

IN-VITRO ANTHELMINTIC ACTIVITY OF HYDROETHANOLIC EXTRACT OF *CALOTROPIS GIGANTEA* LEAVES

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ABSTRACT

Calotropis gigantea a giant milk weed, is known for its pharmacological importance for centuries. The coarse shrub is a very promising source of anticancerous, ascaricidal, schizonticidal, antimicrobial, anthelmintic, insecticidal, anti-inflammatory, anti-diarrhoeal, larvicidal with many other beneficial properties. Plant is described as a golden gift for human kind containing calotropin, calotropagenin, calotoxin, calactin, uscharin, amyirin, amyirin esters, uscharidin, coroglaucigenin, frugoside, corotoxigenin, calotropagenin and voruscharine used in many therapeutic applications. Different compounds like norditerpenic esters, organic carbonates, the cysteine

protease proceraïn, alkaloids, flavonoids, sterols and numerous cardenolides made this plant of scientific attraction for centuries. Different extracts of *Calotropis gigantea* leaves were found to possess invitro anthelmintic activity against Indian earthworms *Pheritimaposthuma*, using piperazine citrate as reference standard. Dose dependent activity was observed in extracts of plant leaves. 70% hydroethanolic extract shown better activity of *Calotropis gigantea* leaves. No work was reported on the part of leaves of *Calotropis gigantea* shows anthelmintic activity.

KEYWORDS: *Calotropis gigantea*, *Pheritimaposthuma*, piperazine citrate, Anthelmintic activity.

INTRODUCTION

From pre-historic times to the modern era in many parts of the world and India, plants, animals and other natural objects have profound influence on culture and civilization of man. Since the beginning of civilization, human beings have worshiped plants and such plants are

conserved as a genetic resource and used as food, fodder, fibre, fertilizer, fuel, and febrifuge and in every other way. *Calotropis gigantea* one such plant.^[1]

In ancient ayurvedic medicine the plant *Calotropis gigantea* known as “sweta Arka”.

Calotropis is drought resistant, salt tolerant to a relatively high degree, grows wild up to 900 meters (msl) throughout the country and prefers disturbed sandy soils with mean annual rainfall: 300-400 mm. Through its wind and animal dispersed seeds, it quickly becomes established as a weed along degraded roadsides, lagoon edges and in overgrazed native pastures. It has a preference for and is often dominant in areas of abandoned cultivation especially disturbed sandy soils and low rainfall. It is assumed to be an indicator of cultivation.

It is a native of India, China and Malaysia and distributed in the following countries: Afghanistan, Algeria, Burkina Faso, Cameroon, Chad, Cote d'Ivoire, Democratic Republic of Congo, Egypt, Eritrea, Ethiopia, Gambia, Ghana, Guinea Bissau, India, Iran, Iraq, Israel, Kenya, Kuwait, Lebanon, Libyan, Arab Jamahiriya, Mali, Mauritania, Morocco, Mozambique, Myanmar, Nepal, Niger, Nigeria, Oman, Pakistan, Saudi Arabia, Senegal, Sierra Leone, Somalia, Sudan, Syrian Arab Republic, Tanzania, Thailand, Uganda, United Arab Emirates, Vietnam.

Helminthiasis is a worldwide disease of all ages and it is common. As per WHO more than two billion people harbor this infection. From the survey of WHO only few drugs are frequently used in the treatment of helminthes in human, due to the cost and the development of resistance against these drugs turned the attention of many researchers towards the evaluation of medicinal plants. After the successful research work of many scientists, the natural sources play a key role in the treatment of the anthelmintic. *Calotropis gigantea* (Ait.) R.Br. (giant milkweed) belong to the family Asclepiadaceae locally known as “aak” in India is being used as herbal medicine by people living the desert areas.^[2] *Arka* (*Calotropis gigantea*) an important drug of Ayurveda is known in this country from the earliest time. It is mentioned by the earliest Hindu writers and the ancient name of the plant which occurs in the Vedic literature was *Arka* alluding to the form of leaves, which was used in the sacrificial rites. There are two common species of *Calotropis*, viz. *Calotropis gigantea* (Linn.) R.Br. and *Calotropis gigantea* (Ait.) R.Br. described by the Sanskrit writers. Both the species are used as substitutes for one another and are said to have similar effects. In

DhanvantariNigantu three varieties of Arkaare mentioned viz. Rajarkah, Suklarkah and Svetamandarah. It has been widely used in the Sudanese, Unani, Arabic and Indian traditional medicinal system for the treatment of various diseases namely leprosy, ulcers, piles and diseases of the spleen, liver and abdomen. In the traditional.

Indian Medicinal system, this plant has been used for a variety of disease conditions including asthma, cold, cough, piles, ulcers, diarrhea, heart diseases, leprosy, rheumatism and diseases of skin, spleen, liver and abdomen.^[3-5] Different parts of the plant have been advocated for use in a variety of disease conditions. The leaf of *Calotropis gigantea* is sessile, 6-15 cm by 4.5-8 cm, broadly ovate, ovate-oblong, elliptic or obovate acute, pubescent, when young and glabrous on both sides on maturity. The lamina which is dorsiventral with mesophyll differentiated into a palisade and spongy tissue.^[6] Extracts from this plant have been found to possess various pharmacological activities.^[7] In search of potential anthelmintic agent and to validate the anthelmintic claim of this plant, this study was designed. Morphologically the plant is erect, tall, large, much branched and perennial shrub or small tree that grows on a height of 5.4m, with milky latex throughout. Bark is soft and corky, branches stout, leaves sessile, opposite, decussate, broadly ovate, oblong, elliptic or obovate, acute, thick, glabrous, green coloured with fine cottony pubescent hair on young. Flowers in umbellate cymes and tomentose on young. Seeds broadly ovate, acute, flattened, minutely tomentose, brown coloured and silky. Fruits tomentose, brown coloured and silky.

Plant profile



Synonym: *Calotropis gigantea* Gauriakavana, Aka, Mandara.

G.S.: *Calotropis gigantea* (Asclepiadaceae) whole plant found all over India upto an altitude of 900m including the Andaman. Also found in dry waste places commonly known as mudar in English.^[10] It occurs throughout India from Punjab and Rajasthan in the north to Kanniyakumari in the south, extending into West Bengal, Assam in the East (K.S. Krishnananmarg)

Scientific Classification

| | |
|---------|----------------|
| Kingdom | Plantae |
| Order | Gentianales |
| Family | Asclepiadaceae |
| Genus | Calotropis |
| Species | Gigantea |

Morphological characters

Parts used: **Habit:** Shrub or a small tree up to 2.5 m (max.6m) height.

Root: Simple, branched, woody at base and covered with a fissured; corky bark; branches somewhat succulent and densely white tomentose; early glabrescent. All parts of the plant exude white latex when cut or broken.

Leaves: Opposite-decussate, simple, subsessile, exstipulate; blade-oblongobovate to broadly obovate, 5-30X2.5-15.5 cm, apex abruptly and shortly acuminate to apiculate, base cordate, margins entire, succulent, white tomentose when young, later glabrescent and glaucous.

Flowers: Bracteate, complete, bisexual, actinomorphic, pentamerous, hypogynous, pedicellate, pedicel 1-3 cm long.

Floral Characteristics: Inflorescence: A dense, multiflorous, umbellate, pedicled cyme, arising from the nodes and appearing axillary or terminal.

Calyx: Sepal 5, Polysepalous, 5 lobed, shortly united at the base, glabrescent, quincuncial aestivation.

Corolla: Petals five, gamopetalous, five lobed, twisted aestivation.

Androecium: Stamens five, gynandrous, antherditheous, coherent.

Gynoecium: Bicarpellary, apocarpous, styles are united at their apex, peltate stigma with five lateral stigmatic surfaces. Anthers adnate to the stigma forming a gynostegium.

Fruit: A simple, fleshy, inflated, subglobose to obliquely ovoid follicle up to 10 cm or more in diameter.

Seeds: Many, small, flat, obovate, 6x5 mm, compressed with silky white pappus, 3 cm or more long.^[8]

Cultivation and collection: The seeds freely float in the air and natural regeneration is very common. Vegetative propagation through stem and root cuttings is very useful in large scale multiplication of the superior genotypes. *Calotropis* has been cultivated in South America and on the Caribbean Islands for the production of fibres at a spacing of 1-1.5m. When cultivated annual yields of up to 500kg/ha are expected. A single harvest per season is preferable to a double or triple harvest; a single harvest would result in a net saving of energy input both on the farm and in the processing plant.^[9]

Side Effects

Interfere with heart function, Vomiting, Diarrhoea, Slow heartbeat, Convulsions, Death.

Uses: Asthma, Abortifacient, Anti-cancer, Anthelmintic, CNS activity, Epilepsy, Eczema, Expectorant, Fever, Leprosy, Migraine. Finally the result of these things useful of ethanol extract of *Calotropis gigantea*.

Need of Work: The active principles responsible for the Anthelmintic activity of ethanolic extract of *Calotropis gigantea* leaves need to be explored and exact mechanism of action need to be studied in detail.

Standard Drugs Used As Anthelmintic Activity: Piperazine Citrate

Objectives: To evaluate anthelmintic activity of Ethanolic extract of *Calotropis gigantea* by using earthworms.

MATERIAL AND METHODS

A. Selection of plant: The fresh leaves of the plant will be collected from the Satara city, Maharashtra.

B. Authentication of Plant: The Plant will be authenticated head of department of Yashwantrao Chavan Institute of Sciences, Satara

C. Extraction of plant material by using combined method of Maceration and Ultrasonication.

D. Plant Material

- 1) The fresh leaves of the plant will be collected from the Satara city, Maharashtra
- 2) The leaves were cleaned by washing with running water and shade dry and then milled to coarse powder by mechanical grinder.

E) Preparation of Extracts

- 1) The dried powder leaves were extracted by Ultrasonication and maceration method combination process.
- 2) Drug macerated for seven days with 70% hydro ethanol and simultaneously, everyday 1hr ultrasonication extraction was carried out on the same extract.
- 3) On the 7th day the solvent portion was evaporated under reduced pressure
- 4) The prepared extracts were kept under refrigeration for screening of anthelmintic activity.

In-vitro Anthelmintic activity

- 1) The anthelmintic activity was evaluated on adult Indian earthworm *Phaeritimaposthuma* due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings.^[10-13]
- 2) The earthworms are collected and washed with normal saline with removal of fecal matter.
- 3) The earthworms are 5 to 6 cm length and 0.2- 0.3 cm widths were used for experiment protocol.
- 4) 70% hydro ethanolic extracts that were prepared from *Calotropis gigantea* leaves were examined systematically for their *in-vitro* anthelmintic activity against *Phaeritimaposthuma*.
- 5) The *in-vitro* anthelmintic assay procedures were carried out. with slight modifications.^[14-16]
- 6) Five groups of equal size Indian earthworm consisting of six earthworms in each groups were released into 15 mg/mL, 30 mg/mL, 60 mg/mL, of desired formulation.
- 7) Each group was treated with one of the following: Vehicle, piperazine citrate (20mg/mL), and different extracts of in normal saline.
- 8) Observations were made for the paralysis time and subsequently for death time of the worms. The mean paralysis and/or death time for each group was recorded (each reading

taken for 6 times). The time taken by the worms to become motionless, consider as paralysis was recorded and the lethal time was recorded by observing the time taken to become motionless on application of external stimuli by pricking with pin. Piperazine citrate (20mg/mL) was taken as reference drug.

RESULT AND DISCUSSION

1. Paramerters of Extracts



70% Hydro Ethanolic extract.

Table No.1

| Characteristics | 70% Hydro Ethanolic extract |
|-----------------|-----------------------------|
| Colour | Brown |
| Odour | Acrid |
| Taste | Bitter |

Phytochemical Analysis

CHMICAL TEST

| A.Test for Alkaloids | Aqueous extract | Ethanolic extract |
|---------------------------|-----------------|-------------------|
| 1.Mayer's Test | + | + |
| 2.Dragndroff's Test | + | + |
| 3.Wagner's Test | + | + |
| 4.Hager's Test | + | + |
| B.Test for Tannins | | |
| 1.5% FeCl ₃ | — | — |
| 2.Lead Acetate | — | — |
| 3.Bromine Water | — | — |
| C.Test for Glycosides | | |
| 1.Cardiac Glycoside | — | — |
| 2.Anthraquinone Glycoside | — | — |
| 3.Saponin Glycoside | — | — |
| 4.Coumarin Glycoside | — | — |

| | | |
|----------------------------------|---|---|
| D. Test for Flavonoids | | |
| 1. Shinoda Test | + | + |
| 2. Sulphuric Acid Test | + | + |
| E. Test for Carbohydrates | | |
| 1. Fehling's Test | = | = |
| 2. Benedict's Test | = | = |
| F. Test for Resin | = | = |
| G. Test for Steroids | | |
| a. Libermann test | = | = |
| b. Salkowski test | = | = |

Anthelmintic screening: Observations were made for the time taken to paralysis and death of individual worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors.



Observation table

Table-1; Anthelmintic activity of 70% hydroethanolic extract of *Calotropis gigantea* leaves

| Treatment | Concentration(mg/ml) | Paralysis Time(min) | Death time(min) |
|----------------------------|----------------------|---------------------|-----------------|
| Vehicle | - | - | |
| Piperazine- citrate | 20 mg/mL | 7.20min | 9.00 min |
| 70% Hydroethanolic extract | 15 mg/mL | 9.37 min | 17.30 min |
| 70% Hydroethanolic extract | 30 mg/mL | 6.45 min | 8.30 min |
| 70% Hydroethanolic extract | 60 mg/mL | 5.30 min | 7.20 min |

RESULT

In the present work, three different concentrations of extract which is prepared by combined extraction technique was used, 70% hydro ethanolic extract conc. at 60mg/ml, 30mg/ml, 15mg/ml used to evaluate in-vitro anthelmintic activity against Indian earthworm.

In-vitro anthelmintic assay the perusal of the data reveals that the 70% hydroethanolic extract at the concentration of 60 mg/mL showed both paralysis and death in 5.30 and 7.20 min. the time of paralysis is less as compared to standard drug. The potency of the extract was found inversely proportional to the time taken for paralysis or nature of extract.

CONCLUSION

C. gigantea (giant milk weed) has been used as traditional folk medicine by many cultures, and it has been the subject of extensive phytochemical and bioactive investigations. It had shown significant pharmacological importance representing as a strong contender in the medical area. In conclusion the 70% hydroethanolic extract of *Calotropis gigantea* leaves had significant anthelmintic activity. It deserves further studies to identify its active components which responsible for anthelmintic activity and investigate their mechanism.

REFERENCE

1. Sastry CST, Kavathekar KY. In: Plants for reclamation of wasteland. Publication and Information Directorate, CSIR, New.
2. "The Ayurvedic pharmacopoeia of India", Government of India Ministry of Health and Family Welfare, Department of Ayush. Part-I, Vol.1, 2007, p. 13. Delhi. 1990: 175-179.
3. Razzak, H.M.A., "Unani System of Medicine in India", Central Council for Research in Unani Medicine, New Delhi, 1991; 29.
4. Kartikar, K.R. and Basu, N., "Indian Medicinal Plants", Lolit Mohan Basu, Allahabad, 1935; 1606.
5. Murti, Y., Yogi, B. and Pathak, D., Int. J. Ayurveda Res., 2010; 1(1): 14.
6. Kumar, V.L. and Arya, S. "Medicinal uses and pharmacological properties of *Calotropis procera*. In: GovilJN, ed. Recent Progress in Medicinal Plants", Texas: Studium Press, 2006; 11: 373.
7. Mueen Ahmed, K.K., Rana, A.C. and Dixit, V.K., Phcog. Mag., 2005; 1(2): 48.

8. Calotropis gigantea - botanical, pharmacological view R Ramasubramania Raja, N Kishore, MSreenivasulu, SK Rasool Bee, SNandini, L Ooha, N Chaitanya ISSN 2320-3862JMPS 2016; 4(2): 87-89© 2016 JMPS.
9. Mueen Ahmed KK, Rana AC, Dixit VK Calotropis species (Asclepiadaceae) comprehensive review. Pharmacognosy Magazine 2005; 1(2): 48-52.
10. Vidyarthi, R.D., "A Text book of Zoology", 14th Edn., S. Chand and Co., New Delhi, 1967; 45.
11. Thorn, G.W., Adams, R.D., Braunwald, E., Isselbacher, K.J. and Petersdorf, R.G., "Harrison's Principles of Internal Medicine", McGraw Hill Co., New York, 1977; 1088.
12. Vigar, Z., "Atlas of Medical Parasitology", 2nd Edn., P.G. Publishing House, Singapore, 1984; 216.
13. Chatterjee, K.D., "Paracetology, Protozoology and Helminthology", 6th Edn., In Guha Ray Sree Saraswati Press Ltd., Calcutta, 1967; 87.
14. Dash, G.K., Suresh P., Sahu S.K., Kar, D.M., Ganapaty, S. and Panda, S.B., J. Nat. Remed., 2002; 2(2): 182.
15. Rastogi, S., Rastogi, H. and Singh V., Indian J. Nat. Prod., 2009; 25(4): 15.
16. Ajayeoba, E.O., Onocha, P.A. and Olarenwaju, O.T., Pharm. Biol., 2001; 39: 217.
17. Deore S.L., et al., Int. J. Chem Tech Res. 2009; 1(2): 178.
18. Practical Pharmacognosy by Dr. K.R. Khandelwal Nirali Prakashan, Page No.25.5,25.6.
19. Chavan, R S; Khadke, A P., Synthesis and Biological Evaluation of Novel Indolyl Isoxazoline Derivatives as Analgesic and Anti-inflammatory Agents, Asian Journal of Chemistry; Ghaziabad 24.6, 2012; 2711-2716.
20. Khadke A P., 2D-QSAR Study of Novel Oxazoline Benzyl Ester Derivatives as Anti-Tuberculosis Agents, IJPBS, OCT-DEC, 2011; 1(4): 501-509.