

## Pharmacological screening of mast cell stabilizing, anti-inflammatory and anti-oxidant activity of *Calotropis procera* extracts

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### Abstract

*Calotropis procera* (Ait.) R. Br., a wild growing plant of family Asclepiadaceae, is well known for its medicinal properties which is widely used in traditional medicine to treat various diseases. *C. procera* flowers showed various levels of preliminary phytochemical screening of extract has revealed the presence of carbohydrates, flavonoids, polyphenols, tannins and saponins, alkaloids, proteins and amino acids. Acute toxicity test has done for the flowers of *C. procera* as per the standard method (OECD No: 423).

The present study was evaluated for phytochemical screening, mast cell stabilizing, anti-inflammatory and antioxidant activity of ethyl acetate extract of *C. procera*

**Keywords:** *Calotropis procera*, mast cell stabilizing, antioxidant and anti-inflammatory activity.

### Introduction

The herbal medicines occupy distinct position right from the primitive period to present day. The ethnobotanical pharmacology is as old as man himself. These medicines have less side effects and man can get the herbs easily from nature. India being a tropical country is blessed with vast 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 to understand the mechanism of action. [1-4]

It is found in most parts of the world in dry, sandy and alkaline soils and warm climate and is more common in south western and central India and western Himalayas. It is found in waste lands and grows as a weed in agricultural lands. In ancient Ayurvedic medicines the plant *Calotropis procera* was known as “Rakta arka”. Different parts of this plant have been reported to exhibit anti-inflammatory, analgesic, and antioxidant properties. *C. procera* has revealed the enormous diversity of its medicinal uses and popular use of the plant for a wide range of common ailments like fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting and diarrhea. Either the whole plant or a plant part used singly or mixed with other plant materials to enhance the efficacy. [5-7]

*Calotropis procera* Linn., also known as Alarka, Surya, Suuryaahvya, Vikirna, Vasuka, Tapan, Tuulaphala, Kshirparna, Arkaparna, Aasphota Aakh, Madaar, Ashar in India, belongs to the Asclepiadaceae family and grows in tropical region and most abundant in Bangladesh, India, Burma, Pakistan and in the sub Himalayan tract. This plant was used first time as a medicinal plant by Ved Sushruta, which is about 800–900 AD. It is used from very ancient period in folk beliefs as well as a drug of choice for different ailments. Different parts of the plant have been used in Indian traditional system of medicine for the treatment of leprosy, ulcers, tumors, piles and diseases of spleen, liver and abdomen. [8-9]

The present study was evaluated for phytochemical screening, mast cell stabilizing, anti-inflammatory and antioxidant activity of the extract of *Calotropis procera* for antiasthmatic potential.

## Methods

### Chemicals

All the chemicals used were of analytical grade, n-hexane, dichloromethane, ethyl acetate, and methanol (Merck lif. Sci. Pvt. Ltd. India)

### Plant material

Flowers of *Calotropis procera* were collected in August 2019, from Nanded Localities, (Maharashtra, India), and the plant was authenticated by Botanical survey of India, Pune, Maharashtra, India. A voucher specimen (CPR2CG) was deposited in the herbarium for further use.

### Extraction

Extraction was carried out with 300 g of the dried powdered flower material of *Calotropis procera*. The plant material was sequentially extracted with hexane, dichloromethane, ethyl acetate, methanol and water using Soxhlet extractor. All the extracts were concentrated in rotary evaporator under reduced pressure.

### Animals

Albino mice of either sex weighing (25-30 g) were housed under standard husbandry conditions having free access to food and water. Animal Ethical Committee of the Institute approved all the protocols of the study (Proposal no. SNIOP/CPCSEA/IAEC/CP-PL/12-2021).

## Phytochemical Screening and Standardization of Plant Extracts.

### Preliminary phytochemicals screening

All the extracts were screened for presence of phytoconstituents viz alkaloids, flavonoids, tannins, steroids, triterpenoids, proteins and sugars<sup>[10]</sup>

The stock solution was prepared from the crude extract (100 mg): and was dissolved in 10 ml of mother solvents. The obtained stock solutions were subjected to preliminary phytochemical screening.<sup>[11, 12]</sup>

### Test for tannins and Phenols:

The following reagents were added separately to the extract solution (prepared by dissolving 500 mg of extract in 25 ml of alcohol)

**Ferric chloride test:** -Extract solution (2-3 ml) was mixed with FeCl<sub>3</sub> solution (2ml; prepared by dissolving 5 g of FeCl<sub>3</sub> in ethanol). Presence of polyphenol was indicated by the appearance of blue or greenish black colour to the solutions shows the presence of tannins.

**Lead acetate test:** - Extract solution (2-3 ml) was mixed with lead acetate (2ml; 10% solution prepared in distilled water). Presence of polyphenol was indicated by the appearance of white precipitate. Precipitate indicates the presence of tannins.

**Potassium permanganate test:** - Extract solution (2-3 ml) was mixed with KMnO<sub>4</sub> solution (3ml; 5% prepared in distilled water). Presence of tannins or polyphenol was indicated by the decolourization of colored mixture.

**Potassium dichromate test:** - Extract solution (2-3 ml) was mixed with  $K_2S_4O_4$  (3 ml; 5% prepared in distilled water). Presence of tannins or polyphenol was indicated by the development of dark red colour to the reaction mixture.

**Acetic acid test-** - Extract solution (2-3 ml) was mixed with acetic acid (3ml).

Presence of tannins was indicated by the formation of red colour.

**Gelatin solution test:** - Extract solution (2-3 ml) was mixed with gelatin solution 3 ml (1% w/v solution was mixed with 10% NaCl), Presence of tannins was indicated by the development of white precipitate.

#### **Test for alkaloids**

Few mg of the sample was taken in 5 ml of 1.5% v/v hydrochloric acid and filtered. The filtrate was then tested using following reagents:

**Dragendorff's reagent**

It is a solution of potassium bismuth iodide. It was prepared by dissolving bismuth nitrate (8 gm) in nitric acid (20 ml), and separately dissolving potassium iodide (27.2 gm) in water (50 ml), mixing the two solutions, and making up the volume to 100 ml. Above Dragendorff's reagent was sprayed on Whatman No. 1 filter paper then the paper was dried. The test filtrate after basification with dilute ammonia was extracted with chloroform and the chloroform extract was applied on the filter paper, impregnated with Dragendorff's reagent, with the help of a capillary tube. Development of an orange red color on the paper indicated the presence of alkaloids.

**Dragendorff's test-** Extract filtrate (2ml) was mixed with Dragendorff's reagent (1ml). Presence of alkaloids was indicated appearance of Orange precipitate.

**Mayer's Reagent**

1.36 gm of mercuric chloride, and 3 gm of potassium iodide were dissolved in water to make 100 ml. To a little of each extract taken in dilute hydrochloric acid in a watch glass, few drops of the reagent was added, formation of cream colored precipitate shows the presence of alkaloid.**Mayer's test-** Filtrate (2ml) and Mayer's reagent (1ml) was mixed in test tube. Presence of alkaloids was indicated appearance of cream colored precipitate.

**Hager's reagent**

It is a saturated solution of picric acid in water. When the test filtrate was treated with this reagent, yellow precipitate was obtained indicating the presence of alkaloids.

**Hager's test-** Filtrate (2ml) and Hager's reagent (1ml) was mixed in test tube. Presence of alkaloids was indicated by appearance of Yellow colored precipitate.

**Wagner's Reagent**

It is a solution of potassium triiodide in water which was prepared by dissolving 1.3 gm iodine in a solution of potassium iodide (2 gm) in water to make 100 ml. Formation of brown precipitate after addition of this reagent in extract indicates the presence of alkaloids.to the extracts (1 g) dilHCl (10-15 ml) was mixed well and filtered. Obtained filtrate was treated with following test reagents for the detection of alkaloids.

**Wagner's test-** Filtrate (2ml) and Wagner's reagent (1ml) was mixed in test tube. The appearance of reddish brown precipitate indicated Presence of alkaloids.

**Test for flavonoids and coumarins:**

**Shinoda test-** To the extracts (25mg), absolute ethanol (5 ml), Conc. HCl (2ml) and magnesium turnings (0.5 g) were added. Presence of flavonoids was indicated by the appearance of pink or magenta colour.

**Coumarins test-** Extracts (20-25mg) was placed in a test tube and covered with whatmann filter paper moistened with dil. NaOH. This test tube was kept in a boiling water bath for 5-10 min and

covered paper was observed in UV chamber for fluorescence. Appearance of yellow or green fluorescence indicated presence of coumarins.

#### **Test for steroids:**

**Salkowski reaction-** To the extracts (15-20 mg) in chloroform (2-3 ml), Conc. H<sub>2</sub>SO<sub>4</sub> (2ml) was added to form a lower layer. Presence of plant sterols was indicated by reddish color to chloroform layer and fluorescence greenish yellow color to acid layer.

**Liebermann- Burchard reaction-** Extracts (15-20 mg) in chloroform (2-3 ml), acetic anhydride (2ml) and conc. H<sub>2</sub>SO<sub>4</sub> (1-2ml) was added to form a lower layer. Presence of steroids was indicated by change in color from red to blue or green.

**Liebermann reaction-** Extracts (25 mg) in chloroform (2-3 ml), acetic anhydride (3ml) and conc. H<sub>2</sub>SO<sub>4</sub> (2-3 drops) was mixed uniformly, Presence of sterol was indicated by appearance of blue colour.

#### **Test for saponins:**

**Foam Test-** Extract (0.25 g) was dissolved in water and shaken vigorously with sodium carbonate. Presence of saponin was indicated by the stable froth/Honeycomb like foam

**Heamolytic test:** - Sample was added to one drop of blood placed on glass slide. Hemolytic zone indicated the presence of saponins.

#### **Test for proteins**

**Biuret test** - Extract solution (3 ml), 4 % NaOH (1 ml) and 1 % CuSO<sub>4</sub> (1ml) was mixed in test tube. Presence of proteins was indicated by violet or pink color.

**Million's test** - Extract solution (3 ml) and million's reagent (5 ml) was mixed in test tube and boiled for 5 min. Presence of proteins was indicated by formation of white precipitate and change in color of precipitate from brick red to red.

**Xanthoproteic test:** - 3 ml of Sample was mixed with 1 ml of concentrated sulphuric acid, formation of precipitate shows positive test.

#### **Test for terpenoids**

##### **Liebermann–Burchard test**

Few mg of the sample was dissolved in 1 ml of chloroform and few drops of acetic anhydride. Concentrated sulphuric acid was added by the side of the test tube. Production of purple color indicates the presence of triterpenoids and blue–green color indicates the presence of sterols.

**Test for triterpenoids:** - The dry crude plant extract (5 mg) was dissolved in chloroform (2 ml) and then acetic anhydride (1 ml) was added to it. One milliliter of concentrated sulphuric acid was then added to the solution. The formation of reddish violate color shows the presence of triterpenoids.

##### **Test for anthraquinone glycosides**

**Borntrager's test:** - To 3 ml of the sample, dilute sulphuric acid was added, boiled, and filtered. To the filtrate equal volume of chloroform was added, and shaken. After separating the organic layer, ammonia was added. Turning pink of ammonical layer indicates the presence of said glycosides.

### Test for carbohydrates

**Molisch's test:** - Extract solution (2 ml), was mixed with alpha-naphthol solution (2-3 drops) and Conc. H<sub>2</sub>SO<sub>4</sub> (1ml) was added to form layer in the tube. A deep violet coloured ring at the junction of two layers indicated the presence of carbohydrates.

**Fehling's test:** -Fehling's solution A and B (1 ml each), were mixed and boiled for 1 min. To this extract solution (2 ml) was added and heated in boiling water for 1 min. To this extract solution (2 ml) was added and heated in boiling water bath for 10 min. The appearances of brick red precipitate indicated presence of carbohydrates.

**Benedict's test:** -Benedict's reagent (2 ml) was added to extract solution (2 ml) And heated water bath for 10 min. The changes in color from yellow to green or red indicated presence of reducing sugar.

**Baefoed's test** – Equal volume (2 ml each) of barfoed's reagent and extract solution was mixed, boiled for 2 min and cooled. The appearance of red precipitate showed presence of monosaccharide.

### Test for Cardiac glycosides

**Keller-Kiliani test:** - To the test extract (15-20 mg) in chloroform (2-3 ml), glacial acetic acid (1 ml) and 5% FeCl<sub>3</sub> (3-% drops) was added. Conc. H<sub>2</sub>SO<sub>4</sub> was added to the mixture so as to form layer and observed for the appearance of reddish brown ring at the junction and bluish green in the upper layer indicated presence of cardiac glycosides.

**Legal's test:** - Extract solution (1ml), pyridine (1ml) and sodium nitropruside (1ml) was mixed in test tube. Appearance of pink or red indicated positive test.

### Standardization

#### Determination of total phenolic content (TPC)

Total phenolic content was determined using Folin-Ciocalteu reagent<sup>[13]</sup>.The reaction mixture consists of 1 ml of extract solution (100 ug/ml) mixed with FC reagent (4 ml,1:10 diluted with distilled water), after 10 min incubation in dark, sodium carbonate (5 ml ,7.5%) was added to the mixture which was followed by 90 min incubation at 30°C. Phenolic content of these samples was determined spectrophotometrically using a UV visible spectrophotometer at 760 nm.

The standard curve was prepared using gallic acid (0.1mg/mL) as standard.

#### Determination of total flavonoid content (TFC)

The total flavonoid content was determined <sup>[14]</sup>.The plant extracts (500µl) was added to 2% aluminium chloride (AlCl<sub>3</sub>) solution in methanol (500µl) and incubated at 30°C for 10 min. Readings were obtained at 368nm in UV-visible spectrophotometer. The standard curve was prepared considering quercetin as standard compound.

### In Vitro Evaluation of Anti-oxidant Activity

#### Determination of DPPH radical scavenging activity

0.3mM DPPH solution (2ml) was prepared in methanol, of which 0.5mL of this solution was mixed with 100µL of extracts. The mixture was kept in dark for incubation at 37°C for 30 min. The absorbance was measured spectrophotometrically at 517nm<sup>[13]</sup>.

The ability to scavenge DPPH radical was calculated using following formula:

$$\% \text{Inhibition} = \frac{\text{Absorbance of the control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

### **ABTS radical scavenging assay**

Stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate/ ammonium persulfate solution. The working solution was prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12h at 30°C in the dark. The solution was then diluted by mixing 1 mL ABTS solution with 60 ml methanol to obtain an OD of 0.706±0.001 at 734 nm using the spectrophotometer<sup>[14]</sup>.

Plant extracts (1 mL) were allowed to react with 1 mL of the ABTS solution and the OD was taken at 734 nm after 7 min using the spectrophotometer. All the readings were taken in triplicates. The ABTS scavenging capacity of the extract was calculated as:

$$\text{ABTS radical scavenging activity (\%)} = \frac{\text{ABTS}_{\text{control}} - \text{ABTS}_{\text{sample}}}{\text{ABTS}_{\text{control}}} \times 100$$

ABTS control is the absorbance of ABTS radical + methanol;

ABTS sample is the absorbance of ABTS radical + sample extract/standard.

### ***In Vivo* Evaluation of Crude Extracts**

#### **Acute toxicity study-**

The OECD guideline # 423 was followed for the acute oral toxicity study for fixing the dose<sup>[15]</sup>. Adult Swiss albino mice of both sexes having weight 20-25gm were randomly selected for acute toxicity tests. The animals were divided into control and test groups containing three animals each. The control group received the vehicle (normal saline) while the test groups got graded doses (200, 400, 600, 800 and 1000 mg/kg) of different extracts orally. The animals were observed carefully up to 4 hours and then occasionally up to 48 hours for seeing any toxic sign or symptom like behavioral changes, locomotion, loss of righting reflex, convulsions etc. and further supervised for a period of 14 days for occurrence of any significant changes in the autonomic or behavioral responses and mortality.

#### **Evaluation of acute anti-inflammatory activity (Carrageenan- induced paw edema in rats)-**

During anti-inflammatory studies, paw edema was induced by injecting 0.1 ml of 1% (w/v) Carrageenan suspension into the sub planter region of the right hind paw of the rats<sup>[19-21]</sup>. The control group was orally administered saline (10 ml/kg) while the standard group was given

Indomethacin (5 mg/kg) and Test drug groups were given 100 mg/kg, 200mg/kg & 300 mg/kg of the test drug extract 1 hour before Carrageenan injection. The measurement of paw edema was carried out by displacement technique using plethysmometer to find out the circumference of paw edema immediately before and after at 1 hr, 2 hr, 3 hr and 4 hours following the Carrageenan injection. The inhibitory activity was calculated according to the formula

$$\% \text{ Inhibition} = \frac{(C_t - C_o) \text{ control} - (C_t - C_o) \text{ treated}}{(C_t - C_o) \text{ control}} \times 100$$

Where  $C_t$  is the paw circumference at time t,  $C_o$  is the paw circumference before Carrageenan injection and  $(C_t - C_o)$  is edema or change in paw size after time t.

### Statistical analysis

The data were statistically analyzed using one-way ANOVA followed by Dunnet's t test for individual comparison of groups with control. Results were expressed as Mean  $\pm$  SEM.  $p < 0.05$  was used to indicate statistical significance.

### Mast cell stabilizing study-

Mice were divided into 17 groups (n=3) animals in each groups for every plant. A three-day drug treatment schedule was followed. Control group treated with 1% Tween-80 (5 mL/kg), intraperitoneally; test groups were treated with Extracts at doses of 100, 200 and 300mg/kg, and standard group received sodium chromoglycate at dose of 50 mg/kg. On the fourth day, entire mice were injected with, 10 mL/kg, 0.9% saline solution, into peritoneal cavity, by gentle massage, the peritoneal fluid was collected after five minute and transferred into test tube containing 7-10 mL RPMI-1640 (Roswell Park Memorial Institute) buffer medium (pH 7.2-7.4) composed with L- Glutamine and 25 mM Hepes buffer, without sodium bicarbonate<sup>[23]</sup>.

This solution was then centrifuged at 400-500 rpm. Pellets of mast cell were washed with same buffer medium twice by centrifugation, discarding supernatant. The cell suspension from treated and control group of rats were challenged with egg albumin (100  $\mu$ g/mL) and incubated at 37°C for 10 min. The cell suspension was stained with 1% toluidine blue and observed under microscope. Degranulated mast cells observed are like burst instead of intact. Total 100 cells were counted from different visual areas and percent protection against degranulation was calculated.

## Results& Discussion

### EXTRACTION OF PLANT MATERIAL

Extraction was carried out with 300 g of the dried powdered material of *Calotropis procera* flower. The plant material was successively extracted with polarity series by using hexane, dichloromethane, ethyl acetate, methanol and water. All the extracts were concentrated in rotary evaporator under reduced pressure.

### Phytochemical screening and standardization of plants extracts

#### Preliminary Phytochemical screening



Phytochemical classes were qualitatively identified in different extracts of the flowers of *Calotropis procera* have been presented table 01.

Preliminary phytochemical screening of extract has revealed the presence of carbohydrates, flavonoids, polyphenols, tannins and saponins, alkaloids, proteins and amino acids.

**Table 1** Preliminary phytochemical screening

Phytochemical Tests	Hexane	DCM	Ethyl acetate	Methanol	Water
Flavonoids	-	+	+	+	+
Tannins	+	-	+	+	+
Alkaloids	+	-	+	-	+
Saponins	+	+	-	+	+
Carbohydrates	-	+	+	+	+
Glycosides	-	+	+	+	-
Proteins and Amino acids	+	-	-	+	-
Fixed oils and Fats	+	+	+	+	-

### Standardization for the contents of total phenolic & total flavonoid

Total phenolic content determined using Folin-Ciocalteu reagent was observed as ethyl acetate extract of flowers showed highest phenolic content ( $189.3 \pm 0.18$  mg/ml). The phenolic content decreased in the order of ethyl acetate > methanol > DCM > water > hexane in the obtained extracts of *Calotropis procera*. Ethyl acetate extract of *Calotropis procera* flowers ( $9.56 \pm 0.12$  mg/ml) exhibited highest flavonoid content. The results indicate that abundance of flavonoid content in the flowers of *Calotropis procera*. Polyphenolics such as tannins and flavonoids in plants have been considered responsible to exert not only positive effect on human health but also possess anti-inflammatory, mast cell stabilizing and anti-allergic activities.

**Table .2 Total Phenolic Content and Total Flavonoid Content.**

Extract	Total phenolic content (mg/ml)	Total flavonoid content (mg/ml)
Hexane	$24.28 \pm 0.63$	$1.3 \pm 0.21$
Dichloromethane	$145.2 \pm 0.24$	$3.15 \pm 0.31$
Ethyl acetate	$189.3 \pm 0.18$	$10.16 \pm 0.18$
Methanol	$161.45 \pm 0.47$	$4.02 \pm 0.16$
Water	$126.64 \pm 0.32$	$2.27 \pm 0.12$

Values represent mean  $\pm$  standard deviation (SD)

## IN VITRO ANTIOXIDANT ACTIVITY

### Estimation of DPPH radical scavenging activity

Electron donation capacity of natural products can be easily measured by 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) purple-colour solution. The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolorizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants<sup>[16, 17]</sup>. In the present study DPPH radical scavenging activity was highest in ethyl acetate extract (95.26%) followed by dichloromethane (86.42±0.28%), water (76±0.24%), methanol (32.13±0.41%) and hexane (24.28±0.12%). Hexane and methanol extracts showed less antioxidant activity as compared to other solvents.

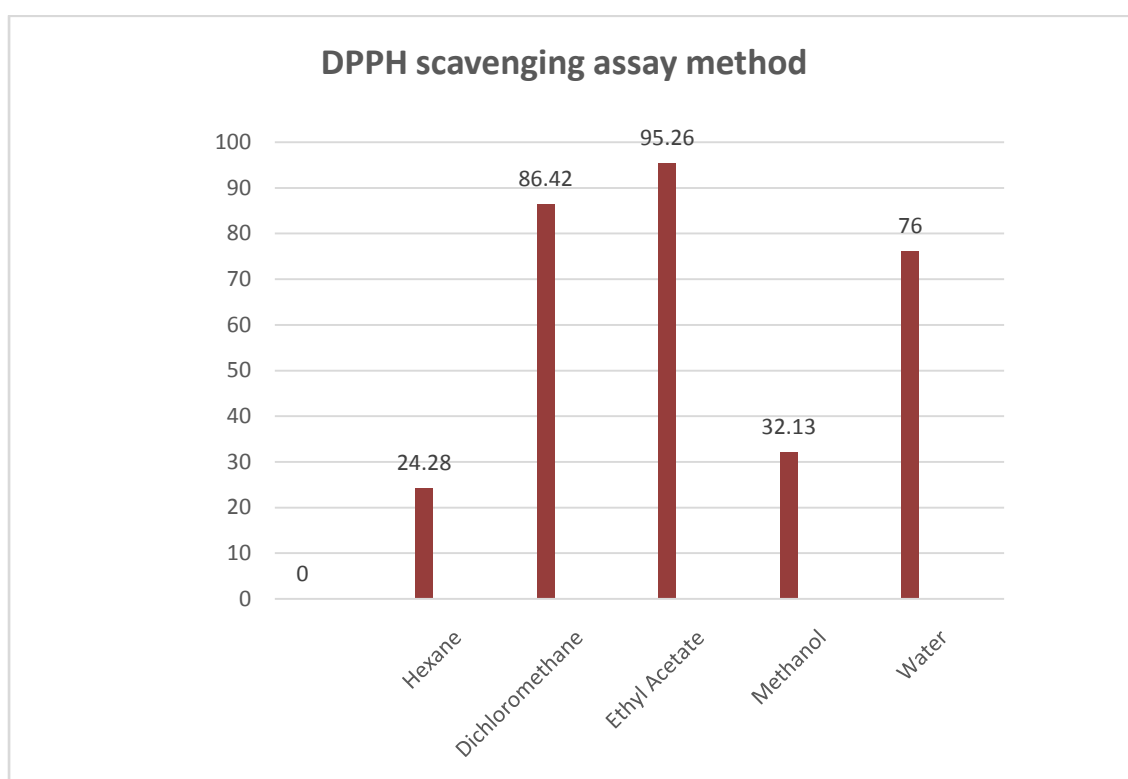
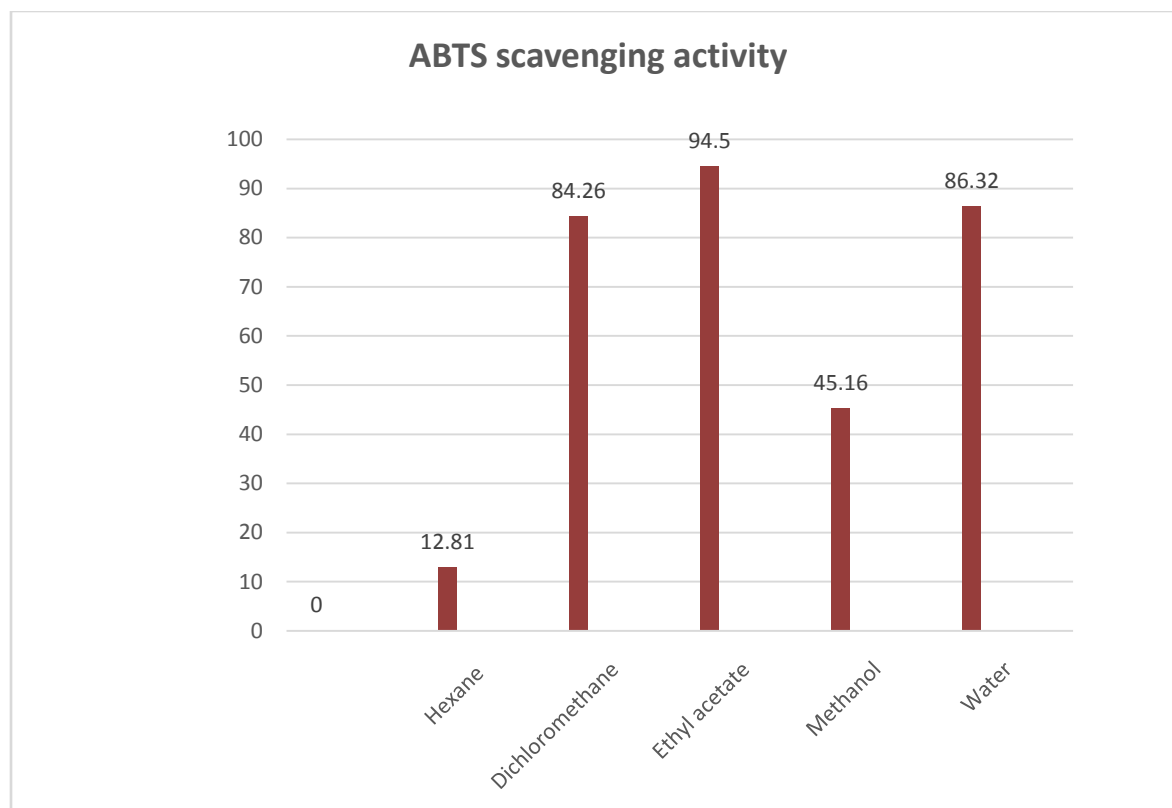


Figure .1 Percent DPPH radical scavenging activity in flowers extract of *Calotropis procera*

### ABTS scavenging activity

The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm<sup>[16, 17]</sup>. The ABTS scavenging activity is highest in ethyl acetate

extract (94.5±0.2%) and their after in water extract (86.32±0.5%) (Figure 5.1.3). The antioxidant activity was observed the lowest in hexane samples.



**Figure 2** Percent ABTS radical scavenging activity of flowers extracts in *Calotropis procera*  
**In Vivo Effect of Crude Extracts**

**Acute Toxicity studies**

Oral administration of different extracts of flowers of *Calotropis procera* up to 1000 mg/kg did not produce any toxic effects in mice during the first 48 hours and even up to the next 14 days. No mortality was observed and all extracts were found to be safe at the given doses.

**In vivo Anti-inflammatory of crude extract**

**Table 3** Anti-inflammatory effect using Carrageenan induced paw edema

Group	Treatment & Dose (mg/kg p.o.)		Paw edema volume (mL) (mean ± SEM) (h)					% inhibition <sup>^^</sup>
			0	1	2	3	4	
I.	Control	10	0.51±0.142	0.68±0.132	0.76±0.142	0.94±0.153	0.91±0.126	00.00
II.	Indomethacin	5	0.50±0.153	0.52±0.148	0.57±0.146	0.63±0.147	0.53±0.246	92.50
III.	HE-CP	100	0.51±0.246	0.64±0.215	0.76±0.128	0.91±0.362	0.85±0.131	15
IV.	HE-CP	200	0.50±0.128	0.61±0.124	0.67±0.244	0.89±0.235	0.81±0.213	22.5
V.	HE-CP	300	0.51±0.378	0.63±0.214	0.75±0.213	0.86±0.168	0.79±0.362	30
VI.	DCM-CP	100	0.50±0.142	0.66±0.235	0.74±0.162	0.91±0.328	0.80±0.362	25

<b>VII.</b>	DCM-CP	200	0.51±0.125	0.62±0.146	0.74±0.144	0.85±0.146	0.73±0.162	45
<b>VIII.</b>	DCM-CP	300	0.49±0.327	0.62±0.148	0.69±0.256	0.75±0.125	0.69±0.153	50
<b>IX.</b>	EAE-CP	100	0.50±0.214	0.61±0.135	0.72±0.142	0.81±0.146	0.74±0.216	40
<b>X.</b>	EAE-CP	200	0.51±0.421	0.63±0.132	0.68±0.429	0.75±0.153	0.64±0.318	67.5
<b>XI.</b>	EAE-CP	300	0.50±0.146	0.59±0.146	0.62±0.261	0.68±0.143	0.56±0.241	85
<b>XII.</b>	ME-CP	100	0.49±0.165	0.68±0.326	0.76±0.241	0.91±0.184	0.86±0.146	7.5
<b>XIII.</b>	ME-CP.	200	0.51±0.186	0.67±0.142	0.75±0.429	0.86±0.153	0.81±0.186	25
<b>XIV.</b>	ME-CP	300	0.50±0.235	0.65±0.254	0.74±0.142	0.82±0.324	0.78±0.143	30
<b>XV.</b>	WE-CP	100	0.50±0.213	0.61±0.146	0.75±0.234	0.84±0.126	0.81±0.264	22.5
<b>XVI.</b>	WE-CP	200	0.51±0.124	0.62±0.213	0.73±0.142	0.82±0.186	0.78±0.136	32.5
<b>XVII.</b>	WE-CP	300.	0.49±0.124	0.58±0.163	0.69±0.253	0.76±0.146	0.71±0.126	45

n=3; values are mean ± SEM

**HE-CP:** - Hexane extract of *Calotropis procera*,

**DCM- CP:** - dichloromethane extract *Calotropis procera*,

**EAE- CP:** -ethyl acetate extract of *Calotropis procera*

**ME- CP:** -methanol extract of *Calotropis procera*,

**WE- CP:** -water extract of *Calotropis procera*.

^^compared with control after 4 hr.

Anti-inflammatory study shows that the ethyl acetate extract of *Calotropis procera* flowers more effective than other extracts

**Mast cell stabilization study**

**Table 4 Effect of *C. Procera* flower extracts administration on egg albumin induced degranulation of mast cell.**

<b>Group</b>	<b>Treatment &amp; Dose(mg/kg)</b>	<b>% degranulation</b>	<b>%Protection</b>
<b>I.</b>	Control 10	74.27±0.132	-
<b>II.</b>	Sodium chromoglycate 50	24.86±0.156	66.52
<b>III.</b>	HE-CP100	72.11±0.362	2.90
<b>IV.</b>	HE-CP200	66.31±0.125	10.71
<b>V.</b>	HE-CP300	61.25±0.161	17.53
<b>VI.</b>	DCM-CP 100	58.82±0.246	20.80
<b>VII.</b>	DCM-CP200	49.11±0.142	33.87
<b>VIII.</b>	DCM-CP300	42.14±0.126	43.26
<b>IX.</b>	EAE-CP 100	41.94±0.114	43.53
<b>X.</b>	EAE-CP 200	34.12±0.162	54.05
<b>XI.</b>	EAE-CP300	28.16±0.135	62.14

<b>XII.</b>	ME-CP 100	68.23±0.145	8.14
<b>XIII.</b>	ME-CP200	59.26±0.165	20.21
<b>XIV.</b>	ME-CP300	51.24±0.132	31
<b>XV.</b>	WE-CP 100	55.16±0.125	20.78
<b>XVI.</b>	WE-CP 200	48.12±0.248	35.20
<b>XVII.</b>	WE-CP 300	44.86±0.132	39.59

n=3; values are mean ± SEM

**HE-CP:-** Hexane extract of *Calotropis procera*, **DCM- CP:-** Dichloromethane extract of *Calotropis procera*, **EAE- CP:-** Ethyl acetate extract of *Calotropis procera* **ME- CP:-** Methanol extract of *Calotropis procera*, **WE- CP:-** Water extract of *Calotropis procera*

### Degranulation of mast cell.

Mast cell stabilizing study shows that the Ethyl Acetate extract of *Calotropis procera* flowers more protective than other extracts.

### Conclusion

In the present study, the successive extraction of *Calotropis procera* flowers powder has been carried out in hexane, dichloromethane, ethyl acetate, methanol and water. Antioxidant study is carried out by estimation of DPPH radical scavenging activity and ABTS scavenging activity method. The ethyl acetate extract shows potent antioxidant activity. Mast cell stabilizing study shows that the ethyl acetate extract of *Calotropis procera* flowers more protective than other extracts. Also the ethyl acetate extract shows good anti-inflammatory effect it may be due to presence flavonoids which is responsible for antiasthmatic potential.

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