0.32 lg mL1) and EtOAc (IC50, 0.55 lg mL1) fractions of the leaves of *Calotropis gigantea*. NMR and MS data were used for elucidating their structure. Among these isolated compounds, compounds 1 and 9 exhibited greater potent for cytotoxicity against PANC-1 human pancreatic cancer cell line under the normoglycemic condition with IC50 values of 3.7 and 3.3 lM, respectively [5]. Methanolic extract of *C. gigantia* shows weak acid and weak base indicators in titration [6]. The milky latex of C. *gigantia* contains hydrocarbons, sterols, fatty acids, and terpenes. Seven spots have been observed on the TLC plates; out of which 3 were identified as calotoxin, uscharin, and calactin. Macroelements and microelements were investigated in the latex and similarly in the leaves and bark from the AA spectra [7].

Materials and Methods
Plant material
Healthy flowers of plants were collected from a different region of Nanded (MS) during their floral growth of the different seasons. Flowers were authenticated and identified by Dr. M. M. Pund (Asst. prof. & Head Dept. of Botany, Indira Gandhi Senior College Nanded MS, India).

Chemicals
All chemicals and reagents were obtained from commercial sources with AR grade (HI Media Pvt. Ltd. Mumbai).

Extraction
Flowers were shaded dried and coarsely grind to a fine powder using a blender (Bosch Pro 1000W Mixer). 15 grams of powder used for extraction in aqueous, methanol, chloroform, and hexane solvents one by one separately, the crude extract was concentrated and evaporated and stored in a deep freezer at 4°C for further use.

Percentage Yield
The obtained yield of extract of flower in different solvents by using Soxhlet Extractor was used. The percentage yield flower extracts in different solvents were determined with the formula

$$\text{Percentage yield} = \frac{\text{Final weight of extract}}{\text{The initial dry weight of the sample}} \times 100 \quad \text{(Table 1)}$$

Phytochemical screening

The flower extracts of plants in different polar and non-polar solvents were screened for phytochemicals indicates the presence of a diverse group of bioactive compounds like flavonoids, tannins, phenols, alkaloids, steroids, anthraquinones, and glycosides by using a standard procedure [8, 9, 10, 14].

Antioxidant activity

DPPH radical scavenging activity

DPPH (2, 2-diphenyl -1-picryl hydroxyl) radical scavenging assay was performed as per the standard method [16] slightly modified by (11). Approximately 100 µl of isolated flower extracts with the concentration of 1000µg/ml was mixed with 100µl DPPH (0.2 mM/L in methanol) in 96 well plates. Plane methanol and Ascorbic acid (1000µg/ml in 100µl) were used as a control and standard respectively. This plate was kept for incubation at 37° C for 30 minutes. The resultant absorbance was recorded at 515 nm. by using an ELISA reader (Erba Elisa Reader). The percentage of scavenging activity was derived using the following formula [11].

Percentage of inhibition (%) = [(A control – A sample) / A control] x 100

Where A control - absorbance of DPPH

A sample - absorbance reaction mixture (DPPH with Sample)

Hydroxyl (OH^-) radical scavenging activity

Hydroxyl radical scavenging activity was determined as per the earlier reported method and slightly modified [12]. We used 3.0 ml of reaction mixture containing 1.0ml of 1.5 mM FeSO4, 0.7ml of 6mM hydrogen peroxide, 0.3 ml of 20mM sodium salicylate, and the flower extract (1mg/ml). The reaction mixture was subjected to incubation at 37°C for 1 hour after the incubation period, the absence of the hydroxylated salicylate complex was measured at 562 nm. by using an ELISA reader (Erba Elisa Reader). Methanol & Ascorbic acid were used as control and standard [13]. The percentage scavenging effect was calculated with the help of the following formula: **Scavenging activity = A0 control– A1 test/A0 control x 100.** Where A0 is the absorbance of the control (without extract), A1 is the absorbance in the presence of the extract.

Statistical Analysis

All results here are evaluated as means ± Standard deviation. ANOVA was used for evaluating the difference between means [15].

Results and Discussion

Extraction and Percentage of the yield of plant samples:

The Soxhlet extraction method was used to obtain flower extract of *Calotropis gigantia*.15 gm of powder form of the flower was used in each solvent for extraction such as water, methanol, chloroform, and hexane respectively [17]. Methanol extract had the highest yield 2.75mg/gm and percentage 18.33, water extract 2.25mg/gm and percentage 15, chloroform extract yield 1.41mg/gm and percentage 9.40 whereas, hexane extract shows 1.43mg/gm and percentage 9.46 mg/gm (Table No.1)

Table 1: percentage yield of flower in a polar and nonpolar solvent

Sr.no	Name of Plants	Solvents	The yield of Extract (mg/gm)	Percentage of Yield*
1	*Calotropis gigantia* Linn.	Water	2.25	15.00
		Methanol	2.75	18.33
		Chloroform	1.41	09.40
		Hexane	1.43	09.46

*indicates experiment performed in triplicates.

Qualitative tests for phytochemical analysis

Phytochemical's study of flowers in polar and non-polar solvents of *Calotropis gigantia* Linn. shown a notable amount of saponins, tannins, terpenoids, glycosides, terpenoids, alkaloids, anthraquinones, and carbohydrates in water, methanol, chloroform, and hexane solvent in different concentrations, aqueous and methanol extract of flower indicating presence of all phytochemicals in variable concentration whereas, less or nil concentration of some phytochemicals were found in non-polar solvent chloroform and hexane respectively [14].

Table 2: Phytochemical Analysis of Different Solvent Systems of Calotropis gigantia Linn. Flower.

Name of plant	Phytochemicals	Test	Solvents			
			Water	Methanol	Chloroform	Hexane
Calotropis gigantia	Flavonoids	Sodium hydroxide	+++	++	+	+
		Lead acetate	++	++	+	-
		Ferric chloride	+++	+	-	+
	Saponins	Frothing	++	++	++	-
	Tannins	Ferric chloride	++	++	++	+
		Chlorogenic	++	++	+	+
		Formaldehyde	+++	+++	+	+
	Terpenoids	Salkowski	+++	++	++	+
	Glycosides	General Test	+++	+++	+	+
	Steroids	Salkowski's	++	+++	+++	+
		Lieberman- Buchard's	++	+	+	+
	Carbohydrates	Free reducing sugar	++	+++	-	+
		Molisch's	++	++	+	-
	Anthraquinones	Free and combined anthraquinones	++	++	++	+
	Alkaloids	Mayer's	+++	+++	+	+
		Dragendorff's	+++	++	++	+

Key: Absent, + Present in low concentration, ++ Present in moderate concentration, +++ Present in high concentration

Antioxidant activity

DPPH radical scavenging activity

The in vitro antioxidant photometric evaluation of *Calotropis gigantia* flower extracts in polar and non-polar solvent was investigated by standard method. Percentage of DPPH radical scavenging activity of flower in hexane extract showed antioxidant activity near to standard ascorbic acid (85.40 ±0.94). Water extract of flower showed percentage antioxidant activity (78.88 ±0.78) Methanol extract of flower showed percentage antioxidant activity (75.46 ± 0.67). chloroform extract of flower showed less antioxidant activity (57.76 ±0.65) as compared to standard ascorbic acid (93.94 ±0.63) which shown the highest antioxidant activity (Table No.3)

Table 3: DPPH radical scavenging activity of Calotropis gigantia Linn. in a different solvent.

Sr. No.	Name of plant	DPPH radical scavenging activity (%) of plant sample in different solvents.				
		Hexane	Chloroform	Methanol	Water	Ascorbic acid (Std.)
01	*Calotropis gigantia* Linn.	85.40 ±0.94	57.76 ±0.65	75.46± 0.67	78.88±0.78	93.94±0.63

The results presented here are the mean values from three independent experiments ± S.D., NR = No reaction under experimental conditions.

Hydroxyl (OH) radical scavenging activity

In-vitro hydroxyl scavenging activity was estimated by the standard model in which standard compound ascorbic acid was used that shown maximum percentage of hydroxyl scavenging activity (57.77 ±0.73) after comparing to it with extracts of flower in different solvents, methanol shows hydroxyl radical scavenging activity (48.22±0.93) chloroform extract(38.22 ±0.90) water extract (22.22±0.79) shows hydroxyl scavenging activity in decreasing order whereas, in hexane extract hydroxyl scavenging activity not detected (Table No.4).

Table 4: OH radical scavenging activity of *Calotropis gigantia* Linn. in different solvent

Sr. No.	Name of plant	OH, radical scavenging activity (%) of plant samples in different solvents				
		Hexane	Chloroform	Methanol	Water	Ascorbic acid (Std.)
01	*Calotropis gigantia* Linn.	NR	38.22 ±0.90	48.22±0.93	22.22±0.79	57.77±0.73

The results presented here are the mean values from three independent experiments ± S.D., NR = No reaction under experimental conditions.

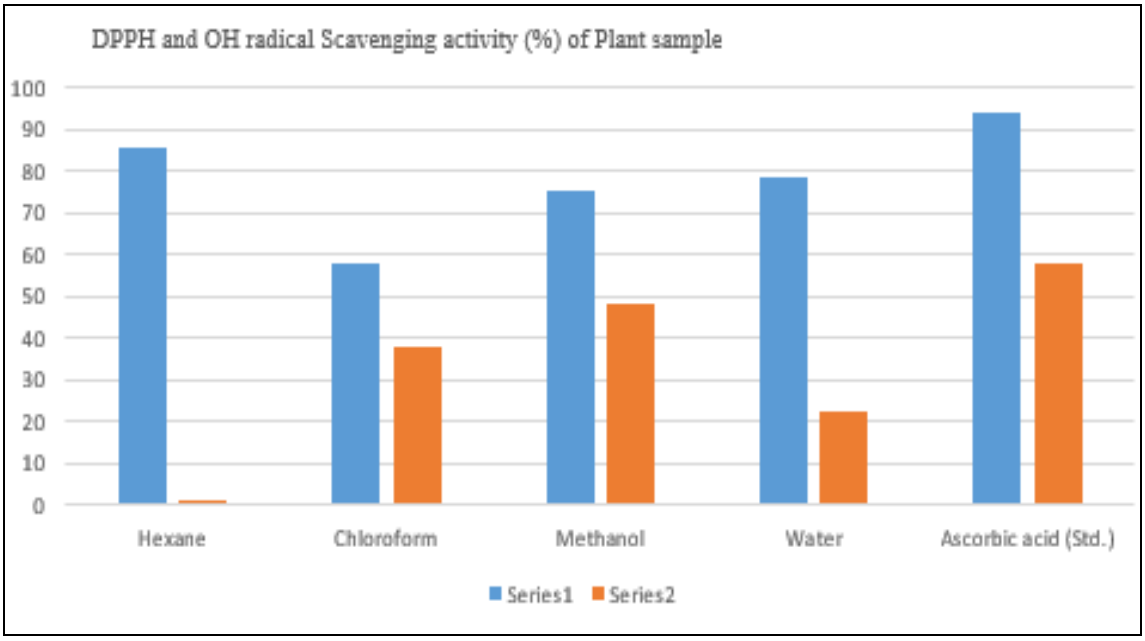

Fig 1: DPPH and OH^- radical scavenging activity (%) of plant sample

Conclusion

The plant *Calotropis gigantia* Linn. Belongs to the family Asclepiadaceae received more attention in concern with an ethnopharmacological view in recent years. Based on review literature, the present research work focused on the flower of *Calotropis gigantia* Linn. For phytochemical analysis in different polar and non-polar solvents by using the Soxhlet extraction method. Diversity in phytochemicals was found after screening flower extracts such as tannins, saponins, alkaloids, terpenoids, flavonoids, and saponins in a different solvent. The obtained extract of flowers screened for antioxidant activity by using standard methods. Although this plant is considered as a notorious wild xerophytic weed as per concern, this plant had certain active biomolecules which are useful against various diseases as therapeutics products. Based on observation and results obtained from current research, flowers of *Calotropis gigantia* Linn. Shows average antioxidant activity particularly in a nonpolar solvent such as hexane and chloroform.

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