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Standardization and Biological activity of *Calotropis gigantea*

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Abstract

Calotropis gigantea (L.) is an important plant drug of Ayurveda from the ancient times of medicinal system? The Sanskrit name of *Calotropis gigantea* is Arka, it is also commonly called as crown flower or gaint milk weed. It is antifungal, anti-diabetic, anti-carcinogenic, expectorant and anti-inflammatory drug. The *Calotropis* (L.) contains anti-diabetic properties that can be identified as having a good influence on diabetes. It has specific properties that are responsible for stimulation of insulin production. The leaves and flower of Arka have certain anti-diabetic agents that improve the sensitivity of insulin and trigger secretion of insulin. The present study is on the determination of pharmacology, preliminary phyto-chemical analysis, fluorescence studies of leaves of *Calotropis gigantean* (L.). It also comprises the biological activities like antioxidant, anti-diabetic and anti-bacterial activities.

Keywords: *Calotropis gigantea* (L.) • Anti-diabetic • Anti-oxidant • Anti-bacterial activity • HPTLC and bio autography

Introduction

Calotropis gigantea is a minor tree or shurb belongs to family Asclepiadaceae, it is 4-10 cm tall. Its stem is straight. The plant has a bunch of the waxy flowers which are white or purple in colour. Its common name is crown flower or gaint milk weed. The leaves are oval, light green and oppositely oriented. The have cylindrical roots about 90 cms long and 2-10 cm in diameter. Stem is smooth. It is growing wide range. The flower has 5 pointed petals [1]. It is not consumed by animals. In axillary cymes with multiple flowers and a fungal tube 2-5 cm long at the base of the tomatoes pedicel makes up the floral inflorescence. The flowers have a yellow and light perfume and can be regular, lilac, bisexual, or pale pink, purple, or green [2]. They are placed laterally on the opposing sides of the nodules or at the tips of the interpetiolar peduncles in simple or infrequently mixed cymose corymbs. Numerous small, *Calotropis* elongated, pointed, protrusions surround each cluster. Oval flower buds appear [3].

This plant contain a chemical constituents, they are numerous glycosides, alkaloids, flavones, tannins and also calotoxin, uschrain, uscharidin and proceroside. Additionally numerous Cardenolides, flavonoids, terpenes and pregnanes are present. *Calotropis, gigantea* and uscharin show digitals like action on heart [4]. *Calotropis* has marked as anti-coagulant which is used to treat coronary thrombosis.

The leaves and Arial parts of the plant are showing the anti-diarrheal activity, anti-oxidant activity, anti-microbial activity, cytotoxic activity and wound healing activity, stem shows hepatotoxic effects [5]. *Calotropis gigantea* is used for the natural remedy for many diseases. The latex of these plants used in different conditions as tumors, analgesic, expectorant, piles and asthma [6].

The leaf juice of *Calotropis gigantea* is rubbed on the body for the body pains, especially if the joints, the fresh leaf juice are used in traditional medicine [7]. They are used as Antivenin and anti-diabetic. Dried roots used to treat asthma. The root juice in India used to reduce labour pain.

In Ayurveda the leaves of *Calotropis gigantea* is used in the treatment of paralysis. In Unani medicine it is used as anti-inflammatory, sedative, wound healer [8]. The root bark constitutes drug. The small doses of the root bark are diaphoretic and expectorant. It useful in the leprosy. The root bark gives relief in diarrhoea and dysentery. It also given in cough and asthma [9]. The latex is used as arrow poison in the Africa. It is an effective fish poison.

Materials and Methods

Collection

Leaf parts of *Calotropis procera* are collected in and around Bangalore and was identified and deposited in raw drug repository of Vriksha Vijnan Pvt Ltd, Bengaluru with the number IV/FP/VVPL/10/06/22. The plant material was screened for the presence of

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admixtures, foreign matters (sand, glass particles and dirt), mold or signs of decay. The plant material was later made into powder using pulverizer and the samples were stored in airtight container for further use.

Organoleptic characters

The present investigation comprises studies on both physical and sensory characteristics such as colour, sensation, taste, and texture of the species under study.

Physico-chemical evaluation

Determination of total ash: Two grams of powdered drug was incinerated in a sintered silica crucible by gradually increasing the heat to 500ºC-600ºC until the drug is free from carbon and then cooled. This kept in a dessicator for 15-20 min and weighed using Anamed electronic balance and noted down the readings.

Total ash (%)=(B-C)/(A) × 100

Where,

A=Sample weight in grams.

B=Weight of dish+contents after drying.

C=Weight of the empty dish in grams.

Determination of acid insoluble ash: Total ash obtained was boiled for 15 min in 25 ml of hydrochloric acid and filtered to collect the insoluble matter on Whatman filter paper and ignited in a sintered crucible. It was allowed to cool and then kept in a dessicator for 15 min. The residue was weighed in Anamed electronic balance and the acid soluble ash was calculated using the formula.

Acid insoluble ash (%) = (B-C/(A) × 100

Where,

A=Sample weight in grams.

B=Weight of dish+content after drying in grams.

C=Weight of empty dish in grams.

Determination of extractive values: Two grams of powdered plant material of both plants understudy were extracted with ethanol and water. Thus obtained extracts were allowed to dry to room temperature. After complete evaporation, weight, nature and colour of the extracts were recorded.

Extractive value (%)=(B-C)/(A) × 4 × 100

Where,

A=Weight of the plant material.

B=Weight of the dish+residue.

C=Weight of the empty dish.

Powder microscopy studies

The powdered plant material was soaked in 10% nitric acid overnight. The sample is washed with distilled water the following day. Slides are prepared by staining the soaked plant material with saffranine and observed under microscope and the images were captured.

Fluorescence studies

The fluorescent examination evaluating the behavior of the powder samples with various chemical regents such as, methanol, ethanol, water, concentrated HCl, H_2SO_4 and HNO_3 were observed in daylight as well as under UV radiation. Fluorescent analyses of all the plant powders were carried out according to the methods of chase and pratt.

Preliminary phytochemical tests

Extract of Illicium verum was subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, tannins and phenolic compounds, flavonoids, steroids, saponins. This examination was done following the methods of Gibbs, Peach and Tracey.

HPTLC studies

Sample solutions were applied to the silica gel 60 F254 pre-coated TLC plates as sharp bands by means of aspire spraylin sample applicator. The spots were dried in a current of air. Chromatography was carried out in a glass chamber (aspire). The mobile phase was poured into a twin trough glass chamber whole assembly was left to equilibrate and for pre-saturation for 30 min. The plate was then developed until the solvent front had travelled at a distance of 80 mm above the base of the plate, at 20ºC and 50% relative humidity. The plate was visualized for detection by observing it under UV 254, 366 nm and after derivatization. The densitometric scan was drawn using just TLC software attached to aspire HPTLC unit.

Bio-autography method

Antioxidant and anti-diabetic activity: A solvent system of toluene, ethyl acetate and formic acid (5:4:1) was taken to develop the chromatogram. Air-dry the chromatogram for the complete removal of solvents. Later spray the chromatogram with a solution of 0.2% DPPH in methanol/ethanol observe antioxidant activity and with iodine solution for anti-diabetic activity. Chromatograms were examined in visible light.

Results and Discussion

Organolopetic evaluation

The organalopetic evaluation of the *Calotropis gigantea* which consists of the testing the smell, taste, texture and etc. the external colour of the flowers identified as the purple and leaves are light green in color. Texture of leaves is coriacious. The stem leaves and other parts of the plant are toxic (Table 1).

Sample	Colour	Texture	Odour	Taste
Calotropis gigantea	Purple	Coriacious	Toxic	Toxic

Table 1. Organoleptic evaluation of *Calotropis gigantea* leaf.

Physicochemical evaluation

Physicochemical analysis of the sample is done in order to study the pharmacology of the sample. It is done by the determining the total ash, acid insoluble ash, alcohol and water extract values (Table 2).

Sample	Moisture	Total ash	Acid in soluble ash	Alcohol extract value	Water extract value
Calotropis gigantea	3.6.2	13.33	3.06	31.48	12.26

Table 2. Physicochemical evaluation.

Fluorescence studies

Florescent studies are done to know that reactivity of the sample to the different chemicals added. We observed the different colors under the UV light (Table 3). This helps us to know that how the sample reacts with different chemicals by showing different colors (Figures 1-3).

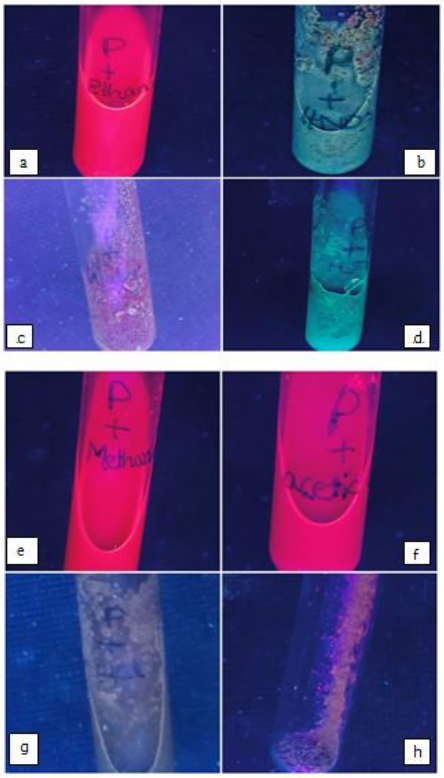

Figure 1. Florescence studies under visible light.

Figure	Chemicals added
a	Powder+ethanol
b	Powder+nitric acid
c	Powder+water
d	Powder+sulphuric acid
e	Powder+methanol
f	Powder+acetic acid
g	Powder+hydrochloric acid
h	Powder

Table 3. Powder sample mixed with different chemicals.

Anatomy of *Calotropis gigantea*

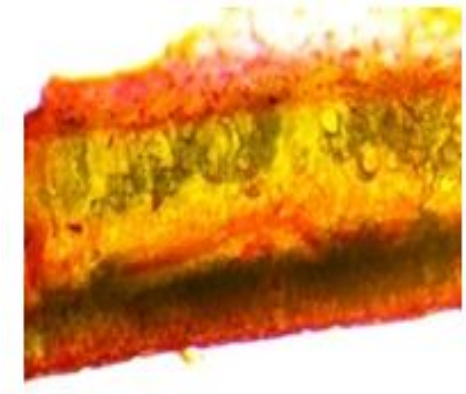

Figure 2. Simple or branched H or Y shaped lactifers are observed (anatomy of stem).

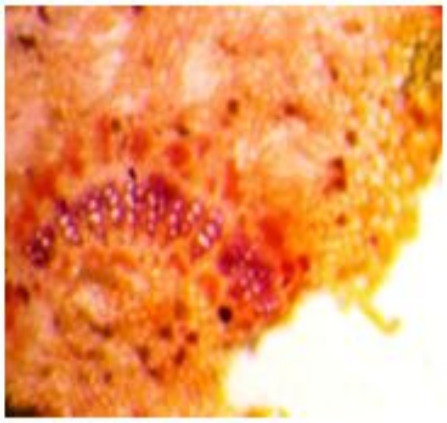

Figure 3. Unbranched and multicellular trichomes are observed in both leaves and stem (anatomy of leaf).

Powder analysis

Powder analysis is done under the microscope. The pinch of powder is taken on clean glass slide which is stained with the sample such as trichomes the saffranin stain. We observed the microscopic characters of stomata (Figure 4).

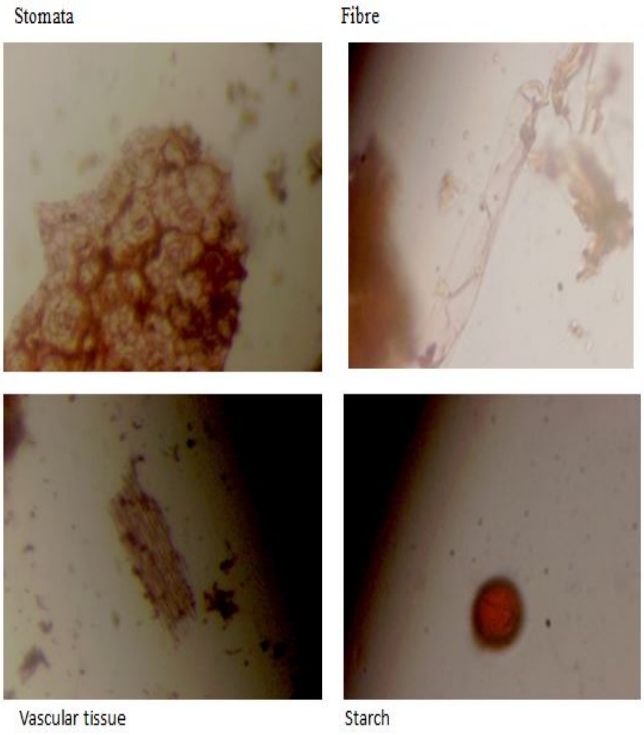

Figure 4. Powder analysis is done under the microscope.

Preliminary phytochemical analysis

The preliminary phytochemical analysis is the study of chemical compounds which are present in the plant (Tables 4-6). It is used to test whether the given leaf extract has following chemicals like alkaloids, saponins, phenolic compounds, steroids, Tannins etc. (Figures 5-7).

S. No.	Phytochemicals	Presence of phytochemicals
1	Alkaloids	+
2	Saponins	+
3	Phenolic compounds	+
4	Steroids	-
5	Tannins	+
6	Flavonoids	+

Table 4. Phytochemical analysis.

HPTLC results

Chromatogram

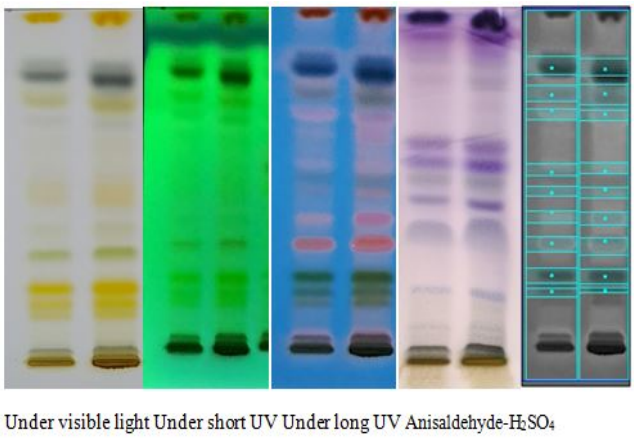

Figure 5. Bands spotted on plate through JUST TLC.

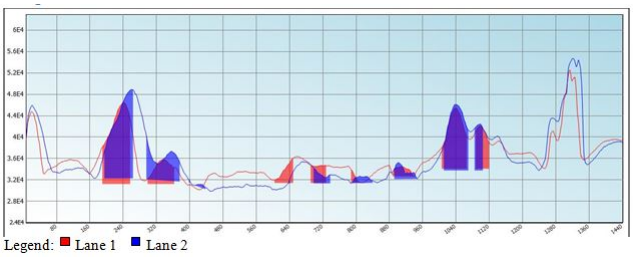

Figure 6. Chromatogram extract of *Calotropis gigantean*.

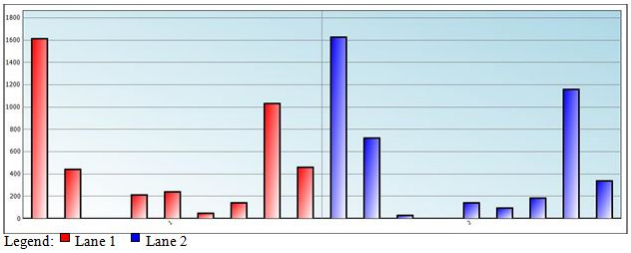

Figure 7. This graph shows HPTLC analysis of *Calotropis gigantean*.

Lanes

ID	Width	Bands	Volume
1	203	9	4163.86
2	199	9	4266.67

Table 5. The lanes observed at long UV.

Bands

ID	Rf	Area	Volume
1_1	0.842	13601	1609.2
1_2	0.77	12992	436.73
1_3	0.728	8526	0
1_4	0.562	9135	210.45
1_5	0.505	7511	238.26
1_6	0.454	9541	46.63
1_7	0.37	11165	136.7
1_8	0.281	12180	1027.2
1_9	0.238	6496	458.69
2_1	0.838	13731	1622
2_2	0.773	15920	721.57
2_3	0.717	7960	23.54
2_4	0.563	7761	0
2_5	0.514	7960	136.11
2_6	0.437	9950	92.54
2_7	0.376	13333	181.4
2_8	0.278	11741	1156.7

2_9	0.241	3980	332.81

Table 6. The bands observed at long UV that is 366 nm with their respective RF values.

The sample was plated with 3 micro litre on TLC plate and was allowed to run in the solvent system toluene: Ethyl acetate (8:2). The bands observed at long UV that is 366 nm with their respective RF values 0.94, 0.83, 0.73, 0, 0.61, 0.29, and 0.14 along with other bands.

Biological activity

Anti-oxidant activity: The plant *Calotropis gigantea* has anti-oxidant activity. The anti-oxidant activity of this is determined by the using the DPPH agent. The formation of the creamish patches on the purple back ground confirms the anti-oxidant activity.

Anti-diabetic activity: The plant *Calotropis gigantea* also has another biological property which is anti-diabetic activity. By using iodine spray we detect the anti-diabetic activity. The appearance of the white patches confirms the plant has an anti-diabetic property (Figure 8-10).

Figure 8. Creamish patches on purple backdrop confirms anti-oxidant activity.

Figure 9. Before iodine spray.

Figure 10. After iodine spray.

Antibacterial activity: The plant also exhibit the anti-bacterial activity we confirm this by using the standard gentamycin, sample and cultured bacteria like *E. coli*, *Bacillus* tenqulensis and *Pseudomonas aeroginosa*. The diameter of the zone formation confirms the anti-bacterial of the plant (Table 7).

	Zone of inhibition in diameter (cms)			
Test organisms	Gentamycin	10 µl	20 µl	30 µl
E. coli	2	0.6	0.7	1.2
Bacillus tequilensis	2.5	0.8	0.8	1
Pseudomonas aeruginosa	3.1	20.8	1	1.1

Table 7. Anti-bacterial activity of the sample.

The zones in the petri plates and the table of inhibition confirms that the sample has an anti-bacterial property

Conclusion

The plant *Calotropis gigantea* can grow in almost all type of soils and different environmental conditions. It does not demand any cultivative practices. It provides many curative and economic benefits. *Calotropis gigantea* used to treat many diseases. The phytochemical analysis of *Calotropis gigantea* revealed bioactive compounds which are responsible for *in vitro* anti-bacterial activity over *Escherichia coli*, *Bacillus tenqulensis*, *Pseudomonas aeroginosa* bacteria in all extracts could be alkaloids, cardiac glycosides, tannins, saponins, flavonoids, steroids, terpenoids and reducing sugars. Saponins the chemical present in *Calotropis gigantea* is responsible for the antioxidant potential and they are identified as anti-diabetic principle from medicinal plants. The plant extract which is rich in phenolic and flavonoids can be used in routine life is used to treat various diseases. From the present study we conclude the leaves of *Calotropis gigantea* exhibit the biological activities like antioxidant and anti-diabetic and anti-bacterial activities.

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