Research Article

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A DETAILED PHARMACOGNOSTICAL AND PHYTOCHEMICAL PROFILE ON STEM OF *TELOSMA PALLIDA* (L.) KURZ.: AN EXTRAPHARMACOPOEIAL DRUG

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ABSTRACT

A traditional medicinal system which lays due emphasis on the preventive and promotive aspects of health by use of peculiar compound preparations and more powerful and potentiates medicinal herbs. Plants which are not mentioned or codified in pharmacopoeia or any other classical text of Ayurveda, yet used traditionally are called as *Anukta Dravya* (extra pharmacopoeial plant). *Telosma pallida* is an unexplored medicinal plant in the Ayurveda, belonging to Asclepiadaceae family. It is traditionally used to treat whooping cough, cold and asthma. The milky latex obtained from fruit is used in leucoderma and other skin diseases. Antioxidant Property of the stem part of the *Telosma pallida* is well established. But, till date, there is no scientific data available regarding the pharmacognostical and physicochemical profile of the stem of the plant. The macro-microscopical characteristics of the stem depict the presence of laticiferous cells and warty trichomes which are the peculiar characters of Asclepiadaceae family and play a major role in authenticating the species. The stem extract indicates the presence of major phyto-constituents such as tannin, alkaloid, glycoside, and flavonoid. Moreover, physicochemical study and fluorescence analysis was performed of the stem as well as its HPTLC profile was generated for authentication purpose.

Keywords: Micromorphology, Histochemical, Phytochemistry, *Telosma pallida*(L.) Kurz., Pharmacognosy

INTRODUCTION

The knowledge of the medicinal plants used in the drugs of the traditional systems of medicine has been of great value especially as leads for the discovery of new single molecular medicines.¹ A Large section of the population of developing countries relies on traditional or herbal folk medicines for their primary health care and has deep faith in it. Since the usages of these herbal medicines have increased, issues regarding their safety, quality and efficacy in industrialized and developing countries have cropped up. Plant-based traditional uses are well known for their potential health benefits in a variety of human ailments since ancient time.² Asclepiadaceae family consists of about 250 genera and over 2000 species, widespread in tropical and subtropical regions.³ *Telosma pallida* (L.) Kurz. belonging to Asclepiadaceae Family, is an extensive, twining under shrub or climber, slender growing up to 3m in height and puberulent. It is available in India, Myanmar, Nepal, Pakistan, Thailand, Vietnam etc. places.^{4,5}

In recent years, Asclepiadaceae family members are reported for pregnane glycosides, acylated derivatives illustration much consideration due to their anti-fungal, immune-stimulants, antitumor, anti-epilepsy, anti-asthmatic properties.⁶ Two new pregnane ester glycosides viz. pallidine and pallidine have been isolated from the twigs of *T. pallida*.^{7, 8} Antioxidant activity of stem part is also reported⁹. Till date, there is no systematic data available regarding the detailed Phyto-pharmacognostical profile of *T.pallida* (L.) Kurz stem. In the

present work, macroscopy, microscopy, histochemical studies and phytochemical study of the stem are taken into consideration as the main aspect of research work.

MATERIALS AND METHODS

Collection and authentication of plant drug

The plant species were identified with the help of literature. Field notes were taken viz. plant's name, habit, habitat and the characteristics of the species along with the plant family. Plants were collected from the natural habit, i.e. Theba village of Jamnagar area. The herbarium sheets were labelled, numbered (Phm-6172/15) and deposited in the Pharmacognosy Lab., IPGT & RA of Gujarat Ayurved University for further reference. Fresh mature stem samples were used for various Pharmacognostical evaluations. The stem was separated and dried in the shed, powdered and passed through 80 # for further phyto-pharmacognostical studies.⁵

Instruments and Chemicals Required

Carl Zeiss microscope, slides and coverslips, Mixer grinder, Weighing machine, glass ware (as per requirements), Precoated silica gel 60 F₂₅₄ aluminium plates (Merck, Germany) with a thickness of 0.1 mm, 10×10 cm, HPTLC Instrument, and U.V Chamber. All chemicals and reagents used were of analytical grade.

Powder Preparation

The plant part (Stem) used in this research work was shade dried and grinded using mixer grinder to obtain fine powder (passed through 80 #sieve). Stem Powder was packed in airtight polybag and used for organoleptic, microscopy, physicochemical study and fluorescence analysis.^{10, 11}

Extract Preparation

The dried stem powder was extracted individually using water, methanol, hydro-methanol and Chloroform. The resulting extracts were filtered and were used for the preliminary phytochemical test. The methanol soluble extract was used for generation of HPTLC profile.^{10, 11}

Macroscopic Study

The collected samples were identified and authenticated by observing characters such as size, shape etc. systematically as per the methods described in the textbooks of pharmacognosy.^{10, 12, 13}

Organoleptic characters of the powder

The organoleptic characters of stem powder viz. colour, odour, taste, and nature of powder were noted by sensory analysis.¹⁰

Microscopic study

Fresh samples were taken for detailed microscopic study. Free hand sections were cleared with chloral hydrate and observed. For observation of lignified components like fibres, vessels etc., the T.S. were stained with phloroglucinol and hydrochloric acid and photographs were taken by using Carl Zeiss Trinocular microscope attached with the camera. Same procedure was followed for detailed powder microscopy.^{10, 12, 13}

Micrometric evaluation

Measurement of the length, width of different cell layers along with their contents including powder characters were taken into consideration for micrometric evaluation.¹²⁻¹⁴

Histochemical evaluation

Thin sections of stem sample and powder samples were subjected to histochemical tests to locate as well as detect starch, tannin etc. by treating various reagents.^{10, 15}

Physicochemical Evaluation

Physicochemical parameters like Loss on Drying (LOD), Total ash, Acid insoluble ash, Alcohol-soluble extractive (90% methanol), Water-soluble extractive and pH value (5% w/v solution at Room temperature) were determined as per the API guidelines for the powdered samples.¹¹

Preliminary Phytochemical Evaluation

For phytochemical analysis the extracts obtained were used as test solutions for preliminary phytochemical examination for Carbohydrate, proteins, Steroids, glycosides, tannins, flavonoids, alkaloids etc. according to standard procedure.¹⁶

HPTLC Profile

Exactly 5µl of Methanolic extract (1mg/ml) of the crude drug was applied to Pre-coated Silica gel (GF₂₅₄) plate as sharp bands at 1cm from the bottom of the plate by means of Camag Linomat V sample applicator. The spots were dried in gentle current of air. Toluene: Ethyl acetate: Acetic acid (7: 2: 1 v/v) was used as mobile phase. The mobile phase (10 ml) was poured into Twin trough glass chamber and the whole assembly was left to equilibrate for 30 min. The plate was then developed in a twin-trough chamber saturated with the above stated mobile phase. The plate was then removed and dried. The detection of spots was done with Camag TLC Scanner 3. The plate was then derivatized by Vanillin – Sulphuric acid as spray reagent.¹¹

Fluorescence Analysis

Fluorescent characteristics of the stem powder as such and after treating them with chemical reagents were observed in daylight as well as under short and long UV. The analysis was carried out as per the method narrated in Indian Pharmacopoeia.¹⁷

RESULTS

Macroscopic study of *T. pallida* stem

The morphological features of the stem are rough in touch and centrally hollow at the mature condition, branched with nodes and internodes. Plenty of lenticels present. Outer creamish brown and inner colour is creamish yellow, short fracture, longitudinally cut fibres.

Transverse section of *Telosma pallida* Stem

Diagrammatic T.S. of the stem is wavy in outline with deep ridges and grooves. Outer epidermis is followed by distinctly furrowed and ridged cork, cortex, hypodermis, vascular bundle and centrally located reduced pith.

Detailed T.S. shows single layered epidermal cell in patches, 10-12 layers of compactly arranged elongated square shaped lignified cork cells. Cortex made up of two types of cells, outer collenchyma and inner parenchyma cells filled with chlorophyll pigments, some of the cells loaded by oil globules and brown content. Single layered endodermis with somewhat elongated parenchymatous cells circularly arranged and loaded with starch grains along with few latex bearing cells. Isolated cortical cells are loaded with rosette and cluster crystal of Calcium oxalate. Pericyclic fibres 2-3 layered discontinuous, circularly arranged all over the section. Vascular bundles open and collateral, radially arranged. Metaxylem faces toward the cortical region while protoxylem towards pith region with xylem parenchyma and fibres. Xylem separated by uni-serrate medullary rays. Phloem is situated above the xylem and made up of sieve elements and its fibres. The Larger region of the section is occupied by pith made up of parenchymatous cells which are filled with oil globules and simple as well as compound starch grains. (Figure 1) All the values were taken in triplicates and mean value is as shown in Table 1.

Organoleptic Characters

Organoleptic study of *Telosma pallida* stem powder was carried out and the results are depicted in the Table 2.

Microscopic and Micrometric evaluation of Powder

Powder microscopy shows brown content, lignified fibres, parenchyma cells, uniseriate multicellular warty trichomes,

prismatic, rosette and cluster crystals of calcium oxalate, starch grains, lignified pitted and border pitted vessels. All the values were taken in triplicates and mean value is depicted in Table 3. (Figure.2)

Histochemical evaluation

T.S. of stem and its powder shows presence of starch grains, calcium oxalate crystals and lignified cells by histochemical tests and results were depicted in Table 4.

Physico-chemical analysis

Physico-chemical parameters of stem powder were performed in triplicate and mean values $\pm$ SEM were calculated and the results were depicted in Table 5.

Preliminary phytochemical evaluation

Preliminary phytochemical tests were carried out in water, methanol, hydro-methanol and chloroform extracts to check the presence or absence of primary and secondary metabolites. (Table 6)

Chromatographic analysis (HPTLC):

Chromatographic techniques were carried out as per the standard methods. Results are depicted in Table 7. (Figure 3)

Fluorescence analysis

Fluorescent characteristics of the Stem powders as such and Powder treating with Different chemical reagents were observed in daylight as well as under short and long UV. Behaviour of powdered plant materials with different chemical reagents is as shown in Table 8.

DISCUSSION

Stem is slender, puberulent, up to 3m in height, with prominent node and internode. Stem T.S shows epidermal cells followed by cork and cortex, cortex made up parenchymatous cells which are filled with chlorophyll pigments, starch grains and oil globules. Laticiferous cells as well as cluster and rosette crystals of calcium oxalate are observed. 2-3 layered discontinuous arranged pericyclic fibres followed by open collateral vascular bundles. Laticiferous cells are mainly related as defence mechanism against herbivores and microorganisms and are a peculiar character of Asclepiadaceae family.¹⁸ 10-12 layered wide, elongated square shaped lignified cork cells. In mature

stem, secondary growth of vascular bundles was seen and cortex as well as pith was found to be reduced.

Starch and Crystals are the basic ergastic substances of the plants, the common location of its accumulation is in parenchymatous cell and the secondary vascular tissues in the stem². Mature stem T.S., as well as powder, shows abundant starch grains and crystals.

Powder depicts the presence of brown content, parenchyma cells, uniseriate multicellular warty trichomes, prismatic, rosette and cluster crystals of calcium oxalate, starch grains, lignified fibres and pitted vessels. While, in matured stem border pitted vessels are found.

The ash value and acid insoluble ash of stem is 7.22 ± 0.27 % w/w and 0.46 ± 0.01 % w/w respectively, which determines the amount of inorganic residue obtained after complete incineration of the drug.¹⁹ A lower value indicates the good collection practices. Hence, it holds a good significance. Alcohol and water-soluble extractive value is 12.24 ± 0.33 % w/w and 16.42 ± 0.02 % w/w respectively. The extractive values signify the presence of chemical constituents which were extracted by the respective and specific solvent. It is notable that in many cases acidic pH (3 to 6) are preferable for stability of phytochemicals and the pH of stem shows 5.16 i.e. acidic in nature.²⁰

Preliminary phytochemical screening of alcohol extract shows the presence of alkaloids, tannin and phenols. While, steroids are found to be present in chloroform extract. Whereas, carbohydrates, saponin glycoside, amino acid are found to be present in water extract. These phytochemicals are active substances with significant physiological action.

HPTLC profile of the methanol extract of the stem shows 9 spots at 254 nm and 3 spots at 366 nm respectively. After spraying with vanillin-sulphuric acid 3 spots were observed under daylight. At both, the wavelengths similarity is seen at 0.03, 0.65 and 0.97 R_f respectively. While, before and after spray similar spots are obtained at 0.65 R_f . This similarity signifies that same class of molecules may be present.

Fluorescence is the behaviour revealed by the chemical constituents present in the plant. Some of them exhibit fluorescence in the visible region, while others fluoresce in the ultraviolet region. The chemical constituents, which do not fluoresce may be converted into fluorescent derivatives by the aid of different chemical reagents. Hence, fluorescence analysis can serve as a useful tool for qualitative assessment of plant drugs.²¹

Table 1: Micrometric measurements of *T. pallida* stem transverse section

Character	Measurement of stem
Section Diameter (4x $\times$ 10x)	10.8 μ m
Epidermis (40x $\times$ 10x)	0.6 μ m
Cork (40x $\times$ 10x)	3.5 μ m
Cortex (10x $\times$ 10x)	1.2 μ m
Diameter of Vascular bundle (4x $\times$ 10x)	1.8 μ m
Pith (40x $\times$ 10x)	2 μ m

Table 2: Organoleptic characters of *T. pallida* stem powder

Characters	Mature stem
Colour	Creamish yellow
Taste	Sweet followed by astringent
Odour	Characteristic
Nature of powder	Fibrous

Table 3: Micrometric evaluation of *T. pallida* Stem Powder

Powder Character (40x × 10x)	Measurements of stem powder
Simple starch grain	0.3×0.5 µm
Prismatic crystals	0.5×0.4 µm
Rosette crystals	1-1.2 × 1 µm
Cluster crystals	0.6-0.8×0.6-0.8 µm
Group of lignified fibres	8-10×1-2.5 µm
Border pitted vessels	0.8 µm
Cork in surface view	2.2-3.2×1.6-2 µm
Warty trichomes	2.5X 0.4 µm

Table 4: Histochemical evaluation of *T. pallida* Stem powder

Reagent	Observation	Characteristics	Results
Phloroglucinol+Conc. HCl	Red	Lignified fibres, vessels and cells	++
Iodine	Blue	Starch grains	++
Phloroglucinol+Conc. HCl	Dissolved	Ca Ox - crystals	++
FeCl ₃ solution	Dark blue	Tannin cells	++

Table 5: Physico-chemical parameters of *T. pallida* Stem powder

Parameters	Result
Loss on drying (% w/w)	15.0±0.41
Ash value (% w/w)	7.22±0.27
Acid insoluble ash (% w/w)	0.46±0.01
Alcohol soluble extractive (% w/w)	12. 24±0.33
Water soluble extractive (% w/w)	16.42±0.02
pH (5% w/v solution)	5.16

Table 6: Preliminary phytochemical evaluation of *Telosma pallida* stem

Name of the test	Water extract	Alcohol extract	Chloroform extract
Alkaloid	++	++	--
Amino acid	++	--	--
Carbohydrates	++	--	--
Flavonoid	++	++	--
Protein	--	--	--
Saponin glycoside	++	--	--
Starch	++	--	--
Steroid	--	--	++
Tannin	++	++	--

++ = Present; -- = Absent

Table 7: HPTLC Studies of Methanol extracts of *Telosma pallida* Stem

Wavelength	Number of spots	R _f values
254 nm	9	0.03, 0.13, 0.19, 0.38, 0.65, 0.68, 0.75, 0.93, 0.97
366 nm	3	0.03, 0.65, 0.97
After spraying	3	0.65, 0.73, 1

Table 8: Fluorescence Analysis of *Telosma pallida* Stem powder

Solvent	Daylight	UV Light(366 nm)
Dry powder	Green	Green
Powder + 5% NaOH	Yellow	Brown
Powder + 5% KOH	Golden yellow	Yellow
Powder + 5% FeCl ₃	Greenish brown	Black
Powder + H ₂ SO ₄	Greenish brown	Bottle green
Powder + dil. NH ₃	Greenish yellow	Bottle green
Powder + Conc. HCl	Bottle green	Black
Powder + Conc. HNO ₃	Brown	Yellow
Powder + 5% HCl	Yellowish brown	Dark brown
Powder + 5% H ₂ SO ₄	Brown	Black
Powder + dil. HNO ₃	Golden brown	Brown
Powder + Na ₂ CO ₃	Greenish yellow	Black
Powder + AgNO ₃	Green	Ash/Gray

Natural habitat of *T. Pallida*

Morphological measurement of stem

Cork, hypodermis, cortex, vascular bundle, pith

Micro- measurement of T.S of *T. pallida* Stem

Lignified cork, pericyclic fibres, and vascular bundles

Medulary rays and pith with Starch grains

Figure 1: T.S of *Telosma pallida* stem

Powder of *Telosma pallida* stem

Lignified cork cells

Group of starch grains

Micro-measurement of rosette crystal

Multicellular warty trichomes

Micro-measurement of border pitted vessels

Micro-measurement of group of fibres

Annular and spiral vessels

Figure 2: *Telosma pallida* stem powder

Figure 3: HPTLC Profile of *T. pallida* stem

CONCLUSION

There is no record of Phyto-pharmacognostical work on the stem of *Telosma pallida*. In the present study - identification, macro-microscopy, phytochemical & physicochemical analysis was carried out in a systemic manner which may be useful for standardization of herbal drugs. In the present era of increasing folk-lore medicine practice the obtained results may enrich the Ayurvedic Pharmacopoeia of India. It will also serve as therapeutic diagnostic tools for the scientists who are keen and truthful to evaluate the herbal medicine of indigenous resources.

REFERENCES

1. Dev Sukh. A Selection of Prime Ayurvedic Plant Drugs Ancient-Modern Concordance: New Delhi: Anamaya Publishers; 2006.
2. Kadam. Histochemical investigation of *Cassia tora* linn. World Journal of Pharmaceutical Research 2017; 6(3); 1008-1015.
3. Sharma OP. Plant Taxonomy. 2nd ed.: Tata Mc-Graw Hill Education Pvt. Ltd.; 2009. p. 350-352.

4. Santpau H, Henry AN. A Dictionary of the flowering plants in India: National Institute of Science Communication(CSIR); p. 168.
5. Shah GL, Flora of Gujarat state. Sardar patel university, Vallabhvidhya nagar; p. 21.
6. Gupta M R, Gupta V, Khare N K. Structural studies of biologically important steroidal pregnane glycosides from medicinal plants. Trends in carbohydrate research2015; 7(3):53-97.
7. Rastogi R P, Mehrotra BN. Compendium of Indian Medicinal Plants New Delhi: CDRI, Publication & Information Director; 1995. p.543.
8. Khare N K, Khare M P, Khare A. Two Pragnane ester Glycoside from Pergularia pallida. Phytochemistry-Elsevier1984; 36(12); 2931-2935.[https://doi.org/10.1016/0031-9422\(84\)83044-7](https://doi.org/10.1016/0031-9422(84)83044-7)
9. Adhikari BS. Medicinal plants diversity and their conservation status in wildlife Institute of India (WII) campus, Dehradun. Ethnobotanical Leaflets 2010; 46-83.
10. Khandelwal KR. Practical Pharmacognosy. 19th ed. Pune: Nirali Prakashan; 2008. p. 20.1-20.5.
11. Anonymous. The Ayurvedic Pharmacopoeia of India. Part-I, Vol. -II, Ministry of Health and Family Welfare, Govt of India, New Delhi: Controller of Publications; 2007. p. 194-195.
12. Evans WC. Pharmacognosy. 16th ed. London: W.B. Saunders; 2009. p. 108.
13. Kokate CK. Pharmacognosy. 2nd ed. Pune: Nirali Prakashan; 2008. p.107 -111.
14. Wallis TE. Text Book of Pharmacognosy. 5th ed. New Delhi: CBS Publishers & Distributors; 2005. p.104 – 158.
15. Krishnamurthy KV. Methods in the plant histochemistry Madras: Vishwanandan Pvt. Ltd.; 1988. p. 74.
16. Baxi AJ, Shukla VJ, Bhatt UB. The method of Qualitative testing of some ayurvedic formulations. Jamnagar: Gujarat Ayurved University; 2001. p. 5-12.
17. Anonymous. The Pharmacopoeia of India. New Delhi: Government of India; 1996. A-60.
18. Metcalfe CR, Chalk L. Anatomy of the Dicotyledons, Vol. II, Oxford; 1950. p. 917-925.
19. Anonymous. Quality control methods for medicinal plant materials, WHO; 1998. p. 4-46
20. Stefan Gafner, Chantal Bergeron. The challenges of chemical stability testing of herbal extract in finish product using State-of-Art analytical methodologies. Current Pharmaceutical Analysis 2005; 1(3): 203-215. DOI: 10.2174/1573412054022734
21. Gayathri V and Kiruba D. Fluorescence Analysis of Two Medicinal Plants – *Psidium Guajava* L and *Citrus Aurantium*. Int J Pharm Sci Res2015; 6(3): 1279-1282. DOI: 10.13040/IJPSR.0975-8232.6 (3).1279-82.

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