Original Research Article **Pharmacognocy**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

IMPROVEMENT OF QUALITY CONTROL PARAMETERS FOR THE STANDARDIZATION OF *CALOTROPIS PROCERA* L. LEAF AND FLOWER

ARSHAD AHMAD NAJAR[1] AND SWATI KHARE[2]*

[1]Department of Botany Govt. Science &Commerce College Benazir, Bhopal (M.P) India
[2]Department of Botany Govt MLB Girls PG Autonomous College, Bhopal (M.P), India*

ABSTRACT

Calotropis procera a key medicinal plant belongs to the family Asclepiadaceae. The macroscopic characters showed that the leaf was alternate, pinnately simple, entire margin, sessile, apex acute etc. The inflorescence possessed lateral and terminal panicles. The leaf showed the presence of open stomata, mesophyll, and vascular bundles etc. While as the flower showed that reproductive part contains anther lobe and 5 petals. The powder microscopy of leaf also revealed epidermal cells, vessels, sclerenchyma cells etc. Flower powder microscopy does not reveal any kind of known structure. Physiochemical analysis of dried leaf powder showed total ash, water soluble ash and acid insoluble ash as 13.44 %, 2.0 % and 0.86% w/w respectively. Preliminary phytochemical screening showed the presence of good amount of flavonoids and alkaloids but steroids were absent. Physiochemical analysis of dried flower powder showed total ash (10.88%)w/w, water soluble ash (1.8%)w/w and acid insoluble ash (0.83%)w/w. Phytochemical screening showed the presence of maximum amount of flavonoids only. The present study was aimed to standardise the pharmacognostic and phytochemical parameters of aerial parts of *C. procera.* It includes comparative, tissue-specific characterization of the species which provides a significant difference in the drug yields of the two parts (leaf and flower) and would also help in its identification from the sister species of this genus. It has a role in quality production of herbal preparations. In nutshell, the determination of these characters will assist future investigators in the pharmacological analysis of this species.

KEYWORDS : Anatomy, Ash value, Calotropis, Medicinal plants.

SWATI KHARE

Department of Botany Govt MLB Girls PG Autonomous College, Bhopal (M.P), India

Received on : 09-08-2016
Revised and Accepted on 24-12-2016
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.b433-439

INTRODUCTION

Plants have been screened for thousands of years to explore medicinal properties. Medicines derived botanically have played a major role in human society throughout history and prehistory. Even today plant derived medicines are preferred over synthetic drugs mainly due to the side effects and high cost of the latter.[1] As per the report, herbal medicines are consumed by 80% of the total world's population especially in developing countries mainly because of their easy availability, and low affordable prices.[2] Hence, to meet these demands, it is necessary to devise a quality control standard protocol to extract medicinally active components from plant tissues.[3] Their pharmacognostic evaluation gives valuable information regarding the morphology, microscopic and physical characteristics of the crude drugs.[4] *Calotropis procera* L. belonging to family Asclepiadaceae is a soft wooded, evergreen perennial shrub and is distributed throughout India and in other warm dry places such as Afghanistan, Egypt and tropical Africa. [5] The decoction of the leaves has been reported to possess ascaricidal[6], schizonticidal, nematocidal[7,8], anti-microbial[9], antihelmintic, molluscicidal[10], insecticidal, anti-inflammatory, anti-diarrhoeal, larvicidal[11-13], anticancer[14] properties. Flowers are reported to possess digestive, tonic, wound healing and antioxidant properties.[15,16,17] Hence, in the present study, an attempt has been made to lay down some standardization parameters for *C. procera* leaf and flowers to extract medicinally important components which includes various pharmacognostic parameters like macroscopic and microscopic phytochemical and physicochemical fluorescence study.

METERIALS AND METHODS

Collection and Authentication

The aerial parts (leaves and flowers) of the plants were collected in the month of March-April, 2014 from M.P Council of Science and Technology Nehru Nagar MPCST 23° 15′ 35.760″ N and 77° 24′ 45.414″ E and P&T Colony Jawahar chowk 23° 13′ 25. 582″ N and 77° 23′ 28.294″ E Bhopal, India. The collected plant samples were identified by Dr Zia ul Hasan (Prof and Head, Faculty of Botany, Safia Science College, Bhopal, India). The specimen samples of *C. procera* were deposited in the departmental herbarium with respective voucher number 481/Bot/Safia/2014.

Macroscopic study

For morphological observations, fresh leaves and flowers were collected from MPCST and P&T Colony Bhopal M.P India in the month of March-April, 2014. The macro morphological features of the leaf and flower were observed under magnifying lens following methods of Tyler V and Brady.[18]

Phytochemical analysis

Qualitative phytochemical analysis

The preliminary phytochemical screening was carried out on the extracts obtained after successive extraction with methanol. The dried extracts were treated with Mayer's reagent, Dragendroff's reagent, Hager's reagents, Wagner's reagents and froth test etc. for the detection of chemical constituents like alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids and saponins.[19]

Physicochemical analysis

The physicochemical analysis of the crude powder of leaf and flower of *C. procera* was carried out as per WHO guidelines.[20] The contents analyzed upon drying were water soluble ash, acid insoluble ash, methanol soluble extract, ethyl acetate soluble extract, ethanol soluble extract, water soluble extract and chloroform soluble extract.

Fluorescence analysis

Fluorescence study of leaf and flower powder was performed as per reported standard procedures.[21] A small quantity of the leaf or flower powder was placed in clean petriplate and 1-2drops of freshly prepared reagent solution was added, gently mixed by tilting and kept as such for few minutes. It was then placed inside the UV chamber and observed in visible light, short (254nm) and long (365nm) UV radiations. The colour observed by application of of different reagents in different radiations was recorded.

RESULTS

Pharmacognostic study

Organoleptic and macroscopic characteristics of leaf and flower

The organolaptic features of *C. procera* leaf are given in Table 1 and Figure 1. The macroscopic study revealed the leaf to be sessile, alternate, simple and glabrous, with entire margin, acute apex and decurrent base. Inflorescence is upright and corolla comprises of 5 elegant, whitish petals having purplish tinge at the apex.

Microscopic characteristics of leaf

The microscopic characters of leaf are shown in Figure 2 (A-E). Transverse sections through the midrib showed an upper and lower, single- layered epidermis externally covered with a few epidermal cells on both lower and upper surfaces, parenchymatous cells that were thin-walled circular. Intracellular spaces were present in ground tissue and open vascular bundles. The xylem consisted mostly of vessels and tracheids, and a strip of cambium was present between the xylem and phloem tissues. Mesophyll cells were differentiated into a palisade and spongy tissue. Central cells were irregular in shape and vascular bundles were also present scattered in this region. Lower surface of leaf showed opened stomata surrounded many prominent gaud cells.

Powder study of leaf

The crude powder of *C. procera* leaf was light green in colour and bitter in taste while as the flower was light pinkish in colour with honey odour and sweet taste. For microscopic examination, the powder was stained with iodine solution, safranin and glycerine for various anatomical features which showed the presence of lignified, epidermal and sclerenchyma cells. (Figure 2: F-H).

Microscopic characteristics of Flower

The microscopic study of the L.S of flower showed ovary has ovules and prominent corolla (5 petals (polypetalous) and 5 sepals (polysepalous). Figure 3 (I).

Table 1
Organoleptic characters of C. Procera leaf and flower

Parameters	*C. procera*
Colour	Pale Green
Lamina	Obovate decussate
Margin	Entire
Apex	Acute
Surface appearance	Glabrous
Venation	Pinnate
Leaf	Simple and sessile
Dimension	3-6 cm and 2-4 cm
Base	Articulate
Leaf Scent	Unpleasant
Leaf persistent	Evergreen
Flower	Pink and erect

Figure 1
Macroscopic characteristics of C. Procera leaf and flower

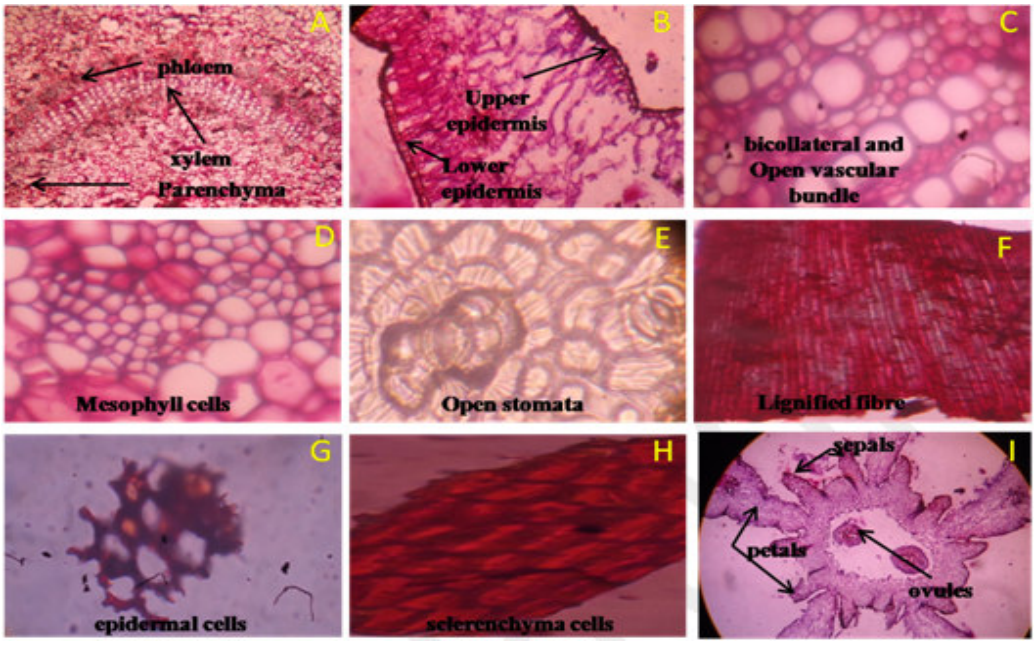

Figure 2
Photomicrograph of microscopic characteristics of C. procera leaf and flower at 10xand 40x.

Phytochemical analysis

The results of phytochemical qualitative analysis of crude drug derived from *C. procera* leaf and flower are shown in Table 2. The leaf contains ample quantity of flavonoids and tannins however; only flavonoids were found in the flower. Minor amounts of glycosides, triterpenes and saponins were present in both leaf and flower with negative values of steroids.

Table 2
Qualitatively phytochemical analysis of C . procera leaf and flower.

Phytochemicals	Reagents	Leaf	Flower
Alkaloid	Dragondroff's , Mayer's , Wagner's	+-+	+-+
Flavonoid	Lead Acetate, Alkaline Reagent, Shinoda	+++	+++
Tanins	Feric chloride, Lead Acetate	-+	–
Terpinoids	Salkowski's , Libermann-Burchard's	-+	++
Steroids	Salkowski's , Libermann-Burchard's	-	-
Saponins	Froth Tests	+	+
Glycosides	Borntrager's , Legal's	- +	-+

Sign ; + : Present, - : Absent

Fluorescence analysis

The fluorescence character of powdered drug plays essential role in resolving the quality of and transparency of the drug material. The fluorescence characteristics of leaf and flower powder of *C. procera* are given in the Tables 3 and 4. Some constituents show fluorescence in the visible range in daylight. The UV light produces fluorescence in many natural products which do not visibly fluoresce in daylight, they may often be converted into florescent derivatives products by applying different reagents.

Table 3
Fluorescence analysis of leaf powder of C. procera.

Solvent	Visible	Short UV(254nm)	Long UV(365nm)
Water	Green	Green	Yellow
Methanol	Green	Green	Red
Ethanol	Green	Green	Yellow
Acetone	Green	Light green	Yellow
Ethylacetate	Green	Light green	Red
Chloroform	Green	Light green	Yellow
N.hexane	Green	Green	Green
Dichloro Methane	Light green	Light green	Yellow
Dil .HCL	Yellow	Green	Brown
Con.HCL	Green	Green	Black
Dil .H_2SO_4	Yellow	Green	Brown
Con. H_2SO_4	Brown	Green	Light green
NH_4 OH . Sol	Green	Green	Yellow
NaOH . Sol	Green	Green	Yellow
$Fecl_3$. Sol	Yellow	Green	Brown
Dil. Acetic acid	Yellow	Green	Black
KOH . Sol	Green	Light green	Yellow

Table 4
Fluorescence anlaysis of flower powder of C. prcera.

Solvent	Visible	Long UV(254nm)	Short UV(365nm)
Water	Yellow	White	Yellow
Methanol	Yellow	Green	Yellow
Ethanol	Yellow	Green	Yellow
Acetone	Yellow	Green	White
Ethylacetate	Yellow	Green	White
Chloroform	Yellow	Green	White
N.hexane	Yellow	Green	Yellow
Dichloro Methane	Yellow	Green	Purple
Dil.HCL	Yellow	Green	Yellow
Con.HCL	Pink	White	White
Dil.H_2SO_4	White	White	Yellow
Con.H_2SO_4	Brown	Purple	Purple
NH_4OH . Sol	Yellow	Green	Yellow
NaOH . Sol	Yellow	Green	Green
$Fecl_3$. Sol	Purple	White	Purple
Dil. Acetic acid	Pink	White	Purple
KOH . Sol	Yellow	Green	Yellow

Physicochemical study

The physiochemical characterization of the leaf and flower of *C. procera* with their extractive values are shown in Table 5. It was found that in case of both leaf as well as flower maximum extractive value was found in water followed by methanol while as least extractive value was found in chloroform. The moisture content of the leaf and flower was 10.32 % and 10.02% respectively. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. The total ash content in leaf, water soluble ash and acid insoluble ash was 13.44%, 2.0% and 0.86% respectively. The total ash in flower was 10.88% while both water soluble ash and acid insoluble ash was 1.8% and 0.83%.

Table 5

Physiochemical parameters of C. procera leaf and flower

Parameter	% Value (w/w) leaf	%Value (w/w) Flower
Methanol soluble extract	13.5	19.82
Water soluble extract	45.4	35.74
Chloroform soluble extract	6.22	7.58
Ethylaccetate soluble extract	5.18	9.42
Ethanol soluble extract	7.42	7.58
Loss on drying	10.32	10.02
Ash value	13.44	10.88
Water soluble	2.0	1.8
Acid in soluble	0.86	0.83

DISCUSSION

Developing standards is an integral part of expanding the correct identity and quality of a crude drug. The microscopic characters, the physiochemical studies and fluorescence analysis can be used for the quality control of the crude drug and these are prime branch for this assessment[22] and are prime step of pharmacognostic evaluation. *C. procera* is an important medicinal plant with many traditional uses; hence it becomes necessary to standardize it for using as a drug. For establishing the correct identity of the source materials microscopic and morphological study are one of the simplest and cheapest methods.[23] The macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken as per WHO guidelines. The previous work has shown that the transverse section of leaf through the midrib contains upper and lower single layer.[24] Spongy parenchyma tissues are almost radially elongated with intracellular spaces and irregular central cells in addition to the presence of laticifers and vascular bundles.[25] Methanol extract of *Calotropis procera* leaf and latex were subjected to phytochemical analysis indicating the presence of various classes of bioactive secondary metabolites.[26] which revealed the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides with very high amount of water extracts.[27, 28] Flowers contain terpenes and flavonoids.[29, 30] Low moisture content indicates less chances of microbial degradation of plant drugs during storage.[31] The general requirement of moisture content in crude drug is that, it should not be more than 14% w/w.[32] The ash values were determined by total ash, acid-insoluble ash and water soluble ash is useful in finding out the genuity of the drug.[33] The extractive values are useful for the determination of exhausted and adulterated drugs in a particular solvent.[34] Fluorescence study of the leaf powder helps in a rapid method for resolution of doubtful specimen.[35] The fluorescent analysis under the visible light and UV light by treatment of different chemical reagents showed different colours. Thus fluorescence is used for qualitative assessment of crude drug.[36]

CONCLUSION

In conclusion, it can be stated that the results of the present work lay down the standard parameters which can be useful for checking the authenticity and adulteration of this important useful medicinal plant, can help in maintaining the quality of crude drug and can be also useful for the preparation of a monograph.

ACKNOWLEDGEMENT

The author thanks Prof. Dr. Swati, Department of Botany Govt. Maharani Laxmi Bai MLB Girls PG P.G. Autonomous College, Bhopal (M.P), India and Prof. and HOD Head Dr. Kirti Jain, Department of Botany Govt. Science & Commerce College Benazir, Bhopal (M.P) India for providing excellent research facilities and guidelines.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Thomas SC. Medicinal plants culture, utilization and phytopharmacology. Boca Raton:CRC Press. 2000 Jun; 119-154.
2. Mukherjee PK. Quality Control of Herbal Drugs An approach to evaluation of botanicals. *1st ed.* New Delhi Business Horizons. 2002 May.
3. Hossan MS, Agarwala B, Sarwar S, Karim M, Jahan R, Rahmatullah M. Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases. Ethnobot Res Appl. 2010 Apr; 8: 61-74.
4. Rakholiya K, Chanda S. Pharmacognostic, physicochemical and phytochemical investigation of *Mangifera indica* L. var. Kesar leaf. Asian Pac J Trop Biomed.1997 Jun; 2: 680-684.
5. Konathala R, Priyadharshni SP, Kumar K.E, Satyanarayana T. Phytochemical Investigation on Stem of *Calotropis Procera* (Ait.) R.Br. Asclepiadaceae. IOSR Journal of Pharmacy and Biological Sciences. 2014 Jun;9(3): 25-29.
6. Chungsamarnyart N, Ratanakreetakul C, Jansawan W. Acaricidal activity of the combination of plant crude extracts to tropical cattle ticks. Kasetsart Journal, Natural Sciences. 1994 ;28(4):649–660.
7. Nandal SN, Bhatti DS. Preliminary Screening of Some Weeds Shrubs for Their Nematocidal Activity against MeloidogyneJavanic. Indian Journal of Nematology.1983 Sep ;13:123-127.
8. Rakesh P, Alok K, Neetu K. Nematicidal activity in flowers of some medicinal and aromatic plants. Indian J Nematol.2001 ;31:96-98.
9. Malik NN, Chughtai MID. Antimicrobial activity of *Calotropis procera*. A preliminary study. Pakistan J Sci. 1979;31:127-129.
10. Hussein HI, Kamel A, Abou-Zeid M, El-Sebae AKH, Saleh M.A. Uscharin, the most potent molluscicidal compound tested against land snails. J Chem Ecol. 1994 ;20:135-140.
11. Girdhar G, Deval K, Mittal PK, Vasudeva P. Mosquito Control by *Calotropis-Procera* Latex. Pesticides. 1984;18:26-29.
12. Jacob S, Sheila MK. A note on the protection of stored rice from the lesser grain borer, Rhozopthera dominica Fabr. By indigenous plant products. Indian J Entomol.1993;55 (3):337-339.
13. Khan SM, Siddiqui MN. Potential of some indigenous plants as pesticides against the larvae of cabbage butterfly *Pieris brassicae* L. *Sarhad* Journal of Agriculture.1994;10:291-297.
14. Moursey LE. Insecticidal activity of Calotropis procera extracts of the flesh fly, Sarcophaga haemorrhoidalis fallen. J Egypt Soc Parasitol.1997;27:505-514.
15. Smit HF, Woerdenbag HJ, Singh RH, Meulenbeld GJ, Labadie RP, Zwaving JH. Ayurvedic herbal drugs with possible cytostatic activity. J Ethnopharmacol.1995;47:75-84.
16. Choedon T, Mathan G, Arya S, Kumar VL, Kumar V. Anticancer and cytotoxic properties of the latex of *Calotropis procera* in a transgenic mouse model of hepatocellular carcinoma. World J Gastroentero 2006;12:2517-2522.
17. Prabha MR, Vasantha K. Antioxidant, Cytotoxicity and Polyphenolic Content of *Calotropis procera* (Ait.) R. Br. Flowers. Journal of Applied Pharmaceutical Science 2011 Sep;01 (07): 136-140.
18. Tyler V, Brady L. *Robbers* J. Pharmacognosy. India: Varghese Company.1977;103-141.
19. Kokate CK. Practical Pharmacognosy, Vallabh Prakashan, 3 rd Ed.2006;493-497.
20. The Indian Pharmacopoeia. New Delhi: Govt. of India Publication; Anonymous.1996.
21. Chase CR, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. Am Pharm Assoc.1949;38:324–31.
22. Minal P, Chanda S. Development of quality control parameters for the standardization of *Limonia acidissima* L. leaf and stem. Asian Pacific Journal of Tropical Medicine 2014;7:244-248 .
23. Ashish S, Zalke, Qureshi MA, Upendra B Gandagule, Duraiswamy B. Pharmacognostical and phytochemical evaluation of the leaves of Ziziphus xylopyrus(Retz) Willd. Ancient Science of Life. 2013 May ;32:(4).
24. Dictonary of India Raw materials and industrial products. The Wealth of India., PID, CSIR. 2000: 78-84.
25. Murti Y, Yogi B, Pathak D. Pharmacognostic standardization of leaves of Calotropis procera (Ait.) R. Br. (Asclepiadaceae), International

Journal of Ayurveda Research. 2010 Jan; 1(1):14-17.
26. Ghias U, Abdur R, Naveed M, Shabana, Nadia M, Mohsina. Phytochemical and Pharmacological Studies of the Whole Plant of *Calotropis procera*. Middle-East Journal of Medicinal Plants Research. 2012 Apr; 1(4): 71-74.
27. Mainasara MM, Aliero BL, Aliero AA, Dahiru SS. Phytochemical and Antibacterial Properties of Calotropis Procera (Ait) R. Br. (Sodom Apple) Fruit and Bark Extracts. International Journal of Modern Botany. 2011; 1(1): 8-11.
28. Raginee V, Satsangi GP, Shrivastava JN. Analysis of phytochemical constituents of the ethanolic and chloroform extracts of Calotropis procerausing gas chromatography-mass spectroscopy (GC-MS) technique. Journal of Medicinal Plants Research. 2013 Oct;7(40): 2986 – 2991.
29. Yahya MA, Meshal IA, Mossa JS, AlBadr AA, Tarig M. Saudi plants: A phytochemical and biological approach. Riyadh: King Saud university press.1990; 31- 34.
30. Ansari SH, Ali M. New oleanene triterpenes from root bark of *Calotropis procera*. *Medicinal and Aromatic Plant Sci*.1999;21(4):978-981.
31. Chumbhale DS, Upasani CD. Pharmacognostic standardization of stems of Thespesia lampas(Cav.) Dalz & Gibs. Asian Pacific Journal of Tropical Biomedicine. 2012 May;2(5): 357-363.
32. Anonymous. African pharmacopoeia. 1st ed. Lago:Organisation of African Unity/The Scientific. Technical and Research Commission (OAU/STRC) 1986;123.
33. Kumar V, Kumar OP. Microscopic evaluation and physiochemical analysis of Dillenia indica leaf . Asian Pac J Trop Biomed. 2011 May;1(5): 337-340.
34. Upendra B, Gandagule B, Duraiswamy, Ashish S, Qureshi MA. Pharmacognostical and phytochemical evaluation of the leaves of Ziziphus xylopyrus Retz. Willd. Ancient Science of Life.2013 Jan; 32 :4,134-136.
35. Pratt RJ, Chase CR. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharm Assoc. 1949 May; 38: 324-333.
36. Zhao Z, Liang Z, Guo P. Macroscopic identification of Chinese medicinal materials: traditional experiences and modern understanding. J Ethnopharmaco. 2011;131(3): 556-561.