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Pharmacognostic standardization of stems of *Thespesia lampas* (Cav.) Dalz & Gibs

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ABSTRACT

Objective: To establish the standardization parameters for complete pharmacognostic evaluation of stems of *Thespesia lampas* (*T. lampas*) (Cav.) Dalz & Gibs (Malvaceae), an important plant in the Indian system of medicine. **Methods:** Morphological, microscopical, physico-chemical evaluations, florescence analysis of *T. lampas* stems were investigated and preliminary phytochemical analysis, GC-MS analysis and HPTLC fingerprinting were carried out for qualitative phytochemical evaluation of various extracts of stems of *T. lampas*. **Results:** Chemo-microscopy revealed the presence of lignin, starch grains and calcium oxalate crystals. Physico-chemical evaluation used to determine numerical standards showed a result with total ash (9.03 ± 0.05) % w/w, acid insoluble ash (1.50 ± 0.01) % w/w, water soluble ash (2.51 ± 0.02) % w/w, sulphated ash (7.50 ± 0.01) % w/w, ethanol soluble extractive (0.24 ± 0.02) % w/w, water soluble extractive (0.08 ± 0.01) % w/w, moisture content (6.03 ± 0.05) % w/w and total crude fibre content of stem powder (47.36 ± 0.32) % w/w. Behavior characteristics of the stem powder showed presence of steroids, starch, alkaloid, flavonoids and proteins. Preliminary phytochemical analysis revealed presence of glycosides, phenolic compounds, tannins, steroids, saponins, flavonoids, carbohydrates and proteins. GC-MS analysis showed the presence of fatty acids such as dodecanoic acid, tetradecanoic acid, n- hexadecanoic acid, 9-tetradecenal and HPTLC fingerprinting revealed the presence of β- sitosterol and quercetin in stems of *T. lampas*. **Conclusions:** The pharmacognostic standardization of *T. lampas* is useful towards establishing standards for quality, purity and sample identification.

1. Introduction

Thespesia lampas (*T. lampas*) (Cav.) Dalz & Gibs, synonym: *Hibiscus lampas* Linn, is a medicinally important plant of the Malvaceae family commonly known as 'Ranbhendi'. It has been found throughout India and in Eastern Tropical Africa[1]. It is an annual undershrub having reddish brown colored bark. Leaves are 7.5-15.0 cm long, cordate or truncate at the base; 3-lobed and lobes triangular, acuminate, finely reticulately veined sometimes with black grandular dots on the lower surface, subglabrous on the upper. Flower is yellow with crimson centre, 7-10 cm, diameter, 1-3 together at the end of long, auxillary or terminal peduncle. Fruits capsules are dull black, 2.5 cm long, ovoid, pointed, woody 4-5 valved, pilose. Seeds are glossy, glabrous, many are dark brown or black, club shaped 3 mm long[2,3]. In the folk medicine, this plant has been considered to be hepatoprotective and traditionally root paste was used to cure jaundice in Korku tribe of Amravati district of Maharashtra and also in Nepal[4,5]. The roots of this plant are reported for anti-diabetic, anti-hyperlipidaemic, hepatoprotective, antioxidant and anthelmintic activity[6-11]. The stem parts are used in the treatment of inflammation, acidity, bleeding nose, bronchitis, cough, dysentery, fever, sun stroke, urinary complaints, anthelmintic and carbuncle[12]. Stems of *T. lampas* have been reported for presence of gossypol[13] and its antimicrobial activity[14]. The literature survey and screening of scientific data revealed that although *T. lampas* stems are traditionally used in the treatment of various diseases for a long time, no systematic pharmacognostic and physico-chemical standardization has been done. The present investigation of *T. lampas* is therefore taken up

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to establish certain botanical and chemical standards like pharmacognostic characterization, physicochemical analysis and preliminary phytochemical testing of stems which would helps to prepare a monograph for the proper identification of the plant.

2. Materials and methods

2.1. Plant material

The plant, *T. lampas* was collected in Trimbakeshwar Hills, Nashik District (Maharashtra) in May 2008. The plant was authenticated and herbarium was deposited in Botanical Survey of India, Pune, Maharashtra under voucher specimen number CDSTL1. (Ref. No. BSI/WC/Tech/2008/79). The stems of the plant were dried, powdered and passed through 40 mesh sieve and stored in an airtight container for further use.

2.2. Macroscopic and microscopic analysis

Macroscopic studies were done by using simple microscope. The color, odour, taste, size and shape of stems were determined. Microscopic studies were done by preparing thin hand section of stems of *T. lampas*. The sections were cleared with chloral hydrate solution, stained with phloroglucinol- hydrochloric acid (1:1) and toludine blue[15,16]. Powder (#60) of the dried stems was used for the observation of powder microscopical characters. The powdered drug was separately treated with phloroglucinol-hydrochloric acid (1:1) solution, acetic acid and iodine solution to determine the presence of lignified fibres, calcium oxalate crystals and starch grains respectively[15].

2.3. Physico-chemical analysis

Physico-chemical parameters of the powdered drug such as total ash, water-soluble ash, acid-insoluble ash and sulphated ash were determined. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content was detected by loss on drying method[17]. Crude fibre content of stem powder was also determined[18].

2.4. Florescence analysis

Powdered stem material was analyzed under visible light, short ultra-violet light, long ultra-violet light after treatment with various organic/inorganic reagents like NaOH, HCl, HNO_3 and H_2SO_4[19,20].

2.5. Behaviors of stem powder

Behaviors of stem powder of *T. lampas* with different chemical reagent were performed to detect the occurrence of phytoconstituents along with color changes under ordinary daylight by standard method[21].

2.6. Quantitative determination of heavy metal and minerals

A total of 500 mg of air-dried stem powder was taken to determine major heavy metal and minerals content. Stem ash was also prepared by taking 2 g of sample and keeping in muffle furnace at 150 degree centigrade till constant weight was obtained. The major inorganic constituents of stem powder and ash were determined quantitatively by atomic absorption spectrometer (ASS) (Perkin Elmer-400), using argon as the carrier gas and flow rate was kept as 1 mL/2 min[22, 23].

2.7. Preparation of extract and preliminary phytochemical analysis

The air-dried stems of *T. lampas* were made into coarse powder. The powdered material was defatted with petroleum ether. The defatted material was extracted with methanol and distilled water using a Soxhlet extractor. Methanolic extract was further fractionated with ethyl acetate to get ethyl acetate soluble and ethyl acetate insoluble fractions. Then the extract was filtered through muslin and the filtrate was evaporated under reduced pressure and vacuum-dried[24]. The qualitative chemical test of various extracts of *T. lampas* was carried out using standard procedure[25].

2.8. GC-MS analysis of saponifiable matter of petroleum ether extract

The petroleum ether extract obtained was processed for separation of the saponifiable and unsaponifiable matter. 1.5 g of vacuum-dried extract was allowed to saponify using alcoholic KOH with reflux and then it was extracted with solvent ether for separation of unsaponifiable matter. The aqueous phase was acidified with concentrated H_2SO_4 and then again extracted with the solvent ether for separation of the saponifiable matter. All the three part were separated, *i.e.* petroleum ether, unsaponifiable matter and saponifiable part. The saponifiable matter of the petroleum ether extract was subjected to gas chromatography - mass spectroscopy (GC-MS)[26].

2.9. HPTLC fingerprinting of different extracts of *T. lampas* stems

20 mg/mL solution of petroleum ether extract, methanolic extract, ethyl acetate soluble fraction of methanolic extract and aqueous extract was prepared in petroleum ether, methanol, ethyl acetate and water respectively. Dissolve 10 μg of standard drug, β-sitosterol and quercetin in 1 mL methanol. Take 1 mL of above solution and diluted to 10 mL with methanol (0.1 μg/mL). Then 20 μL was applied on the silica gel GF254 HPTLC plates (10×10). For β-sitosterol, toluene: methanol: ethyl acetate (9:1:0.5) and for quercetin, toluene: ethyl acetate: formic acid (9:1:0.5) was used as mobile phase. After development the plates were scanned in ultraviolet range at 254 nm and 366 nm and then the plates were derivatized by using 10% ethanolic sulphuric acids[27].

3. Results

Table 1
Physicochemical parameters of *T. lampas* stems.

Parameters	Mean ± SD (% w/w)
Total ash	9.03 ± 0.05
Acid-insoluble ash	1.50 ± 0.01
Water-soluble ash	2.51 ± 0.02
Sulphated ash	7.50 ± 0.01
Ethanol soluble extractive	0.24 ± 0.02
Water soluble extractive	0.08 ± 0.01
Moisture content	6.03 ± 0.05
Total crude fibre content	47.36 ± 0.32

Table 2
Fluorescence analysis of powdered stems of *T. lampas*.

Reagents	Color observed in ordinary light	Color observed under ultraviolet light	
		Short (254 nm)	Long (365 nm)
1 N NaOH in methanol	Brown	Black	Blue
1 N NaOH in water	Brown	Bluish brown	Blue
1 N HCl	Brown	Green	Blue
50% HNO_3	Brown	Black	Brown
50% H_2SO_4	Brown	Black	Brown

Table 3
Behavior analysis of powdered stem of *T. lampas*.

Reagents	Colour/ppt	Constituents
Powder as such	Brown	–
Concentrated H_2SO_4	Brownish red	Steroid (+)
Aqueous ferric chloride	No black color	Tannins (–)
Aqueous iodine solution	Blue color	Starch (+)
Aqueous mercuric chloride	Brown	Alkaloid (+)
Picric acid	Yellow ppt	Alkaloid (+)
Magnesium HCl	Pink	Flavonoids (+)
Aqueous silver nitrate solution	No change	Protein (+)
Ammonia solution	No change	Anthraquinone glycosides (–)
Aqueous KOH	Greenish	Anthraquinone glycosides (–)

"+"– Present; ""–Absent

Table 4
Heavy metal and mineral content analysis of *T. lampas* stems.

Heavy metals and mineral content	Values* in stem ash (ppm)	Values* in stem powder (ppm)
Lead	4.290	4.960
Aluminum	183.000	N. D.
Magnesium	N. D.	N. D.
Zinc	27.000	27.300
Copper	12.600	11.600
Iron	142.000	158.000
Nickel	3.610	3.590
Chromium	0.148	0.297

N. D. – Not Detected; * – Average of three determinations.

Table 5
Extractive values of *T. lampas* stem extracts.

Extract	Yield (% w/w)	Color of extract
Petroleum ether	3.75	Yellow
Methanolic	8.00	Reddish brown
Ethyl acetate soluble	3.80	Reddish brown
Ethyl acetate insoluble	4.20	Brown
Aqueous	8.44	Brownish black

3.1. Macroscopic characteristics

The stems of *T. lampas* have reddish brown colour. Outer bark of stem consisting of reddish brown colour with yellowish white wood. Stems have characteristic odour, bitter in taste and straight, erect, stout, smooth textured with varying length from 3–6 cm and 0.5–1.5 cm in thickness.

3.2. Microscopic characteristics

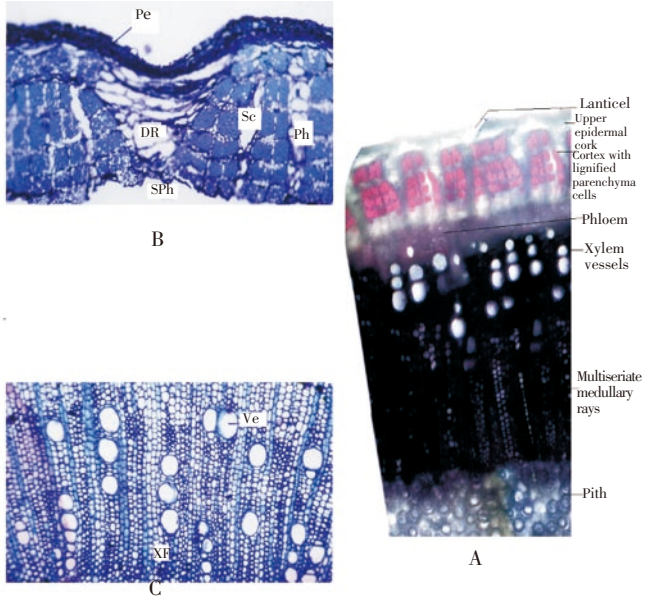

Figure 1. Microscopy of of *T. lampas* stems.
A: Transverse section of the stem; B: Transverse section of the stem bark; C: Transverse section of the stem showing secondary xylem. (Pe – Periderm, DR – Dilated ray, Sc – Sclereids, Ph – Phloem, SPh – Secondary phloem, Ve – Vessel, XF – Xylem fibres)

The plant, *T. lampas* showed the general characteristics of a dicot plant. The microscopical study of stem showed very large pith and vascular bundles arranged in a ring. The stem has thick hallow, continuous cylinder of secondary xylem and secondary phloem. A narrow periderm is evident. The lenticels present in a periderm which are similar in function to stomata, having open pores with absence of guard cells. The upper epidermal cork consists of thick walled tangentially elongated cells. Below that the presence of thin walled parenchyma with very small intracellular spaces of cortex contains bundle of acicular calcium oxalate crystals. The phloem consists of isodiametric parenchyma with small intercellular spaces. Multiseriate, thin walled, elongated medullary rays with presence of acicular and cluster form of starch grains. Pith consists of thin walled

Table 6

Preliminary phytochemical analysis of *T. lampas* stems extracts.

Chemical Constituents	Chemical tests	Petroleum ether extract	Methanol extract	Ethyl acetate soluble fraction	Ethyl actate Insoluble fraction	Aqueous extract
Alkaloids	Dragendorff's test	–	–	–	–	–
	Mayer's reagent	–	–	–	–	–
Carbohydrates	Molisch's test	–	–	–	+	+
	Barfoed's test	–	–	–	+	+
Glycosides	Borntrager's test	–	–	+	+	+
	Keller-killianin test	–	+	–	–	–
Saponin glycosides	Foam test	–	–	–	–	–
Flavonoids	Shinoda test	–	+	+	+	+
	Sodium hydroxide test	–	+	+	+	+
	Lead acetate test	–	+	+	+	+
Tannins	Ferric chloride test	–	+	+	+	+
	Phenazone test	–	+	+	+	+
Steroids	Salkowski test	+	+	–	–	–
	Libermann-burchard test	+	+	–	–	–
Proteins	Biuret test	–	–	–	–	+

+: Present –: Absent.

Table 7

CG– MS analysis of saponifiable matter of petroleum ether extract of *T. lampas* stems.

Compound	Molecular formula	Retention time	Concentration (%)	M^+	Base peak
Dodecanoic acid	$C_{12}H_{24}O_2$	9.134	10.31	200	73
Tetradecanoic acid	$C_{14}H_{28}O_2$	11.514	8.85	228	73
n– hexadecanoic acid	$CH_3(CH_2)_{14}COOH$	13.715	28.33	256	73
9-tetradecenal	$C_{14}H_{26}O$	15.524	7.68	210	55

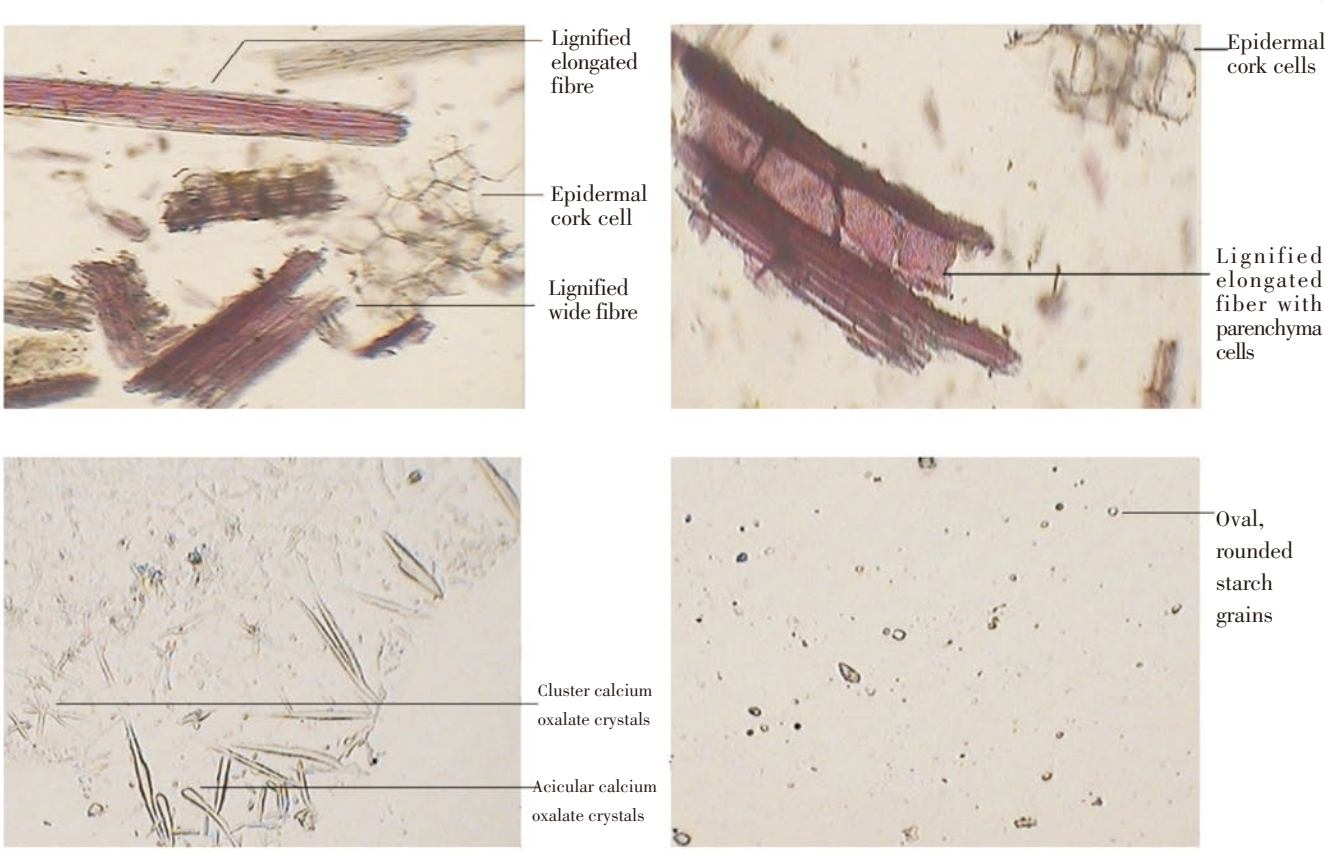

Figure 2. Powder Microscopy of of *T. lampas* stems.

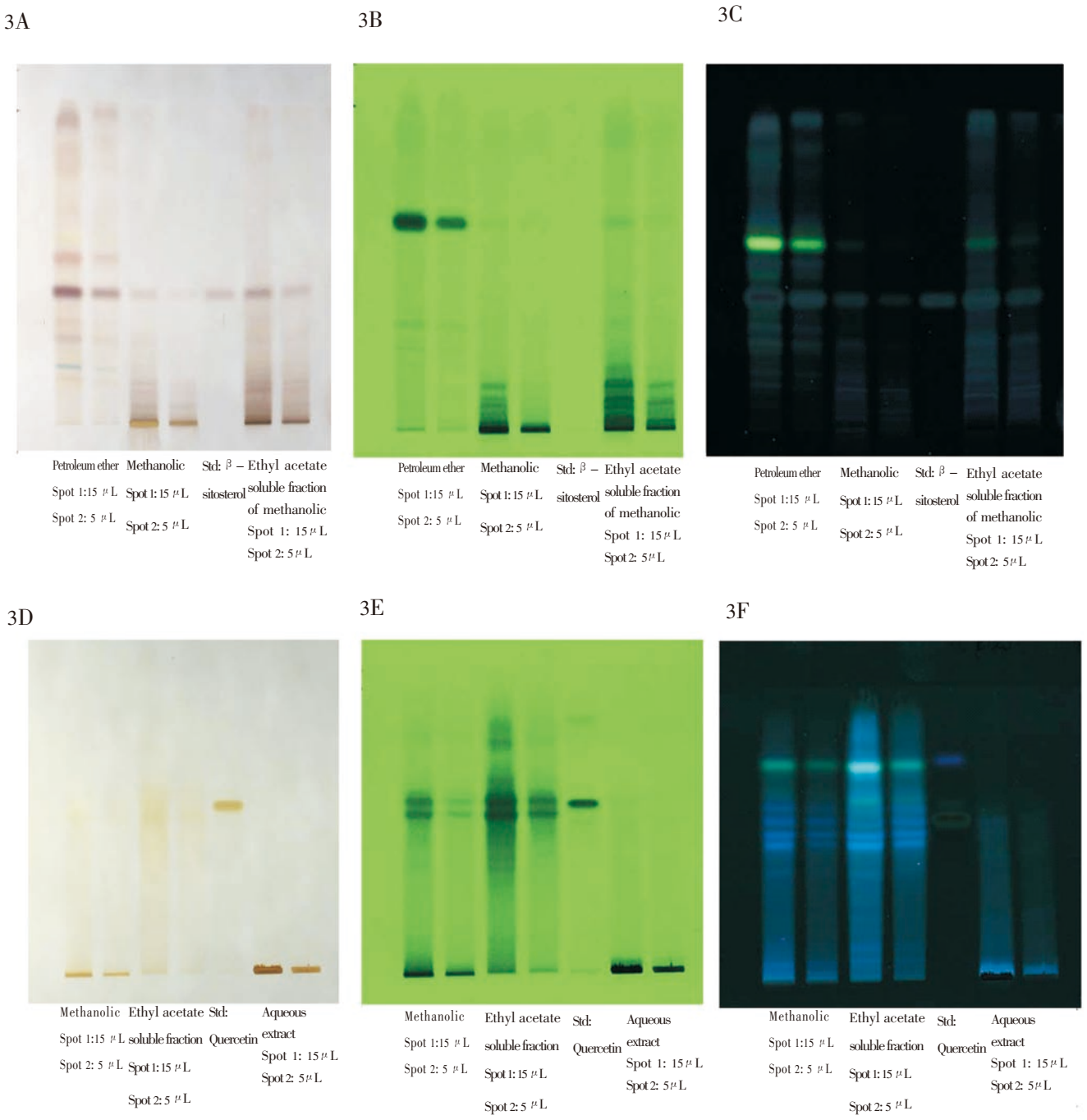

Figure 3. HPTLC fingerprinting of different extracts of of *T. lampas* stems
3A: Chromatogram spraying by 10% H2SO4; 3B: Chromatogram at scanned 254 nm; 3C: Chromatogram at scanned 366 nm; 3D:Chromatogram at daylight; 3E: Chromatogram at scanned 254 nm; 3F: Chromatogram at scanned 366 nm

parenchymatous mucilage cavities (Figure 1a). The stem bark of *T. lampas* consists of two distinct regions. They are outer bark and the inner bark. Outer bark constitutes the periderm and the inner bark comprises of the secondary phloem region. Periderm consists of the outer 4 or 5 thick walled, suberised phellem cells and inner 2 to 3 layers of thin walled phelloderm cells. The periderm is crushed and appears as a dark thick band. Inner to the periderm is the secondary phloem region which consists of cone shaped dense solid bands of fibers extending up to the periderm region. The phloem consists of thick walled sclereid masses alternating with narrow band of thin walled cells of sieve elements. Phloem rays are wide and alternate with in phloem sclerenchyma cones (Figure 1b). The secondary xylem consists of vessels, xylem fibers and thin radial lines of xylem rays. The vessels are elliptical, thick walled, either in radial multiples or solitary. The fibers are thick walled and lignified. The primary xylem occurs in radial rows around the pith region (Figure 1c).

3.3. Powder characteristics

Powder microscopy of the stem of *T. lampas* showed the presence of lignified long, elongated and also short, wide fibres with centrally consisting of parenchyma cells, polygonal thin walled epidermal cork cells, narrow, cylindrical, vessel elements, cluster and acicular form of calcium oxalate crystals and also presence of oval and rounded starch grains. Chemo-microscopy revealed the presence of starch grains, lignin and calcium oxalate crystals (Figure 2).

3.4. Physicochemical constants

The ash values, extractive values, moisture content and total crude fibre content of stems were determined. The total ash, acid insoluble, water soluble and sulphated ash values were observed to be (9.03 ± 0.05) % w/w, (1.50 ± 0.01) % w/w, (2.51 ± 0.02) % w/w, (7.50 ± 0.01) % w/w respectively on dry weight basis whereas extractive values of ethanol and water were found to be (0.24 ± 0.02) % w/w and (0.08 ± 0.01) % w/w respectively. The moisture content and total crude fibre content of stem powder were (6.03 ± 0.05) % w/w and (47.36 ± 0.32) % w/w respectively (Table 1).

3.5. Fluorescence characteristic

Fluorescence characteristic of powdered stem of *T. lampas* were observed for resolution of doubtful specimen. The stem powder of *T. lampas* was observed in visible light, short and long ultra-violet light (Table 2).

3.6. Behaviors of stem powder of T. lampas with different chemical reagent

Behaviors of stem powder of *T. lampas* with different chemical reagents showed presence of steroids, starch, alkaloid, flavonoids and proteins (Table 3).

3.7. Heavy metal and mineral analysis

ASS analysis of stem powder showed presence of heavy metals namely lead, and minerals such as zinc, copper, irons, nickel and chromium whereas in stem ash showed presence of heavy metals namely lead, aluminum and minerals such as zinc, copper irons, nickel and chromium (Table 4).

3.8. Extractive values and preliminary phytochemical analysis

The extractive values of petroleum ether, methanolic, ethyl acetate soluble, ethyl acetate insoluble and aqueous extract were found to be 3.75%, 8.0%, 3.8%, 4.2%, 8.44% w/w respectively (Table 5). Preliminary phytochemical analysis mainly revealed the presence of carbohydrates, glycosides, flavonoids, tannins, steroids and proteins (Table 6).

3.9. GC- MS analysis of saponifiable matter

GC-MS analysis of saponifiable matter of the petroleum ether extract gives the idea about chemical characters of compounds present in the extract. The result showed the presence of fatty acids such as dodecanoic acid (10.31%), tetradecanoic acid (8.85%), n- hexadecanoic acid (28.33%), 9-tetradecenal (7.68%) (Table 7).

3.10. HPTLC fingerprinting of T. lampas stems extracts

In HPTLC fingerprinting, it was observed that one of the constituent of petroleum ether extract appears at Rf 0.35, 0.34, methanolic extract at Rf 0.34, Standard β- sitosterol at Rf 0.34 and ethyl acetate soluble fraction of methanolic extract at Rf 0.35. In another plate, it was also observed that one of the constituent of methanolic extract appears at Rf 0.52, 0.56, ethyl acetate soluble fraction of methanolic extract at Rf 0.52, 0.56 and standard quercetin at Rf 0.55 and aqueous extract at Rf 0.56. Hence from the above observation, β-sitosterol and quercetin might be the constituents of stems of *T. lampas* (Figure 3).

4. Discussion

Pharmacognostic standardization including physico-chemical evaluation is meant for identification, authentication, detection of adulteration and also compilation of quality control of crude drugs. Since the plant, *T. lampas* is useful in traditional medicine for the treatment of some ailments, it is important to standardize it for use as a drug. Physico-chemical evaluation used to determine numerical standards reported in this work could be useful for the adulterants resolution of doubtful specimen or improper handling of drugs and compilation of a suitable monograph. The total ash is particularly important in the evaluation of purity of drugs, *i.e.* the presence or absence of foreign inorganic matter such as metallic salts and/or silica. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content of the drug is not too high, thus it could discourage bacterial, fungi or yeast growth, as the general requirement for moisture content in crude drug is not more than 14% w/w[28]. Fluorescence characteristic is a rapid method for resolution of doubtful specimen. When physical and chemical methods are inadequate, the plant material may be identified from their adulterants on the basis of fluorescence characteristics. Behaviors of the powdered drug with different chemical reagents and preliminary phytochemical analysis are helpful for detection of various phytoconstituents. Heavy metal analysis of powdered drug showed presence very low amount of toxic elements whereas presence of considerable amount of mineral elements of drug may be directly or indirectly helpful in the management of many diseases. All herbals are standardized for active constituents. Extract refers to a concentrated preparation of active constituent of a medicinal herb. The concept of standardized extracts definitely provides a solid platform for scientific validation of herbals. Phytochemical evaluation is one of the tools for

the quality assessment of plants, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques. GC-MS detection, has found a variety of analytical uses, which performing quality control analysis in both the pharmaceutical and food product industries[29]. In the last two decades HPTLC method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations[30]. This analysis is the first step towards understanding the nature of active principles and their detailed phytochemistry.

In conclusion, the present study was undertaken with an aim of pharmacognostic standardistion and preliminary phytochemical evaluation of stems of *T. lampas* providing useful information, which may help in authenticating the genuine plant along with nature of phytoconstituents present in it. This work could be useful for the adulterants resolution of doubtful specimen and compilation of a suitable monograph for its proper identification.

Conflict of interest statement

We declare that we have no conflict of interest.

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