June - July 2017; 6(4):2706-2710

International Journal of Research and Development in Pharmacy & Life Science
An International Open access peer reviewed journal
ISSN (P): 2393-932X, ISSN (E): 2278-0238
Journal homepage:http://ijrdpl.com

Original Article

Standardization of roots of *Calotropisprocera* and *Calotropis gigantean* via evaluation of morphological and physicochemical parameters

Pratibha Mishra*, Khushwant S. Yadav and Girendra Gautam

Institute of Pharmaceutical Sciences and Research Centre, Bhagwant University, Ajmer, Rajasthan, India

Keywords:*Calotropis gigantean*, *Calotropisprocera*, roots, standardization, physio-chemical parameters

Article Information:

Received: March 30,2017;
Revised: April 20, 2017;
Accepted: May 01, 2017

Available online on:
15.06.2017@http://ijrdpl.com

http://dx.doi.org/10.21276/IJRDPL.2278-0238.2017.6(4).2706-2710

ABSTRACT: Both*Calotropis gigantean*and *Calotropisprocera* are medicinal shrub, belongs to the family Asclepiadaceae. Both plants are used in Ayurvedic system of medicine for healing various diseases. However, the present study was aimed to evaluate the parameters to determine the identity, purity and strength of the plant. This study comprises of Morphological and Preliminary physio-chemical investigations of the shrub. Organoleptic characteristics such as color, taste, odour, powder microscopy,etc. were studied. The fluorescent behavior of the powdered root part of *Calotropis gigantean*and *Calotropisprocera* in different solutions towards ordinary light and ultraviolet light (both long and short wavelengths) were found out. Total Ash Value, Acid insoluble ash, Water & Alcohol soluble extractive values were determined.

⇑ Corresponding author at:
Pratibha Mishra, Institute of Pharmaceutical Sciences and Research Centre, Bhagwant University, Ajmer, Rajasthan, India
E-mail address:mithun211@gmail.com

INTRODUCTION

*Calotropisgigantea*and*Calotropisprocera*is native to Indonesia, Malaysia, Philippines, Thailand, Sri Lanka, India and China. It is a common wasteland weed. Calotropis belongs to Asclepiadaceae or Milkweed or Ak family which includes 280 genera and 2,000 species of world-wide distribution but most abundant in the sub-tropics and tropics, and rare in cold countries. Collection of plant material will be done from surrounding fields of bhopal region and collected material will be subjected for shade drying and powdered. Physicochemical paraneters for collected material will be also be evaluated to estabilise quality control parameters[1].Literature survey reveals that *Calotropisgigantea and Calotropis*procerais having wide range of therapeutic importance, however, no exensive physicochemical parameter has been reported so far.

Hence, the present study was aimed to scientifically develop a standard monograph on the basis of physicochemical aspects that would be beneficial in the authentication of *Calotropisgigantea and Calotropis*procera for future work and use[2].

Authentication, Collection and Drying of plant material

The Roots of *Calotropisprocera and calotropisgigantea*Linn. was collected from in and around bhopal city, Madhya pradesh. It was authenticated by Dr. Zia ul Hassan, HOD, Dept. of Botany, Saifia Science College, Bhopal. A voucher specimen was submitted and preserved in the Department of Pharmacy, Bhagwant University, Ajmer.

STANDARDIZATION PARAMETERS

Organoleptic characters and Morphology[3-6]

Organoleptic evaluation can be done by means of sense organs, which provide the simplest as well as quickest means to establish the identity and purity to ensure quality of a drug. Organoleptic characters such as shape, size, colour, odour and taste.The collected plant materials were washed thoroughly with water separately, dried under shade at room temperature and Root samples were subjected for morphological examination based on nature, colour, odour, taste, Shape and Size.

Determination of physio-chemical parameters

Determinations of physiochemical constants are important for crude drugs. The quality parameters of the crude drugs were established with the help of several official determinations based on physiochemical studies. These studies were aimed at ensuring standardization of herbal drugs under investigation. Several physiochemical parameters were established for both roots of *calotropisprocera and calotropisgigantean.*

After the morphological examination, the collected plant materials were subjected to size reduction to get coarse powder and then passed through sieve no.40 to get uniform powder. Then they are stored in well-closed light resistant container until further use.

The uniform powder was subjected to standardization with different parameters as per pharmacopoeias/ literature.

A) **Loss on Drying (%) (LOD)/Moisture Content (%) [**1, 3-6**]:**The percentage of active chemical constituents in crude drugs is given in terms of air-dried drugs. The presence of moisture may lead to microbial contamination and loss of chemical constituents. Hence the moisture content of a drug should be determined.About 1 gm of powdered drug was accurately weighed in a petridish and kept in a hot-air oven maintained at 105^0C and weighed at different time intervals until a constant weight was obtained. After cooling in a desiccator, the loss in weight was recorded. This procedure was repeated till constant weight was obtained.

Loss on drying (% LOD) =

$$\frac{\text{Loss in weight (gms)}}{\text{Weight of drug (gms)}} \times 100$$

B) Ash Values[2, 7-11]:

i. **Total ash value:**About 2gm of powdered drug was weighed accurately into a tarred silica crucible and incinerated at 450°C in muffle furnace until free from carbon. The crucible was cooled to room temperature and weighed. Percentage of total ash was calculated with reference to air-dried substance.

$$\text{Total ash value in percentage} = \frac{(z-x)}{Y} \times 100$$

z = weight of the dish + ash (after complete incineration)

x = weight of the empty dish

y = weight of the drug taken

ii. **Acid insoluble ash:** Ash obtained from total ash was boiled with 25ml of 2N HCl for few minutes and filtered through an ashless filter paper. The filter paper was transferred into a tarred silica crucible and incinerated at 650°C in muffle furnace until free from carbon. The crucible was cooled and weighed. Percentage of acid insoluble ash was calculated with reference to air-dried substance.

iii. ***Water-soluble Ash:*** Boiled the ash for 5-10 min with 25 ml of water, collected the insoluble matter on an ashless filter paper in a crucible, washed with hot water and ignited at temperature not exceeding 450°C for 2 hours. Subtracted the weight of insoluble matter from the weight of the ash. The difference in weight-represents the water-soluble ash. Calculated the percentage of water soluble ash with reference to the air-dried drug.

C) **Extractive Values** [2, 7-11]:

i. ***Determination of Alcohol-soluble Extractive:*** Macerate 5gm of the shade dried drug coarsely powdered, with 100 ml of methanol of the specified strength in a closed flask for twenty-four hours shaking frequently for six hours and allowed to stand for 18 hrs. Filter rapidly taking precautions against loss of methanol. Evaporates 25ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at 1050, and weigh. Calculate the percentage of alcoholsoluble extractive with reference to the air-dried drug.

ii. ***Determination of Water-soluble Extractive:*** Proceed as directed for the determination of alcohol-soluble extractive using chloroform water instead of alcohol.

iii. ***Determination of Chloroform-soluble Extractive*:**Proceed as directed for the determination of methanol-soluble extractive using chloroform instead of methanol.

iv. ***Determination of Benzene-soluble Extractive*:**Proceed as directed for the determination of methanol-soluble extractiveusing benzene instead of methanol.

v. ***Determination of Petroleum-ether soluble Extractive*:** Proceed as directed for the determination of methanol-soluble extractive,using petroleum ether (40-60^{o}C) instead of alcohol.

D) **Fluorescence Analysis of the Drug**[3, 12-13]:Many crude drugs show the fluorescence when the sample is exposed toUV radiation. Evaluation of crude drugs based on fluorescence in day light is notmuch used, as it is usually unreliable due to the weakness of the fluorescent effect.Fluorescence lamps are fitted with suitable filters, which eliminate visible radiation from

the lamp and transmits UV radiation of definite wavelength.

Several crude drugs show characteristic fluorescence useful for their evaluation. The powdered parts of *Calotropisgigantea*and *calotropisprocera*in different solutions shows differentfluorescence which are tabulated in table No.:

RESULTS

***Morphology*:** Morphology and Organoleptic evaluation of root part of *C.gigantea and C.procera.*

S.NO.	PARAMETERS	OBSERVATION	
		ROOT OF *C.PROCERA*	**ROOT OF *C.GIGANTEA***
1	COLOUR	Whitish grey	Whitish grey
2	ODOUR	Pungent	Pungent
3	TASTE	Bitter	Bitter
4	SHAPE	Outer surface is wrinkled inthe fresh condition	Outer surface is wrinkled inthe freshcondition
5	SIZE	0.51-2.2 cm in diameter	0.50-2.0 cm in diameter

Physicochemical Parameters

S.NO.	PARAMETERS	OBSERVATION	
		ROOT OF *C.PROCERA*	**ROOT OF *C.GIGANTEA***
I	**PHYSICAL TESTS**		
	Nature	Coarse powder	Coarse powder
	Color	Yellowish cream	Yellowish cream
	Odour	Odourless	Odourless
	Taste	Bitter	Bitter
II	**LOSS ON DRYING/ MOISTURE CONTENT(% w/w)**	3.48±0.23	3.26±0.19
III	**ASH VALUES**		
	Total ash (% w/w)	3.15±0.31	2.45±0.43
	Acid insoluble ash (% w/w)	0.85±0.53	0.71±0.45
	Water soluble ash (% w/w)	3.61±0.18	3.46±0.15
IV	**EXTRACTIVE VALUE**		
	Alcohol-soluble (% w/w)	3.75±0.61	2.15±0.44
	Water-soluble (% w/w)	11.50±0.49	10.81±0.38
	Chloroform-soluble (% w/w)	1.25±0.56	0.39±0.51
	Benzene-soluble (% w/w)	0.28±0.33	0.19±0.42
	Petroleum-ether soluble (% w/w)	0.42±0.42	0.38±0.27

Values are expressed as mean ±SD, n=3

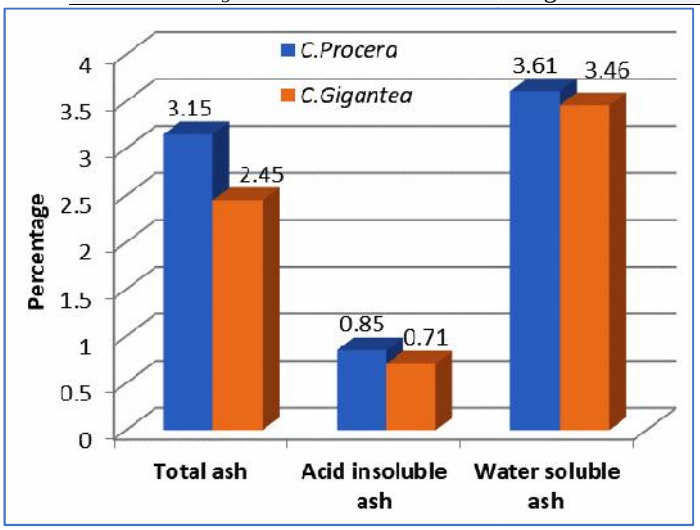
Table : Fluorescent analysis of root of *CalotropisProcera*

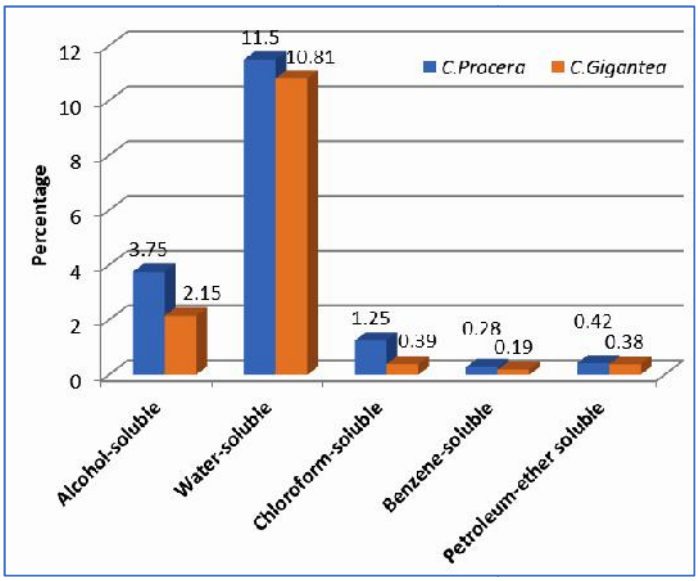
Powder drug	Observation		
	OrdinaryLight	**UV (254 nm)**	**UV (366 nm)**
Dry powder	Dark Green	Light green	Light green
H_2SO_4	Cherry red	No change	No change
H_2SO_4 + water	Yellow	Light green	Green
HCl	Light Green	No change	No change
HCl + water	No change	Light green	Dark green
HNO_3	Yellow	green	Green
HNO_3 + water	Light green	dark green	dark green
Acetic acid	dark green	No change	Blood red
Methanol	dark green	No change	Blood red
Ethanol	Light green	No change	Light red
Chloroform	Green	Yellow	Red
Petroleum ether	Dark green	Dark Yellow	Light yellow
Dist. water	Light yellow	Dark Yellow	Green
10% NaOH	Blood red	No change	Green
5% Iodine	blood red	No change	No change
Picric acid	light green	No change	Dark Green
$FeCl_3$ solution	Cherry red	No change	No change

NH_3 solution	Blood red	Dark green	Dark green

Table : Fluorescent analysis of root of *Calotropisgigantea*

Powder drug	Observation		
	OrdinaryLight	UV (254 nm)	UV (366 nm)
Dry powder	Green	No change	No change
H_2SO_4	Blood red	No change	No change
H_2SO_4 + water	Yellowish green	Light green	Green
HCl	Green	No change	No change
HCl + water	No change	Light green	Dark green
HNO_3	Yellow	green	Green
HNO_3 + water	No change	Yellow	Light green
Acetic acid	Light green	No change	Blood red
Methanol	Light green	No change	Blood red
Ethanol	Light green	No change	Light red
Chloroform	Green	Yellow	Pink
Petroleum ether	Dark green	Dark Yellow	Dark Yellow
Dist. water	Light yellow	Dark Yellow	Green
10% NaOH	Light brown	No change	No change
5% Iodine	Cherry red	Brick red	No change
Picric acid	Yellowish green	No change	Green
$FeCl_3$ solution	Dark brown	No change	No change
NH_3 solution	Light brown	Grayish black	Black





DISCUSSION

Standardization of crude drug is an integral part of establishing its correct identity. The results of the physicochemical constants of raw material lie within the limit, signifies that the quality and purity of raw material is good enough.

Determination of physiochemical constants is important for crude drugs. The quality parameters of the crude drugs as raw materials were established with the help of several official determinations based on physical and phytochemical studies. These studies were aimed at ensuring standardization of herbal drugs under investigation. Thus, the process of standardization can be achieved by stepwise physio-chemical studies as stated above. These studies help in identification and authentication of the plant material. Such information can act as reference information for correct identification of plantand also will be useful in making a monograph of the plant. Further, it will act as a tool to detect adulterants and substituent and will help in maintaining the quality, reproducibility and efficacy of natural drugs.

The moisture content (loss on drying) of the drug should be controlled and minimized in order to prevent decomposition of crude drugs either due to chemical change or microbial contamination. Insufficient drying favors spoilage by molds and bacteria and makes possible the enzymatic destruction of active principles. Not only the ultimate dryness of the drug is important, equally important is the rate at which the moisture is removed and the condition under which it is removed thus the determination of moisture content also provide the method of preparation of drug; and it is observed that the moisture content of the drug was found to be 03.86 ±0.72 which signify that the drug is properly dried and properly stored.

The organic and inorganic constituents present in a drug or plant play a significant role in the identification of crude drug. The physical constants like ash and extractive values help in establishing the Pharmacopoeial standards of the drug. The fluorescence analysis helps to identify the drug in powder form. The organic analysis helps to understand the organic constituents of the drug while chromatographic profile may be compared with authentic samples which in turn helps in the identification of the drug.

Ash values are helpful in determining the quality and purity of crude drugs in powdered form according to the standard procedure. Ash value of a drug gives an idea of the earthy matter of the inorganic composition and other impurities present along with the drug. Significant amount of acid insoluble ash detected which indicates presence of various silicacious substances. Cellulosic substances also contributed significantly in total ash as indicated by water soluble ash.

Extractive values are useful for evaluation of crude drugs and gives idea about the nature of chemical constituents present in them. In some cases, the amount of drug soluble in each solvent is an index of purity. Extractive values are primarily useful for the determination of exhausted or adulterated drug. Extractive values are useful for evaluation of crude drugs and gives idea about the nature of chemical constituents present in them. In some cases, the amount of drug soluble in each solvent is an index of purity. Extractive values are primarily useful for the determination of exhausted or adulterated drug.

Fluorescence is a type of luminescence in which the molecule emits visible radiation passing from a higher to lower electronic state. Fluorescence provided by a drug is one of the several methods used for analyzing crude drugs. Fluorescence analysis of both dried root powder, on treatment with various reagents showed fluorescence. The fluorescent behavior of the powdered drug in different solutions towards ordinary light and ultraviolet light (both long and short wavelengths) gives an idea about various phytoconstituents present in plant drugs. These results indicate the presence of some phytoconstituent in the respective root powder which was later confirmed by the phytochemical work.

Above studies concludes that both the species have almost similar Physio-chemical profile and falls in the range as mentioned in ayurvedic pharmacopoeia of India. Various physiochemical parameters were evaluated for root as per W.H.O. guideline.

CONCLUSION

The present research study was successfully completed for developing a standard monographbased on physicochemical aspects that would be beneficial in the authentication of *Calotropisgiganteaand Calotropisprocera* for future work and use.

REFERENCES

1. Ahamed M, Rana AC and Dixit VK. Plant Review *Calotropis*species (*Ascelpediaceae*): A comprehensive review. *Pharmacognosy Magazine*. 2005; 2: 48 – 52.
2. Yelne MB, Sharma PC, Dennis TJ. Database on medicinal plants used in ayurveda, central council for research in ayurveda and siddha, New Delhi; Vol. 2,69-73 (2000)
3. Kokate C. K. et.al. Pharmacognosy.NiraliPrakashan, Pune, 1999, pp 109-114.
4. Anonymous. Indian Pharmacopoeia, Vol-II, Ministry of Health and Family welfare, Govt of India, New Delhi, Controller of Publications; pp. 1996, A - 53 – 54, A-95, A-97, A-109.
5. Tatiya A, Surana S, Bhavsar S, Patil D, Patil Y. Pharmacognostic and preliminary phytochemical investigation of *Eulophiaherbacea*Lindl. Tubers (Orchidaceae). Asian Pac J Trop Disease 2012; 2(Suppl 1): S50-55.
6. Zhao Z, Liang Z, Guo P. Macroscopic identification of Chinese medicinal materials: Traditional experiences and modern understanding. J Ethnopharmacol 2011; 131:556-561.
7. Quality Control Methods for Medicinal Plant Materials. World Health Organization, Geneva. AITRS Publisher &Distributors, New Delhi. 2002, 14-17, 33-36, 51-52.
8. James, C. S. (1995) Analytical Chemistry of Food. Chapman and Hill, London, pp. 64 – 65.
9. Mukherjee P.K., Quality control of Herbal drugs-An approach to evaluation of botanicals. Business Horizons, New Delhi. 2002, pp 390-403.
10. Siddiqui & M.A. Hakim, Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, anuary, Central Council for Research in Unani Medicine (CCRUM), New Delhi, (appendix), 1995, 24-25.
11. Quality Control Methods for Medicinal Plant Material, By WHO Geneva, A.I.T.B.S. Publishers and Distributors, New Delhi, 2002, p. 8-24, 51
12. Kokoshi C J, Kokoshi R J and Sharma F T, Fluorescence of powdered vegetable drugs under ultraviolet radiation, *J Amer Pharm Assoc*, 1958, 47, 715-717.
13. Chase CR and Pratt RS, Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification, *J AmerPharmacolAssoc*, 1949, 38, 32.

How to cite this article

Mishra P, Yadav KS and Shrivastava P. Standardization of roots of *Calotropisprocera* and *Calotropisgigantea* via evaluation of morphological and physicochemical parameters. *Int. J. Res. Dev. Pharm. L. Sci. 2017; 6(4)*: 2706-2710.doi: 10.13040/IJRDPL.2278-0238.6(4).2706-2710.