IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
April 2017 Vol.:9, Issue:1

Calotropis gigantea (L) Root: Pharmacognostic Evaluation

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
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Submission: 2April 2017
Accepted: 7 April 2017
Published: 25 April 2017

www.ijppr.humanjournals.com

Keywords: *Calotropis gigantea* (L); pharmacognostic evaluation; phytochemical studies.

ABSTRACT

Calotropis gigantea (Family-Asclepiadaceae) frequently known as Madar in Hindi is a perennial herb with a long history of use in traditional medicines. *Calotropis gigantea* is used as a traditional medicinal plant. The Plant root shows Nootropic activity in methanolic extract. It can be also used in many diseases like anxiogenic activity, expectorant, antihelmintic, sedative, leprosy, ulceration, coughs, ringworm of the scalp, piles, explosion on the body, asthma etc.*Calotropis gigantea* (L) root: Quantitative Pharmacognostic analysis gave moisture content 18.7%, total ash value 6.5%, acid insoluble ash 4.0% and water soluble ash 0.08%, water insoluble ash 2.5%. Different extractive values are: In Petroleum ether 4.16%, Chloroform 1.2%, Methanol 4.52% and water soluble extractive value 10.12%. Water soluble extractives are more than alcohol soluble extractives show more water soluble constituents in the roots.

1. INTRODUCTION

We hereby reported our findings related to cognitive dysfunction activities of plant *Calotropis gigantea* (Asclepiadaceae).*Calotropis gigantea* is a large shrub plant. It has clusters of waxy flowers that are either white or lavender in color. Whole plant found allover India (up to an altitude of 900m) including the Andaman. Also found in dry squander places. It occurs throughout India from Punjab and Rajasthan in the north to Kanniya kumara in the south, extending into West Bengal, Assam in the East (2,1,3).*Calotropis gigantea* (Asclepiadaceae) containa long history of use in traditional medicines. Roots are externally whitish-grey in color. Transverse section of mature root shows cork zone composed of 30–50 or rows of polyhedral to nearly cubical thin walled cell. Very small sized cubical crystals are found in the inner row (5,6).

Figure 1.1:*Calotropis gigantea* (L) Plant, Plant root and Root powder

The root bark of *Calotropis gigantea* resembles Ipecac in its action and is for its substitute for it. In small doses, the root bark is diaphoretic and expectorant. It acts as a mild stimulant and is given with carminatives in dyspepsia. It is useful in leprosy. The powdered root bark gives relief in diarrhea and dysentery. It is also given in asthma and as a febrifuge (7).It is very nutritious. It mainly contains moisture (18.7%), protein (1-3%), fat (1-2%), minerals (Copper, lead, nickel, chromium, cadmium, iron and zinc etc), and alkaloid percentage in roots ranges from 0.11 to 0.31%(8,4).

2. MATERIALS AND METHODS

2.1. IDENTIFICATION, COLLECTION, AUTHENTICATION OF ROOTS:

The root of *Calotropis gigantea* (L) collected from botanical garden of Prasad Institute of technology, Jaunpur, U.P. India July 2016. The roots were dried under normal environmental conditions and were authenticated by Dr. G.P. Sinha Principle Scientist, Botanical Survey of India Central Regional Center, 10 Chatham Lines, Allahabad- 211002.

Figure 2.1:*Calotropis gigantea* (L.) plantHerbariumforauthentication letter

The specimen with voucher number SIP/2016/189 has been deposited at the Department of Pharmacy, SIP, Allahabad and Botanical Survey of India for future reference. The roots were washed properly and dried in shade. Dried plant material was subjected to reduction to coarse powder using mechanical grinder, passing through sieve #40 and stored in a tight container these fine powders are analyzed for following Pharmacognostic parameters(9).

2.2. PRELIMINARY PHYTOCHEMICAL INVESTIGATION:

2.2.1. DETERMINATION OF EXTRACTIVE VALUES:

2.2.1.1. SUCCESSIVE SOLVENT EXTRACTION METHODOLOGY:

The dried coarse powder material of *Calotropis gigantea* (25 g) was subjected to Soxhlet extraction separately and successively withpowdered material was defatted with petroleum ether (60-80ºC) and successively extracted for 24 hr using chloroform (61.2ºC), methanol (64.7ºC) and distilled water. Solvents are used in the increasing order of polarity. The solvent was distilled under reduced pressure, controlled temperature (reference of solvents) and the

resulting semisolid mass was vacuum dried using rotary evaporator to yield a solid residue put in airtight container and stored in refrigerator (8).

Solvent – Petroleum ether, chloroform, methanol and aqueous.

Method – Soxhlet extraction

The yield of the extracts was calculated using the following formula (12):

Formula:

$$\text{Extractive value \%} = \frac{\text{Residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

2.2.1.2.Petroleum ether soluble extractive value:

The roots of *Calotropis gigantea* coarse and air dried drug material were washed and allowed to dry in shade for a week and then grounded into fine powder in mixer grinder. 25 grams of dried powder of roots was subjected to Soxhlet extraction with 200 ml of solventsfor 4 hr starting from petroleum ether (60-80ºC). Extraction was concentrated by distilling off the solvent and then evaporated to dryness on water bath.The extract obtained by solvent was weighed.Its percentage was calculated in terms of air-dried weighed of powdered drug. The powdered drug was dried in desiccator or in hot air oven (below 150ºC), for next step(10,11)(Table no.1)

Calculation:

$$\text{Extractive value \%} = \frac{1.4}{25} \times 10$$

$$= 4.16\%$$

2.2.1.3. Chloroform soluble extractive value:

Each time before extracting with the next solvent, the powdered material was dried in hot air oven (below 150ºC). Then the dried powderroots were subjected to Soxhlet extraction with 200 ml of solvent using chloroform for 4hr. The residue was evaporated using a rotator evaporator and then evaporated to dryness on water bath. Average extractive value in

percentage w/w (on the dry basis) was calculated with reference to air dried drug. (10, 11) (Table no.1)

Calculation:

$$\text{Extractive value \%} = \frac{0.3}{25} \times 100$$

$$= 1.2\%$$

2.2.1.4. Methanol soluble extractive value:

Each time before extracting with the next solvent, the powdered material was dried in hot air oven (below 150 ºC). Then the dried powder roots were subjected to Soxhlet extraction with 200 ml of solvent using methanol for 4 hr. The residue was evaporated using a rotator evaporator and then evaporated to dryness on water bath. Average extractive value in percentage w/w (on the dry basis) was calculated with reference to air dried drug. (10,11) (Table no.1)

Calculation:

$$\text{Extractive value \%} = \frac{1.13}{25} \times 100$$

$$= 4.52\%$$

2.2.1.5. Water soluble extractive value:

Each time before extracting with the next solvent, the powdered material was dried in hot air oven (below 150ºC). Then the dried powder roots were subjected to Soxhlet extraction with 200 ml of solvent using distilled water for 4 hr. The residue was evaporated to dryness on water bath. Average extractive value in percentage w/w (on the dry basis) was calculated with reference to air dried drug. (10, 11) (Table no.1)

Calculation:

$$\text{Extractive value \%} = \frac{2.53}{25} \times 100$$

$$= 10.12\%$$

2.3. DETERMINATION OF ASH VALUE:

2.3.1. Total Ash value:

2 grams of dried, powdered plant material was taken in the pre-weighed clean sintered silica crucibles. Then, they were incinerated by gradual increasing of the temperature (400-500 ºC) in the muffle furnace till white ash obtained until constant weight of ash obtained. The crucible was cooled to room temperature in a desecrator and weighed the ash and calculated the % of total ash with reference to the air-dried sample of the crude drug using following formula (Table-2) (12, 13).

Formula:

$$\text{Total ash Value (\%)} = \frac{Z-X}{Y} \times 100$$

Where, X= Weight of the crucible, Z = Weight of the crucible with ash,
Y = Weight of the powder taken (g).

Calculation:

$$\text{Total ash Value (\%)} = \frac{18.75-18.62}{2} \times 100$$

$$= 6.5\%$$

Where, X= 18.62gm, Z = 18.75gm, Y = 2gm.

2.3.2. Acid insoluble ash value:

The total ash content of the plant material obtained was boiled for 15 min, after adding 25ml of 25%(v/v) HCl into a 100 ml beaker and was allowed to cool. It was filtered through a Whatman filter paper no. 44 (ashless) and wash the residue twice with hot water. The insoluble ash thus retained on filter paper along with paper was ignited in a preweighed sintered crucible (1000ºC). Then the crucible along with the residue was weighed and calculated the acid insoluble ash content using the following formula (Table-2) (12, 13).

Formula:

$$\text{Acid insoluble ash Value (\%)} = \frac{X}{Y} \times 100$$

Where, X= weight of the residue; Y= Weight of powder taken (g).

Calculation:

$$\text{Acid insoluble ash Value (\%)} = \frac{0.08}{2} \times 100$$

$$= 4.0\%$$

Where, a= 0.08gm, Y= 2gm.

2.3.3. Water soluble ash value:

The total ash value was determined using 2 g of the air-dried powdered sample. The total ash was boiled for 5 minutes with 25 ml of distilled water; the insoluble matter was collected on an ashless filter paper, washed with hot distilled water and ignited for 15 minutes at a temperature not exceeding 450 ºC. The weight of the insoluble matter was subtracted from the weight of the total ash; the difference in weight represents the water-soluble ash. The percentage of the water-soluble ash was calculated with reference to the air-dried powdered plant sample. It was calculated by using following formula (Table-2) (14).

Formula:

$$\text{Water insoluble ash Value (\%)} = \frac{X}{Y} \times 100$$

Where, X= Weight of the residue; Y= Weight of powder taken (g)

Calculation:

$$\text{Water insoluble ash Value (\%)} = \frac{0.05}{2} \times 100 \quad = 2.5\%$$

Where, a= 0.05gm, Y= 2gm.

Water soluble ash Values (%) = Total ash value ─ Water insoluble ash value.

= 0.13 – 0.05

= 0.08%

2.4. FLUORESCENT STUDIES OF POWDER DRUGS:

A group of herbs illustrates fluorescence when the powder is showing in UV light and this can be helpful in their identification.The fluorescence nature of the powder drug (40 mesh) was studied both in daylight and UV light (254 nm and 366 nm) and after treatment with different reagents like hydrochloric acid, picric acid, petroleum ether, chloroform, and methanol etc. (15) (Table-3).

2.5. PHYTOCHEMICAL SCREENING:

The roots were collected and dried in shade and reduced to coarse powder. The root powder was extracted with Petroleum ether, Chloroform, Methanol and Distilled water in Soxhlet apparatus. The extracts were filtered and solvent was removed by distillation under reduced pressure (using rotatory evaporator). The percentage yields were calculated and the extracts were further subjected to phytochemical tests for Alkaloids, Flavonoids, Glycosides, Carbohydrates, and Tannins (Table-4) (19, 11).

THIN LAYER CHROMATOGRAPHY (TLC):

The method is used for separation of the natural products such as steroids, terpenes, alkaloids, and flavonoids present in the mixture and various solvent systems are used for this purpose TLC analysis was performed following method (11).A thin film of silica gel is coated on the glass plate. A mark is made by a pencil about 1cm height from the lower edge of the end of plate. The extract sample of *C. gigantea* roots was spotted on this line, with the help of fine capillary.

Sample using Methanolic extract of *C. gigantea* roots, Solvent systemusing Chloroform and Methanol. The distance traveled by the solvent is marked with a pencil and the spots are seen by help of iodine crystals vapor kept in a closed chamber and UV-lamp and marked as the shape the make (21). The distance traveled by the spots and distance traveled by the solvent is noted. The Rf value of each spot is calculated. TLC analysis showed result:

Formula:

$$Rf = \frac{\text{Distance traveled from baseline to the spot}}{\text{Distance from baseline to solvent}}$$

Rf values when a combination of chloroform, ethanol was used:

Rf values are 0.05; 0.15; 0.37; 0.49; 0.68; 0.83; 0.86; 0.90.

3. RESULTS AND DISCUSSION:

On the basis of these investigations, I candiscuss the *Calotropis gigantea* (L.) plant, which has the many medicinal properties like plant root extract have flavonoids which are very useful for neuroprotective and nootropic substance so also useful in memory loss (amnesia) condition.The results of Pharmacognostic studiessuch as percentage of extractive yields of roots extract of *Calotropis gigantea*(L.), ash values and phytochemical screening.

Table 1: The Extractive values of *Calotropis gigantea* (L.) root powder by hot extraction method

Sr.No.	Nature of Extract	Values (% w/w) by hot extraction
1	Petroleum ether	4.16
2	Chloroform	1.08
3	Methanol	4.52
4	Aqueous	10.12

Table 2: Ash value of *Calotropis gigantea* (L.) root powder

Sr.No.	Physical Contents	Values (% w/w)
1	Moisture contents	18.7
2	Total ash value	6.5
3	Acid insoluble ash	4.0
4	Water soluble ash	0.08
5	Water insoluble ash	2.5

Table 3: Fluorescent studies of powder drug of *Calotropis gigantea* (L.) root

Sr.No.	SolventTreatment	Visible light	Short UV (252 nm)	Long UV(366nm)
1.	Drug as such	Yellowish white	Light yellow	Alice blue
2.	Drug and 1M.HCL	Wheat	Khaki	Dark khaki
3.	Drug and picric acid	Yellow	Greenish	Black
4.	Drug and petroleum ether	Light cream	Yellowish green	Cream
5.	Drugand Chloroform	Light yellowish	White	Blue violet
6.	Drug and methanol	Light yellow	Yellowish green	Khaki

Table 4: Phytochemical Screening of Methanol and Aqueous extract of *Calotropis gigantea* (L.) root.

Sr.No.	Constituents	Methanolic extract	Aqueous extract
1.	Alkaloid	Present	Easily seen/noticed
2.	Glycoside	Absent	Easily seen/noticed
3.	Tannins	Present	Present
4.	Saponins	Easily seen/noticed	Easily seen
5.	Flavonoids	Easily seen	Easily seen
6.	Terpenoids	Absent	Absent

4. FUTURE ASPECTS OF *CALOTROPIS GIGANTEA*:

Medicinal plants are used widely for the encouragement of primary health care requirements of the people existing in the rural areas. The traditional medicines were derived from the plants, minerals and other organic matters. But the herbal drugs are obtained from the medicinal plants alone (17).

Calotropis gigantea is a common desert weed with remedial properties. Studies conducted indicates that the plant has antibacterial, wound healing, antiasthmatic, free radical scavenging, vasodilation, anticancer, antifertility, analgesic, cytotoxic, antipyretic, antidiarrheal activities and anticonvulsant also. The plant was also found in Ayurvedic preparations, for the treatment of asthma and for the treatment of other diseases. Legacy of the consumption of plant as the source of medicine plays an essential piece of the health care scheme in India (18).

5. CONCLUSION:

The Pharmacognostic, Physico-chemical and preliminary phytochemical analysis of the root of *Calotropis gigantea*(L.) evolved from the present investigation which provides useful information and authentication of the plant.Flavonoids are present in the methanolic root extract which is more useful for the protection of brain and also work as a nootropic agent. The phytochemical investigation can further be isolated and undergo further pharmacological evaluation of the active principles present in the*Calotropis gigantea* (L.) which will be of considerable use for the researchers and also in the field of home-grown system of medicine.

ACKNOWLEDGEMENT:

I am thankful to the Shambhunath Institute of Pharmacy (SIP), Allahabad for providing chemicals and other infrastructure for doing this research work. The work is dedicated to my entire guide, Mr. Mayank Srivastava, also include my father who gives me support for my research work,thankyou papa.

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