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RESEARCH ARTICLE

A Diagnostic approach for same looking plants for their Pharmacognosy value

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Abstract

Objective: To establish diagnostic features of same looking plants (*C. gigantea* and *C. procera*) and also to explore the diurnal influence on their Pharmacognosy values. **Methodology:** To meet the objectives, the characteristics of these plants were explored by macroscopic, microscopic (light microscopy), and also by physio-chemical parameters. The physiochemical analysis was performed with air dried leaves and flowers of *C. gigantea* and *C. procera*. The collected samples were used for the quantitative determination of ash value (water soluble, acid soluble and sulphate ash values), extractive values, loss on drying, swelling index, and foaming index through standard methods. The leaf and flower extracts (with different solvents) were subjected to qualitative phytochemical screening using the fluorescence test. Further, to explore the diurnal influence, the samples were plucked at different time intervals (morning, afternoon and evening) and fixed immediately for further processing. **Results:** It was observed that the macroscopic, microscopic and physiochemical characteristics analysed could serve as diagnostic features to distinguish these closely related species. Phytochemically, these plants are rich in constituents like carbohydrates, alkaloids, cardiac glycosides, flavonoids, saponins, phenolic compounds and terpenoids. Moreover, physio-chemical parameters with methanolic extracts provided higher bioactive constituents than other solvents. Besides this, total ash values were found to be maximum i.e. PLA (15.33 ±0.050%) and GFE (14.15 ± 0.031%) than other acid insoluble and water-soluble values which were under 2-10%. Pertinently, the moisture content was found little higher in *C. gigantea* GLA (10.60± 0.200%) and GFA (11.06± 0.100) than in *C. procera* PLA (8.81± 0.598%) and PFA (9.92± 0.244, while a considerable amount of foaming content was present in both the species was less than 100. On the basis of observed pharmacognosy, *C. procera* was found more promising in drug prospective bioactive constituents than *C. gigantea* and thereby offers more contribution toward establishment of pharmacognostic profile of this medicinally effective plant species. **Novelty:** Our approach pays a way for the inclusion of an important factor (diurnal

factor) in assessing the medical efficacy of desired plant species that could help in sampling the specific plant material with desired chemical profile and enhanced pharmacognosy potential.

Keywords: Anatomy; Ash value; Calotropis; Medicinal plants; Stomata; Trichomes

1 Introduction

The variables, like geographical distribution and environmental factors (climate, temperature, altitude, season, and other conditions) and time of harvesting/collection of medicinal plants seem to be important factors related to variation in the active compounds of medicinal plants [1]. The ancient physicians were aware about relation between time of collection and distribution of active plant constituents, and impact of different variables on availability of active components in medicinal plants besides the variation in therapeutic efficacy during different times or seasons of the year [2].

The Main hurdle in this context is availability of data/approach for proper characterization of these medicinal plants, in particular, to define variation present between same looking species. The factors like time/place of collection, and way of collection, etc. are important variables that have prime effect on the presence of various bioactive components qualitatively and quantitatively in test samples.

The two same looking plants *Calotropis gigantea* L. and *C. procera* L. belong to the family Asclepiadaceae [3]. *C. procera*, locally known as "Aak" in India besides being commonly known as Sodam of apple (Swallow wort in English and Akundia in Hindi) [4] is having enormous medicinal properties which include: use of leaves as pain relieving agents [5], anti-helminthic [6], analgesic, antipyretic, and anti-jaundice effects [7], antimicrobial [8], anti-diarrhoeal, larvicidal, anticancer, cytotoxic [9], and anti-inflammatory [10]. The flower extracts have antipyretic [11], spermicidal, and anti-fungal properties [7], besides being used for constipation, fever, joint pain and muscular spasm [10], digestive, tonic for asthma and catarrh [4]. The other congeneric species studied is *C. gigantea*, locally known as "Rakta Arka"in India, and commonly as milkweed or wasteland weed [12,13]. Its leaves are reported for anti-diarrhoeal, anti-candida, and antibacterial activities [14], besides for fever, rheumatism, paralysis, nausea, vomiting, indigestion, cough, cold, eczema and diarrhoea [15]. It is also used against asthma and arthralegia swellings [16,17]. The flowers are reported to possess analgesic, antimicrobal and cytotoxic effects [18,19] in addition to the stomachic, bechic, and anti-asthmatic [20] problems.

The pharmacological and phytochemical research efforts are underway to extraction and characterization of plants for active compounds such as alkaloids, tannins, flavonoids, resins, phenols, fatty acids and steroids which play an important role in human health, and may also be nutritionally important to give birth to high activity profile of new pharmaceutical drugs [21]. One of the most difficult tasks in establishing the ethno-botanical value of herbal plants lies in its correct botanical identification [22]. However, and comparatively, a lesser amount of research has been done that focuses on identification of same-looking species for their pharmacognosy differences. Further, sunlight plays a key role in initiating a cascade of chemical synthesis starting from photosynthesis itself.

The photosynthates thus generated nourish the plant and also aid in the synthesis of important secondary metabolites that can protect and defend the host against various environmental perturbations. Also, the role of edaphic factor/soil types influencing the pharmacological value have been explored well (Ghasemzadeh et al. 2018 [23–25]. Indeed, good amount of research work is available on exploring these useful secondary metabolites and emphasizing the importance of their extraction procedures [26]. Nonetheless, and so far, the least attention has been given on the influence of diurnal

changes (plucking/harvesting –time) of medicinal herbs as otherwise the diurnal variation (largely influenced by sub-light) is expected to impact the plant physiology including the accumulation of chemical constituents.

In this context, and with regard to the documented medical efficacy of the two congeneric species of Calotropis viz *C. procera* and *C. gigantea*, present study was undertaken to document the information on micro-morphological features of these plants which would help in their authentication, adulteration and identification. It will also provide the basic pharmacognostic figures required for the herbal pharmacopoeia combination and at the same time to find out the possible diurnal importance on pharmacognosy application. The time of collection of the plant species is anticipated to affect the yield and physio-chemical profile of Calotropis. Nevertheless, published data on such variations is lacking. Therefore, present study has been planned to screen out the effect of time of collection in physio-chemical profile of two same looking *Calotropis* spp. Thus, this study explores the macro-/micro- characters and phytochemistry of *C. gigantea* and *C. procera* leaf and flower vis-à-vis time of collection and their specific physical and chemical standards. The study may be useful as a quality control parameter in the Indian herbal pharmacopoeia.

2 Materials and methods

2.1 Plant 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; 1050m asl) and P&T colony, Jawahar chowk (23° 13′ 25. 582″N and77° 23′ 28.294″ E; 900m asl), Bhopal, India. The collected plant samples were identified by Dr. Z.H.(Saifia Science College, Bhopal, India). The specimen samples of *C. procera* and *C. gigantea* were deposited in the departmental herbarium with respective voucher numbers 481/Bot/SC/2014 and 482/Bot/SC/2014.

2.2 Macroscopic study

The macro-morphological features of the collected leaf and flower samples were observed under magnifying lens [27].

2.3 Microscopic study

Selected samples of the leaf and flower were stored in FAA solution containing 5 ml formalin, 5 ml acetic acid, and 90 ml of 70% (v/v) ethyl alcohol. Fixed for 24 hr, the specimens were dehydrated with a graded series of tertiary-butyl alcohol as per the method of Sass [28]. The dehydrated samples were casted into paraffin blocks and sectioned with the help of a Rotary Microtome (RMT-30, Radical Instruments, India). The dewaxing of the sections was carried out as per the method described by Johnson [29]. The generated sections were finally stained with safranin and mounted in glycerine for microscopic observations

2.4 Physicochemical analysis

The physiochemical analysis of air dried leaf and flower of *C. gigantea* and *C. procera* was used for the quantitative determination of ash value, extractive value, loss on drying, swelling index, and foaming index through standard methods [30]. The total ash value for a crude drug is not always trustworthy since there is a chance of presence of non-physiological substances, such as soil contents, etc. So, the parameters viz water soluble, acid soluble and sulphate ash values were performed. The extractive values with methanol, ethanol, ethyl acetate, chloroform and water were also determined as previously reported [31].

2.5 Fluorescence analyses

Fluorescence study of leaf and flower powder was performed as per reported standard procedures [32]. A small quantity of the leaf or flower powder was placed in clean Petri plate and 1-2drops of freshly prepared reagent solution was added, gently mixed by tilting and kept as such for a 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 adding of different reagents in different radiations was recorded.

2.6 Qualitative phytochemical analyses

The crude powder of leaf and flower was subjected to qualitative phytochemical analysis [33]. The phytochemicals analysed were carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, glycosides, terpenoids, steroids and saponins.

3 Results

3.1 Organoleptic and macroscopic characters

The organoleptic feature/s of aerial parts of *C. gigantea* and *C. procera* is/are given in Table 1 and Figure 1.

Table 1. organoleptic characters of Calotropis of leaf and flower

Plant parts	Colour	Taste	Odour
C. gigantean leaf	Dark green	Aromatic bitter	Characteristic
C. gigantean flower	Whitish	Aromatic sweet	Characteristic
C. procera leaf	Light green	Aromatic sweet	Characteristic
C. procera flower	Light brown	Aromatic bitter	Characteristic

Fig 1. Physical evaluation of Calotropis leaf and flower

The comparative macroscopic characters showed that studied species of Calotropis are perennial shrubs of 5.4 metres height and highly branched. Leaves are opposite, entire, simple and sessile; leaf blade is oblong having short pointed apex and heart shaped base. *C. gigantea* is large shrub or small tree, 1-5 m tall, young branches covered with white cottony tomentum, leaves are 3-10 inches long, 2-6 inches wide, thin and elliptic-oblong or obovate-oblong, base cordate, tip acute or shortly acuminate. On the other hand, *C. procera* is an erect shrub or small tree up to 3m tall, much branched from the base, leaves are 3-6 inches long, 2-4 inches wide, thick and elliptic-oblong or obovate-oblong, base cordate, tip acute or shortly acuminate (Figure 2).

Fig 2. (A-B) *C. gigantea* with reverse corolla and large thin leaf, (C-D) *C. procera* with upright corolla and small thick leaf

The comparison of the flowers of these congeneric and closely related species can be used for their identification. The petals in *C. gigantea* are reflexed (bent in turn round) and 3-4.5 cm in diameter with white colour, whereas *C. procera* is distinguished by its erect and divided 2/3 the way down corollas; glaborous; lobes acute and white petals with dark purple tips. Flowering can be observed all the year round (Flora of Madhya Pradesh western part, V.P Singh) (Figure 2).

3.2 Microscopic characters

3.2.1 Leaf anatomy

Transverse section of leaf midrib of *Calotropis* spp. at ×10×40× showed single layered upper and lower epidermis covered with a thick cuticle. Mesophyll was seen differentiated into palisade and spongy tissue. Below the upper epidermis were three rows of elongated and closely arranged parenchyma palisade cells. Spongy parenchyma cells had intercellular spaces and ground tissue composed of bicollateral and open vascular bundles. The lower epidermis showed paracytic open stomata surrounded by many gaud cells as shown in Figure 3 (A-D).

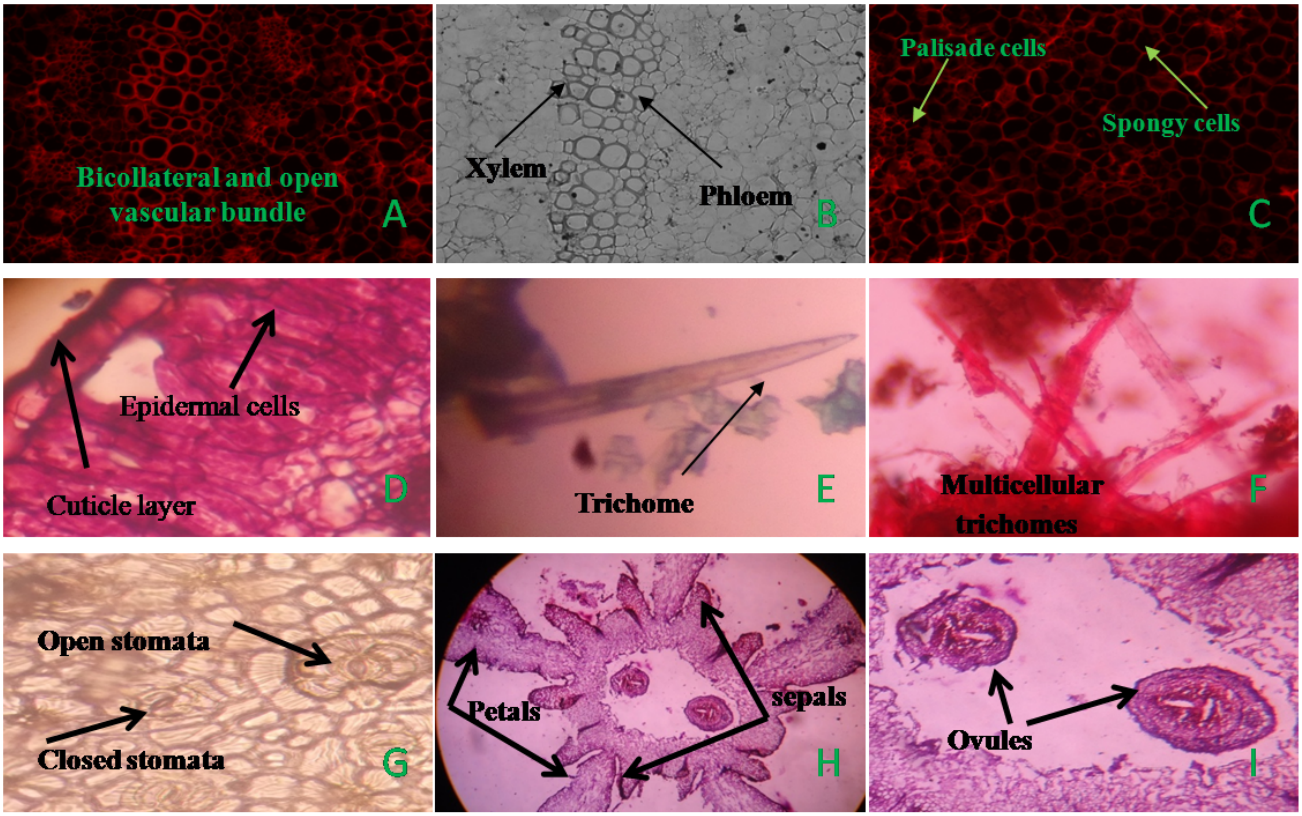

Fig 3. Anatomy of *Calotropis* Leaf and flower tissues: T.S. of adaxial leaf surface of *Calotropis* (A-D); Leaf powder showing unicellular (E) and multicellur (F) trichomes in *C. procera* and *C. gigantea*, respectively, Paracytic stomata of *Calotropis* (G); Floral parts of *Calotropis* (H-I)

3.2.2 Flower anatomy

The transverse section of *C. gigantea* and *C. procera* flower at ×10×40× showed ovary having ovules (future seed), petals 5 in number (gamopetalous) and sepals also 5 in number (gamosepalous) as shown in Figure 3 (H and I).

3.2.3 Powder microscopy

The powder microscopy of leaf of both the *Calotropis* species at ×10×40× also revealed epidermal cells, vessels, and sclerenchyma cells, besides unicellular and multicellular trichomes in *C. procera* and *C. gigantea*, respectively as shown in Figure 3 (E and F). However, the flower powder microscopy did not reveal any kind of structure except the trichomes.

3.3 Physico-chemical analyses

The extractive values for various solvents such as ethanol, ethyl acetate, chloroform, methanol and water were assessed here. Efficient extraction was found in the methanol after water for extractive value. Indeed, using different solvents to select good solvent for extraction was our first method to determine which solvent gives the good amount of specific constituents. The

maximum extractive value with methanolic extracts was found in PFM (19.82%) and GFA (24.04%) respectively (Figure 4 A-B).

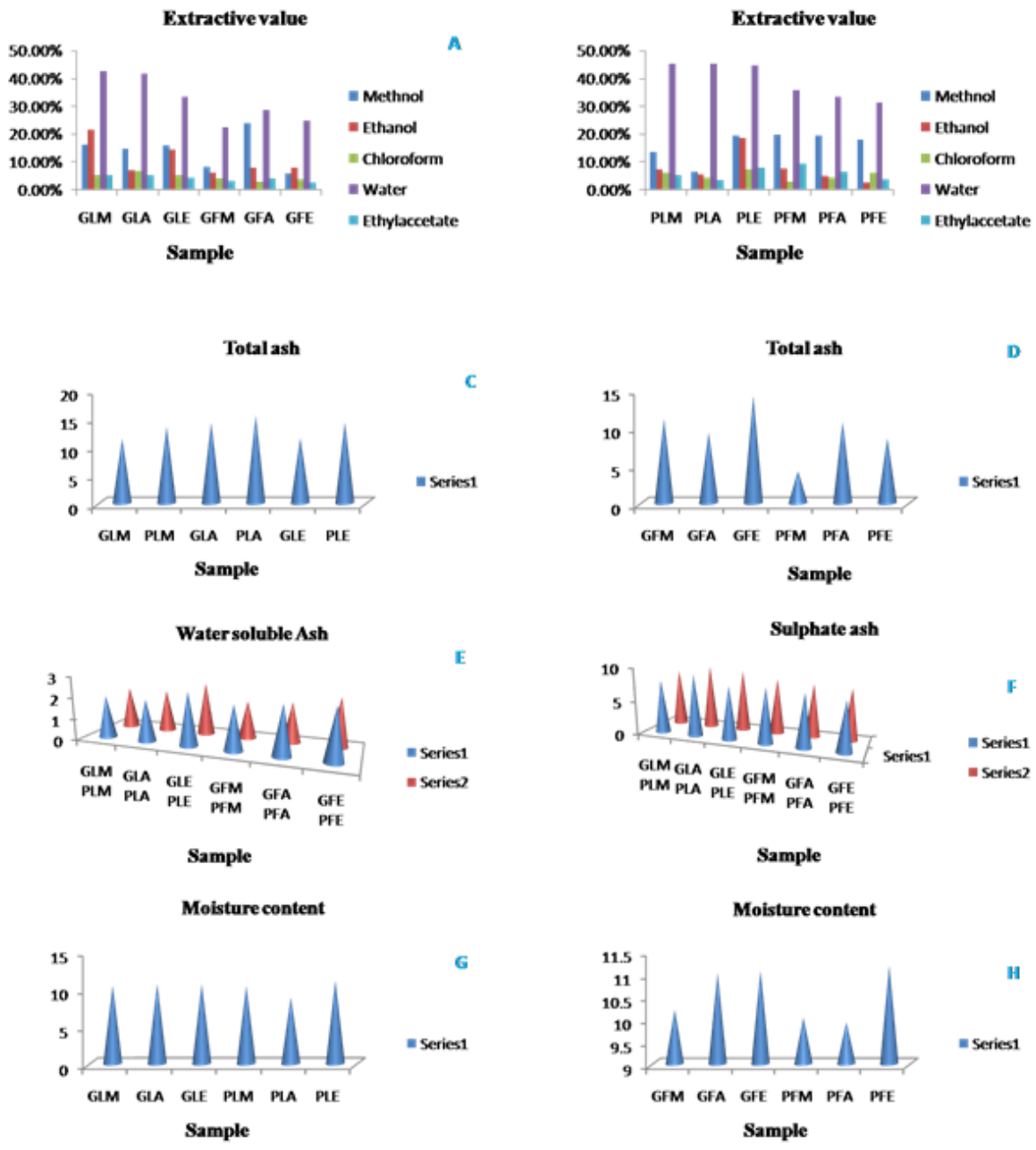

Fig 4. Physiochemical analyses and extractive value of *C. gigantea* and *C. procera*; A: extractive value of *C. gigantea* leaf and flower, B: extractive value of *C. procera* leaf and flower, C-D: total ash of Calotropis leaf and flower, E-F: water soluble ash and sulphate ash of Calotropis leaf and flower, and G-H: moisture content of Calotropis leaf and flower.
Abbreviations: GLM (*Gigantea* leaves Morning), GLA (*Gigantea* leaves Afternoon), GLE (*Gigantea* leaves Evening) GFM (*Gigantea* Flowers Morning), GFA (*Gigantea* Flowers Afternoon), GFE (*Gigantea* Flowers Evening) PLM (*Procera* leaves Morning), PLA (*Procera* leaves Afternoon), PLE (*Procera* leaves Evening) PFM (*Procera* Flowers Morning), PFA (*Procera* Flowers Afternoon), PFE (*Procera* Flowers Evening).

Spatial and temporal measurements of different parameters of *C. gigantea* and *C. procera* showed variable results. The leaf and flower at different intervals of time including morning, afternoon and evening revealed different physio-chemical parameters

such as ash value (total ash, water soluble ash and sulphate ash), loss on drying and foaming index. Ash constitutes the inorganic residues obtained after complete combustion of a drug. Ash value is a validity parameter to describe and to assess the degree of purity of a crude drug, especially in the powdered form. Total ash was found maximum in PLA (15.33 ±0.050%) and GFE (14.15 ± 0.031%). Similarly, total ash was found lowest for GLE (11.45 ± 0.035%) and PFM (4.22 ± 0.053%) (Figure 4 C-D).

Water soluble ash was found maximum (2.5%) both in *Calotropis* leaf evening (PLE, GLE) and GFE (2.4%). Similarly, water soluble ash was found lowest in PFM (1.9%) (Figure 4 E). Sulphate ash was found maximum in PLA (9.5%) and PFM (8.3%). Similarly sulphate ash was found lowest in GLE (7.9%) and GFE (7.3%) (Figure 4 F). The microbial growth (fungi and bacteria) is promoted when medicinal plants are having access of water. Loss on drying is the loss of mass expressed as percent (w/w), and the test for loss on drying determines both water and volatile matter in the crude drug. Further, moisture is an unavoidable component of crude drug which must be eliminated as far as possible. The moisture content was found maximum in GLA (10.60± 0.200%) and GFA (11.06± 0.100), and lowest in PLA (8.81± 0.598%) and PFA (9.92± 0.244) (Figure 4 G-H). The foaming ability of an aqueous decoction of herbal materials and their extracts is measured in terms of a foaming index. Foaming index was found more or less same in all samples of *C. gigantea* and *C. procera* and was less than 100.

3.4 Fluorescence analyses

The phytochemical constituents of the powder material are fluorescent in different wavelengths when illuminated by light. Some phyto-constituents show fluorescence in the visible range[34]. Similarly, the ultraviolet light produces fluorescence of natural products (alkaloids) that are not visible in day light. Some crude drugs have been regularly assessed using the fluorescence parameter for their quality. Therefore, the powdered material was made fluorescent by adding various reagents.

Table 2. Fluorescence analysis of powdered leaf and flower of *Calotropis* spp.

Reagent	Colour observed in Ordinary Light	Colour observed under UV Light	
N NaOH	Green /Green	Green/ Green	Green / yellow
N HCL	Yellow /Green	Green/ Green	Yellow/ Black
0% NO4OH	Green /Green	Light Green/ Green	Green/ Yellow
0%H2SO4	Yellow/Yellow	Green/ Green	Brown/ Brown

Owing to its importance in Pharmacognosy[35], the fluorescence studies of leaf and flower powder of *Calotropis* spp. at the time of collection showed marked colouration at different wavelengths using different regents due to the light remitted by the molecules during return from the excited to non-excited state. Excited molecules dissipate the absorbed light energy by driving photochemical energy convergent as heat or by emission as fluorescence radiation[36].

3.5 Preliminary phytochemical screening

The results of phytochemical qualitative analyses of the crude drug of leaf and flower tissues of *Calotropis* spp. are as under:

Table 3. Preliminary phytochemical screening of *Calotropis* spp.

Phytochemical compounds	Methanolic Extracts Leaves and Flowers of *Calotropis gigantea* and *Calotropis procera*					
Plants Parts	Time of collection (Leaves)			Time of collection (Flowers)		
1.Tests for carbohydrates	Morning GLM/PLM	Afternoon GLA/PLA	Evening GLE/PLE	Morning GFM/PFM	Afternoon GFA/PFA	Evening GFE/PFE
I.Molish Test	+/+	+/+	+/+	+/+	+/+	+/+
II.Fehling's Test	-/-	-/-	-/-	-/-	-/-	-/-
III.Benedict's Test	+/+	+/+	+/+	-/+	+/+	+/+
2.Tests for proteins and aminoacids :						
I.Biuret'sTest	-/-	-/-	-/-	-/-	-/-	-/-
II.Ninhydrin Test	-/-	+/-	+/+	-/-	-/+	-/+
3.Tests for Glycosides:						
I.Borntrager'sTest	-/-	-/-	-/-	-/-	-/-	-/-
II.Legal's Test	+/+	+/+	+/+	+/+	+/+	+/+
III.Keller–killiani Test	-/-	-/-	-/-	-/-	-/-	-/-
4.Tests for Alkaloids:						
I.Mayer'sTest	-/-	-/-	-/-	-/-	-/-	-/-
II.Dragendrof's Tests	-/-	-/+	-/-	+/-	-/+	-/-

Continued on next page

Table 3 continued

III.Hager's Test	-/-	+/+	-/+	-/-	+/+	-/+
IV.Wagner's Test	-/+	+/+	+/+	+/+	+/+	+/-
5.Tests for Saponins:						
I.Froth Tests	+/+	+/+	+/+	+/+	-/+	+/+
6.Tests for Flavonoids:						
I.Lead Acetate Test	+/+	+/+	+/+	-/+	+/+	+/+
II.Alkaline Reagent test	-/+	+/+	-/+	+/-	+/+	-/+
III.Shinoda Test	+/+	+/+	+/+	-/+	-/+	+/-
7.Tests for Triterpenoids and Steriods:						
I.Salkowski's Test	-/-	-/+	+/-	-/-	+/+	-/-
II.Libermann-burchard's Test	-/-	-/+	+/+	+/-	-/+	-/+
8.Tests for Tannin, Phenoliccompounds:						
I.Feric chloride Test	-/-	-/-	-/-	-/-	-/-	-/-
II.Lead Acetate Test	-/-	-/+	-/-	-/-	-/+	-/-
III. Gelatin Test	-/-	-/-	-/-	-/-	-/-	-/-
9.Tests for fats and oils:						
I.Solubility Test	+/+	+/+	+/+	+/+	+/+	+/+

*Sign indication: Presence (+) and Absence (-)

4 Discussion

Standardization is an essential tool for ensuring the quality control of herbal drugs which is of paramount importance in justifying their acceptability in modern system of medicine[37]. The morpho-anatomical variation observed in the *C. gigantea* and *C. procera* from the family Asclepiadaceae could be used to distinguish the species for identification[38]. Anatomical studies of the *Calotropis* spp. leaf and flower showed prominent variation in shape and types of stomata and trichomes, shape of epidermal cells, vascular tissue, size and shape of floral parts, all of which could be used in identification of the two plants. Correct identification of medicinal plants is important to detect the adulteration necessary for the sustainable and effective utilization of plant drugs[39]. Powder microscopy of *Calotropis* spp. leaf also showed prominent unicellular and multicellular, epidermal cells and mesophyll cells, but, flower does not show any characters except the trichomes. From current observation of *Calotropis* spp. that standardization and pharmacognostic analyses will provide the correct identification and authentication which is of the paramount importance in justifying their acceptability in modern system of medicine. Unicellular and multicellular trichomes and paracytic and actinocytic stomata were found on adaxial surfaces in *C. procera* and *C. gigantea*, respectively. Thus stomata and trichome character can thus be used as classification and identification parameters in taxonomy as a diagnostic identity tool for this species[40,41]. Physicochemical parameters and quantitative screening methods such as aqueous and non-aqueous extract value, total ash value, acid insoluble ash, water-soluble ash, and moisture content are regarded as critical parameters involved in the standardization procedures while evaluating the quality of herbal drug. All these parameters were determined in this study to assess the drug values of *Calotropis* spp. Further, the maximum extractive value found in PFM and GFA is in agreement with the earlier reports of other researchers as methanol is known to be the efficient solvent for extraction of the phytochemicals from various plant materials[42]. Total ash value, water-soluble ash and sulphate ash of *Calotropis* leaf samples were found maximum in PLA (15.33 $\pm$ 0.050%), PLA (2.5%) and PLA (9.5%) respectively while the Calotropis flower samples the total ash value, water soluble ash and sulphate ash found maximum in the GFE (14.15 $\pm$ 0.031%), 2.4% and PFM (8.3%) respectively in time of collection. The highest concentration of physiochemical compounds in test samples were detected in afternoon in leaves of *Calotropis procera* as compared to *Calotropis gigantea*. Considering that stimulation factors (light and geography) may play an important role in biosynthesis of plant bioactive compounds. These findings support the observations that the increase in the production of bioactive constituents and variation in percentage to maximum of solar radiation and sample collected seasons[43] . Lower moisture content is reported to indicate less chance of microbial degradation of plant drug during storage[44]. This is the demarcation with our observation. The general requirement of moisture content in crude drug should not be more than14%[45]. Moisture content value was found maximum in the sample of leaf GLA (10.60$\pm$ 0.200%) and lowest in the PLA (8.81$\pm$ 0.598%) whereas, in the flower sample, moisture content was found maximum in GFA (10.60$\pm$ 0.200%) and lowest in the PFA (9.92$\pm$ 0.244). Fluorescence analyses showed different colours by different regents between three UV ranges of different plant extracts used for the qualitative assessment of crude drugs[46]. The *Calotropis* spp. appears to be rich in phytochemical constituents widely used in traditional medicines to cure various dreadful diseases. Phytochemical screening showed that the plants contain secondary metabolites such as alkaloids, cardiac glycosides, tannins, terpenoids, and saponins in all samples but particularly was found maximum in the samples collected in the afternoon of

calotropis. The phytochemical biosynthesis is mediated by environmental factors and even could be seasonal variations. Factors like precipitation, temperature, light, and humidity are critical in affecting the overall yield and major bioactive constituents [47]. In this study phytochemicals obtained from collected sample in accordance with the time of collection in afternoon showed maximum yield as compared to other collecting time.

The secondary metabolites are known exhibit various activities against different ailments, and include anti-inflammatory, analgesic, antimicrobial, anticancer and anti-diuretic activities, etc. The present study is purely preliminary attempt to find the conformation based on Ayurvedic principles. Also, it is a qualitative phytochemical study, but, more extensive works on quantification of bioactive components is needed to establish a concrete conclusion about the time of collection. This attempt may bring druggists nearer in achieving specific time of collection and suitable preparation of drug.

5 Conclusion

Yield of Calotropis species depends upon the time of collection (morning, afternoon and evening). Maximum yield was obtained in C. *procera* (leaf and flower parts) as compared to *C. gigantea*. More variation in phytochemical extraction is due to the time of harvest at which different samples were collected in a day. Pertinently, maximum contents were found a bit higher in afternoon supporting the classical statement for timely collection of different parts of *Calotropis* species. The *Calotropis procera* could be feasible harvested in the afternoon for medicinal yield. In nutshell, the current study reveals collection time variation in physio-chemical profile of *Calotropis* spp. which may serve some purpose to the Indian pharmacopeia.

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Abbreviation

GLM (*Gigantea* leaves Morning), GLA (*Gigantea* leaves Afternoon), GLE (*Gigantea* leaves Evening) GFM (*Gigantea* Flowers Morning), GFA (*Gigantea* Flowers Afternoon), GFE (*Gigantea* Flowers Evening) PLM (*Procera* leaves Morning), PLA (*Procera* leaves Afternoon), PLE (*Procera* leaves Evening) PFM (*Procera* Flowers Morning), PFA (*Procera* Flowers Afternoon), PFE (*Procera* Flowers Ev)

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