Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 11, 2015

Original Article

PHARMACOGNOSTICAL AND PRELIMINARY PHYTOCHEMICAL INVESTIGATIONS OF AERIAL PARTS OF *TRICHODESMA INDICUM* R. BR.

MANANI LATA M.*[1], SALUJA AJAY K.[2], PRAJAPATI PARIXIT R.[3]

[1]Department of Pharmacognosy, SSR College of Pharmacy Sayli, Silvassa, DNH 396230, India, [2]Department of Pharmacognosy, A. R. College of Pharmacy, and G. H. Patel institute of Pharmacy, Vallabh Vidyanagar, Anand 388101, India, [3]Department of Pharmaceutical Chemistry, SSR College of Pharmacy Sayli, Silvassa, DNH 396230, India
Email: latamanani_20@yahoo.com

Received: 16 Apr 2015 Revised and Accepted: 22 Sep 2015

ABSTRACT

Objective: This study was undertaken to carry out pharmacognostical studies and phytochemical studies on aerial parts of *Trichodesma indicum* R. Br belonging to the family Boraginaceae.

Methods: The aerial parts of plant leaf and stem were evaluated for pharmacognostical studies such as macroscopy, microscopy, powder study, and quantitative microscopy. The powder was evaluated for proximate analysis like ash vale, extracting value, moisture content, swelling index, elemental analysis, fluorescence analysis, and preliminary phytochemical studies.

Results: Transverse section of leaf of *T. indicum* R. Br. showed presence of covering trichomes with bulbous base upper and lower epidermis, collenchyma, prisms of calcium oxalate, vascular bundle and palisade cells. Surface preparation showed the presence of wavy epidermal cells, anomocytic stomata, anisocytic stomata, trichomes**.** Transverse section of Stem of *Trichodesma indicum* R. Br showed the presence of trichomes with, epidermis, hypodermis, cortex, xylem and pith**.** Powder study of aerial parts of *T. indicum* R. Br showed the presence of trichomes, xylem vessels, parenchyma, epidermal cells, fibres, calcium oxalate crystals. The powder of aerial parts was evaluated for proximate analysis such as ash value, extractive value, moisture content, total solid content, and the swelling index, which give idea about the presence of siliceous material, and amount of constituents extracted into different solvent. The elemental analysis of aerial parts showed that plant was free from heavy metal contamination i.e. arsenic, lead. The fluorescence analysis of plant powder showed that plant contains phenolic compounds. Qualitative chemical examination showed that the aerial parts of Trichodesma indicum R. Br, is credited with phytosterol, triterpenoids, tannins, phenolic compounds, carbohydrates, fixed oil, fatty acids mucilage.

Conclusion: The study reveals specific identifying characteristics for the particular crude drug which will be of significant use in identification and control to adulteration of the raw drug and can serve as a reference for any further investigations.

Keywords: *Trichodesma indicum* R. Br. Boraginaceae, Undhaphuli.

INTRODUCTION

Plants play a vital role for the existence of human and animal life on earth. Tribal medicine or traditional medicine plays an important role in the primary health care of tribal as well as rural people. In some developing Latin American and Asian countries, more than half of the population is using traditional medicine in primary health care. In most of these cases, the use of traditional medicine is the most affordable and accessible route for the cure of disease. In developed countries, there has been an increasing use of traditional and alternative medicine, even though western medicine is readily available. Therefore, it is essential to preserve the unique properties of plants and to give a deeper scientific understanding of how these plants can cure certain ailments. The Pharmacognosy group is concerned with natural substances and their chemical and pharmacological basis for their biological activities. There is a revival of interest in the pharmacy, both from the pharmaceutical industry as a source of new lead molecules and from the general public who are using plant extracts in many ways in conventional and complementary therapies. The use of plants for healing purpose predates recorded history and forms the origin of much of modern medicine. The plant based traditional knowledge has become a recognized tool in the search for new sources of drugs and Nutraceuticals.

Trichodesma indicum R. Br is in Gujarati known as Undhaphuli. The whole plant is used as diuretic, as an emollient poultice; in diseases of the eye; as a cure for fever, in ear pain. Some references are available in the literature on the plant of *Trichodesma indicum* R. Br. However, systemic and exhaustive information is lacking. Anti-inflammatory activity, antitussive activity, antidiarrheal activity, diuretic activity and antimicrobial activities of the plant are proven by the experimental models. *Trichodesma indicum* R. Br has been explored for varied pharmacological and phytochemical investigations, however, there are no reports published for standardization of *Trichodesma indicum* R. Br. [1-6].

Hence, an attempt was made to carry out the pharmacognostical and preliminary phytochemical evaluation of aerial parts of *Trichodesma indicum* R. Br that can serve as a reference material for any further studies related to adulteration, pharmacognostical and phytochemical investigations.

MATERIALS AND METHODS

Authentication, collection and preparation of plant material

Fresh and fully grown plants of *Trichodesma indicum* were collected from the medicinal plant garden of A. R. College of Pharmacy, Vallabh Vidyanagar of Gujarat State in the month of October-November. The plant was authenticated by Dr. Bhanu Kakrani, Lecturer, Dept. of Botany, Tolani College of Arts and Science, Adipur (Kutch) and voucher specimen LMM/Ti-1/37/ARGH-11 was deposited to the Department of Pharmacognosy of A. R. College of Pharmacy. After Authentication of plant the aerial parts were collected from plant garden of A. R. College of Pharmacy, Vallabh Vidyanagar, Anand and dried under shade. The material was powdered to 60# separately and stored in airtight containers and used for further studies.

Pharmacognostical studies

Macroscopical determination of leaf and stem

Macroscopic evaluation of the fresh leaves and Stems of *Trichodesma indicum* was carried out for identification and the character were compared with the literature [2-6].

Microscopical determination and powder characteristics

For the preparation of transverse section of leaf and stem, Boiled T. S. of leaf and stem in test tube with chloral hydrate for several minute till completely clarified and then examined. After clarification put a drop of glycerin solution and mount the slide under the microscope in 10x and 45x objective lens. With another clarified piece of leaf and stem stained with phloroglucinol and concentrated HCl for 2 min and mount the slide for lignified tissue identification. Different cell components were observed and studied. The presence/absence of epidermal cells, covering trichomes, xylem, phloem, stomata and collenchyma were observed.

For surface preparation of the leaf, Small portion of leaf (2 mm square) was placed in chloral hydrate solution in test tube. Epidermis was exposed by scrapping of the tissues with sharp edge of razor on the glass slide. Water was added slowly and continuously scrapping was done till transparent. The portion was mounted in a mixture of equal portion of glycerin.

For powder preparation of leaves and stems, the aerial parts of *Trichodesma indicum* were dried under shade. They were powdered by grinding and passed through the sieve number 60. Finally, from this coarse powder, microscopical examination was done. Slides were prepared same as mentioned in above method [7, 8].

Quantitative microscopy

Quantitative microscopy including stomatal number, stomatal index, palisade ratio, vein-islet number, veinlet termination number was also determined using prescribed methods [7-10].

Proximate analysis

Proximate analysis aids to set up the certain standard for dried crude drugs in order to avoid batch-to-batch variation and also to judge their quality. Their studies also give an idea regarding the nature of phytoconstituents present. Proximate analysis of these crude drug powders was carried out using methods prescribed in the Ayurvedic Pharmacopoeia of India by subjecting them to various determinations like: Total Ash, Acid insoluble ash, Water soluble ash, Alcohol soluble extractive value, Water soluble extractive value, Moisture content, Swelling Index [7-9].

Fluorescence analysis

A small quantity of dried and finely powdered leaf was placed on a grease free clean microscopic slide and 1-2 drops of the freshly prepared reagent solutions were added and mixed by gentle tilting the slide and kept a side for 1-2 min. Then the slide was placed inside the UV chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations. The colors observed by application of different reagents at different wavelength were recorded [7-8].

Elemental analysis

The determination of elements comprising arsenic, lead, cadmium and trace elements (selenium and zinc) was carried out by subjecting the samples to inductively coupled plasma optical emission spectrophotometer (ICP-OES, Make: Perkin Elmer, USA, Model: optima-3300 rl).

For sample preparation 2 g of the powder aerial parts of *trichodesma indicumr. br.*, 7.5 ml of concentrated HNO_3 was added. It was then heated at 200 °c for 15 min. In microwave digestion, then cooled it. After cooling, made 100 ml of solution by de ionized water. This solution is used for elemental analysis by inductively coupled plasma optical emission spectrophotometer (ICP-OES, Make: Perkin Elmer, USA, Model: optima-3300 rl) and concentration of each element in the sample was calculated as the percentage of dry matter. These results are tabulated.

Preliminary phytochemical studies

Successive solvent extraction

10g of the air-dried powdered plant material was successively extracted using different solvents of increasing polarity in a soxhlet apparatus. All the extracts were concentrated and were dried in an oven at 50 °C. The consistency, color, appearance of the extracts and their percentage yield were noted and tabulated [11].

Qualitative chemical tests

The extracts were then subjected to various qualitative test using reported methods, to determine the presence of various phytoconstituents such as alkaloids, glycosides, flavonoids, carbohydrates, amino acids, saponins, sterols and terpenoids, cardiac glycosides, coumarins, carotenoids, tannins, phenolic compounds, fixed oils and fats etc [11-20].

RESULTS AND DISCUSSION

Pharmacognostical studies

Pharmacognostic evaluation is the first and foremost step to determine identity and to access the quality and purity of the crude drug. The selected plant is a crude drug therefore it was first subjected to pharmacognostic evaluation in reasonable details.

Macroscopic characteristics

The Macroscopic studies of leaves and stem are depicted in fig. 1. Leaves are green in color, tasteless odorless. The leaves were observed to be simple, sessile, with acuminate apex, entire margin, and pinnate venation. Surface of leaves are covered with prominent white tubercles reach with hairs. They are 3.8–10.0 cm long and 0.6–5.0 cm in width. The shape of leaf is ovate or oblong with cordate base. The stem are found to be green in color, light brown when dry surface, odourless, tasteless hairy surface, branches clothed with spreading trichomes. They are 50 cm long and 0.2 to 0.3 cm in width.

Fig. 1: Morphology of *T. indicum* R. Br.
(A) Aerial parts of *T. indicum* R. Br. (B) Macroscopy of leaf (C) Macroscopy of stem

Microscopic characteristics

Free hand transverse sections (T. S) of fresh leaf and stem of *Trichodesma indicum* were taken and studied for their histological characters.

Transverse section of leaf

As observed in fig. 2. A transverse section of leaf shows lamina and midrib portion. Lamina is isobilateral type, differentiated into palisade and spongy parenchyma (fig.3A). Upper and lower epidermis shows the presence of long unicellular or multicellular uniseriate covering trichomes with bulbous base (fig. 3B). Upper epidermis is the single layer, polygonal and covered with cuticle. Lower epidermis is similar to upper epidermis, has many stomata and more trichomes. The Palisade cells are single layered, compact with radially elongated cells, present up to the midrib. Below palisade cells 2 to 3 layer of spongy parenchymatous cells are

present having xylem vessels. In midrib portion collenchymas is well developed, which is present bellow the upper epidermis and above the lower epidermis (fig. 2). Rest of midrib is filled with cortical parenchyma. In the middle region presence of arc shaped vascular bundle and is present more towards the ventral surface (fig.2). Prism shaped calcium oxalate crystals are present.

Surface preparation of leaf

As observed in fig. 3C and fig. 3D in the surface view of the leaf, the lower epidermal cells are more wavy in outline, shows anomocytic and anisocytic stomata, simple covering unicellular or multicellular trichomes with bulbous base and upper epidermal cells are less wavy in outline, both type of stomata and trichomes are present.

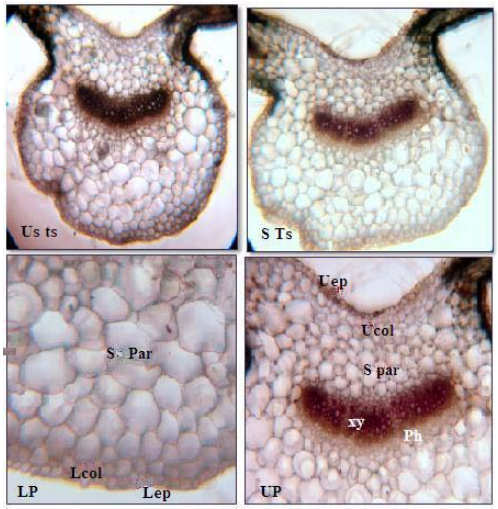

Fig. 2: Transverse section of leaf of *Trichodesma indicum* R. Br. US ts-Unstained T. S. of leaf [100x], S Ts-Stained T. S. of leaf [100x], LP-Lower portion of leaf [100x], S par-spongy parenchyma, Lcol-lower collenchymas, Lep-lower epidermis, UP-Upper portion of leaf showing Vascular bundle [100x], Uep-upper epidermis, Ucol-upper collenchymas, xy-xylem, Ph-phloem

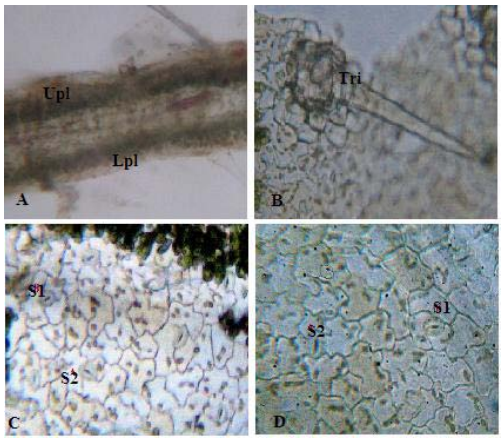

Fig. 3: Surface preparation of *T. indicum* R. Br (A) Lamina portion of leaf [100x], Upl-upper palisade layer, Lpl-lower palisade layer, (B) Trichome [100x], Tri-Trichome, (C) Upper surface preparation [100x], (D) Lower surface preparation [100x], S1-Anisocytic stomata, S2-Annomocytic stomata

Transverse section of stem

As observed in fig. 4. Transverse Section of stem of *Trichodesma indicum* is circular in outline having numerous long multicellular or unicellular covering trichomes. It is dicot stem. In outermost single layer epidermis is present. It is flattened tangentially and fitting closely along their radial walls with well defined cuticle extending over it. Hypodermis is the outer collenchymatous tissue forming a narrow zone of 2 to 3 layers of tangentially elongated or cubical to rectangular cells. General Cortex consists of 8 to 10 layers of parenchymatous cells. The vascular bundles are arranged in a ring. Phloem lies externally. Phloem is followed by lignified xylem. Metaxylem outer side to the cortex region and protoxylem inner side to the pith, so endarch type of vascular bundle. Inner to xylem big parenchymatous pith is present. Prism shaped calcium oxalate crystals are present in the cortex as well as in the pith region.

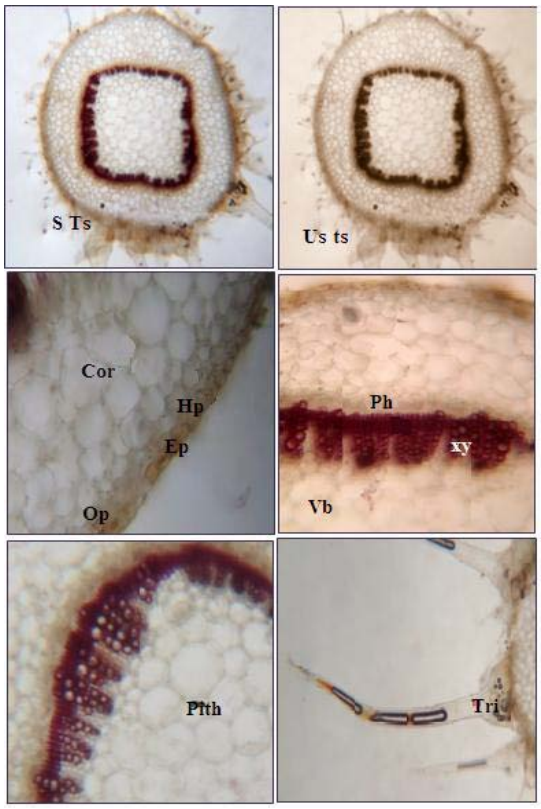

Fig. 4: Transverse section of stem of *Trichodesma indicum* R. Br S Ts-Stained T. S. of stem [100x], Us ts-Unstained T. S. of stem [100x], Op-Outer portion of stem, Ep-epidermis, Hp-hypodermis; Cor-cortex, Vb-Vascular bundle, Xy-xylem, Ph-phloem, Tri-trichome

Powder characteristics

The diagnostic characters revealed in the study of powder of aerial parts of *Trichodesma indicum* R. Br were Lignified spiral xylem vessel, Lignified pitted xylem vessel, Parenchymatous cells, Epidermal cells, Long unicellular uniseriate covering trichomes, Long multicellular uniseriate covering trichomes, Prism shape calcium oxalate crystals, Lignified fibre as in fig. 5.

Quantitative microscopy

Quantitative microscopy of leaf was done and stomatal number, stomatal index, veinislet number, the vein termination number, palisade ratio were determined. The results are given in table no.1. The surface parameter of leaf of Trichodesma indicum can be used to assess the purity and identification of the plant. From the above data, it can be concluded that a number of stomata is higher on the lower surface as compared to an upper surface.

Proximate analysis

Proximate analysis aids to set up certain standards for dried crude drugs in order to avoid batch to batch variation and to judge their quality. Their studies also give an idea regarding the nature of

phytoconstituents present in the crude drug. Different standardization parameters such as total solids content, moisture content, ash value, extractive values and swelling index were determined for aerial parts of *Trichodesma indicum* R. Br. and results are presented in table no.2. Ash values of the drug give an idea of earthy matter or the inorganic composition and other impurities presents along with the drug. Total ash values were high due to the presence of more silicaceous matter, calcium oxalate crystal and it suggests the presence of minerals in plants. Acid insoluble value indicated presence of silica especially sand and siliceous earth. Water soluble ash values indicate an amount of sugar and salt present. Extractive values are also useful to evaluate the chemical constituents present in the crude drug and help in estimation of specific constituents soluble in particular solvents. Alcohol soluble extractive value was high indicating presence of more alcohol soluble constituents. Moisture content of the drug was not too high, thus it could not encourage bacterial or fungal growth. Swelling index showed the presence of mucilage. The evaluated physicochemical parameters will be helpful in assessing the quality of the raw material for further research. This phytochemical analysis can be useful for detection of exhausted and adulterated drug.

Fluorescence analysis

In fluorescence analysis, behavior of the powder by treatment with different reagents can provide preliminary idea about the presence of various types of constituents present in the sample. The fluorescent properties of the powder of aerial parts of *Trichodesma indicum* R. Br. treated with several reagents were examined under ordinary light and U. V. light (254 nm and 365 nm). These results are presented in table no.3. The powder of *Trichodesma indicum* when treated with 5% $Fecl_3$ solution showed green and black color respectively indicating the presence of phenolic compounds. Treatment with other reagent showed different coloration or no change can serve as the simple tool for evaluation of the powdered samples of the crude drug under study.

Elemental analysis

Contamination of medicinal plants with arsenic and heavy metals can be attributed to many causes including environmental pollution and trace of pesticides. The element content of aerial parts of *Trichodesma indicum* R. Br. was determined by ICP-OES. It showed absence of heavy metals. The results were as summarized in table no.4.

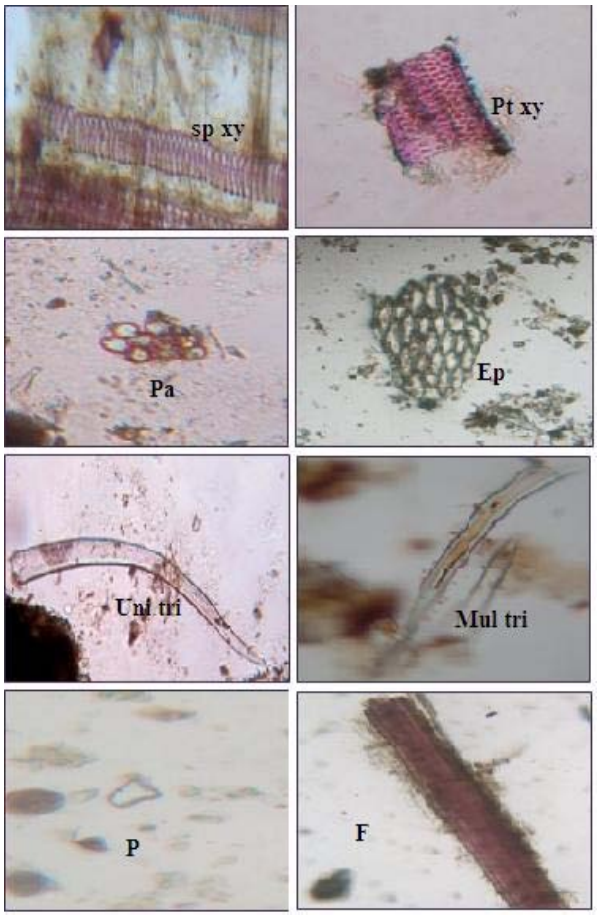

Fig. 5: Powder microscopy of aerial parts *T. indicum* R. Br. sp xy-Spiral xylem vessels, Pt xy-Pitted xylem vessels, Pa-Parenchymatous cells, Ep-Epidermal cells, Uni tri-Unicellular covering trichome, Mul tri-Multicellular covering trichome, P-Calcium oxalate prism, F-Lignified fibre

Table 1: Quantitative microscopy of Leaf

S. No.	Determination	Value per square mm
1.	Stomatal number	
	i) Upper surface	100
	ii) Lower surface	400
2.	Stomatal index	
	i) Upper surface	11.11
	ii) Lower surface	26.66
3.	Veinislet number	10.5
4.	Vein termination	4.5
5.	Palisade ratio	
	i) Upper surface	7.5
	ii) Lower surface	5.5

Preliminary phytochemical studies

Successive solvent extraction

Powder of aerial parts of *Trichodesma indicum* R. Br. were separately extracted with Petroleum ether (40°-60°), Toluene, Chloroform and Methanol in Soxhlet apparatus respectively. At the end drug was extracted with Water in vessel. The extract obtained were then dried completely and kept in vacuum desiccator. The results of colour, consistency and extractive value are presented in table no.5. Highest yield was found in water followed by methanol indicating presence of more polar constituents. The consistency of water extract was solid as compared to other extracts which are sticky.

Qualitative chemical evaluation of extracts obtained by successive solvent extraction

Preliminary qualitative chemical tests were performed on these extracts which showed that the aerial parts of *Trichodesma indicum* R. Br, is credited with phytosterol, triterpenoids, tannins, phenolic compound, carbohydrates, fixed oil, fatty acids and mucilage. The results are summarized in table no.6. The results and observations can provide evidences for establishing the botanical identity of the aerial parts and can serve as routine quality control tests for monitoring the quality of the aerial parts of *Trichodesma indicum* R. Br. These simple and reliable pharmacognostic standards will help the manufactures for identification and selection of the raw material for the production of drugs.

Table 2: Standardization parameters of aerial parts of *Trichodesma indicum*

S. No.	Determination	Percentage W/W
1.	Total Ash	20.05
2.	Acid insoluble Ash	6.65
3.	Water Soluble Ash	8.7
4.	Alcohol Soluble Extractive value	6.5
5.	Water soluble Extractive value	2.54
6.	Moisture content	5.5
7	Total solid content	94.5
8	Swelling Index	1.69

Table 3: Fluorescence analysis of powder of aerial parts of *Trichodesma indicum*

Reagent	Day light	UV 254	UV 365
1M NaOH in H_20	Light yellow	Green	Brownish yellow
1M NaOH in MeOH	Light green	Green	Green
1 M H_2SO_4	No color	Light bluish	No color
1 M HCl	No color	No color	Light blue
Picric acid	Yellow	Green	Yellow
10% KDichromate	Orange	Green	Light brown
Methanol	Dark Green	Green	Light red
50% HNO3	No color	No color	Purple
Dil. HNO_3	No color	No color	Purple
Dil NH3	Light Brown	Green	Dark Brown
Acetic acid	Green	Brown	Brown
5% $FeCl_3$	Brown	Green	Black
5% iodine	Yellow	Green	No color

Table 4: Elemental analysis of powdered aerial parts of *Trichodesma indicum*

Sample	Element	Wavelength	Instrument Detection mg/l	Sample Results (ppm) mg/kg
Plant Powder	Zinc (Zn)	206.200	0.0059	29.672
	Arsenic (As)	193.696	0.0530	Not detected
	Lead (Pb)	220.353	0.0420	Not detected
	Selenium(Se)	196.026	0.0750	Not detected
	Cadminum (Cd)	228.802	0.0027	0.1147

Table 5: Preliminary phytoprofile of aerial parts of *Trichodesma indicum* R. Br.

S. No.	Solvent	Color and consistency after drying	Average value(%w/w)
1.	Petroleum ether (60-80ºC)	Yellowish brown sticky mass	0.63
2.	Toluene	Dark green sticky mass	0.72
3.	Chloroform	Brownish black sticky mass	0.23
4.	Methanol	Green sticky mass	4.75
5.	Water	Dark Brown solid mass	7.49

Table 6: Qualitative evaluation of extracts obtained by successive solvent extraction.

S. No.	Tests of Phytoconstituents	P. ether extract	Toluene extract	Chloroform extract	Methanol extract	Water extract
1.	Alkaloids					
	a)Mayer's reagent	*	*	-ve	-ve	-ve
	b)Dragendroff's reagent	*	*	-ve	-ve	-ve
	c)Hager's reagent	*	*	-ve	-ve	-ve
	d)Wagner's reagent	*	*	-ve	-ve	-ve
2.	Test for flavonoids					
	a)Shinoda test	*	*	*	-ve	-ve
	b)Fluroscence test	*	*	*	-ve	-ve
3.	Tests for saponins					
	a)Froth test	*	*	*	-ve	-ve
4.	Test for Carbohydrate					
	a)Molisch's test	*	*	*	-ve	+ve
	b)Fehling's test	*	*	*	-ve	+ve
	c)Barfoed's test	*	*	*	-ve	+ve
	d)Benedict's test	*	*	*	-ve	+ve
	e)Test for gums	*	*	*	-ve	-ve
	f)Test for mucilage	*	*	*	-ve	+ve
5.	Test for Sterols and triterpenoids					
	a)Libermann-Buchard test	+ve	+ve	-ve	-ve	*

	b)Salkowski reaction	+ve	+ve	-ve	-ve	*
6.	Test for Tannins and phenolic compounds					
	a)Test with $Fecl_3$	*	*	-ve	+ve	+ve
	b)Test with Lead acetate	*	*	-ve	+ve	+ve
	c)Test with $KMnO_4$	*	*	-ve	+ve	+ve
	d)Test with Br_2 in water	*	*	-ve	+ve	+ve
7.	Tests for cardiac glycoside					
	a)Baljet's test	*	*	*	-ve	-ve
	b)Legal's test	*	*	*	-ve	-ve
	c)Keller killani test	*	*	*	-ve	-ve
8.	Tests for coumarins					
	a)with ammonia	*	*	*	-ve	-ve
	b)with Hydroxylammine hydrochloride	*	*	*	-ve	-ve
9.	Test for fixed oil					
	a) Spot test	+ve	-ve	*	*	*
	b) Saponification test	+ve	-ve	*	*	*

*: Not done-ve: Absent+ve: Present

CONCLUSION

As there is no pharmacognostical anatomical work on records for this traditionally much valued shrub, present work is taken up in the view to lay down the macroscopic, microscopic and Phytochemical standards, which could be used in deciding the genuineness of the herb, irrespective of their collection from different sources. The colored photographs of the above mentioned plant might facilitate the researcher for identification. The results of the phytochemical screening, histological tests can be considered as distinguishing parameters to identify and decide the authenticity of *T. indicum* and thus can be used as standards for reference purpose also. The outcome of the quantitative parameters described on the above-mentioned plant parts might be useful in determining the authenticity In future, the plant extract containing an active compound can be explored for detailed pharmacological activity and if the result are promising then, it can be exploited commercially.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Gupta R, Variable MG, Deshmukh RR, Chaudhary PR, Wate SR. Ethanomedicinal uses of some plants used by Gonel tribe of Bhandara district, Maharastra. Indian J Traditional Knowledge 2010;9:713-7.
2. Kirtikar KR, Basu BD. Indian medicinal plants. Vol. 3. Edn 2. Lalit Mohan Basu, Allahabad; 1975. p. 1672-73, 1691-5.
3. Dutta AC. Botany for degree students. Edn. 6. Oxford University Press, Calcutta; 1999. p. 558-60.
4. Shah GL. Flora of gujarat state. Edn 1. Sardar Patel University Press, Vallabh Vidyanagar; 1978. p. 455-7.
5. Chopra RN, Nayar SL, Chopra IC. Glossary of indian medicinal plants. Edn 1. National Institute of Science Communication, New Delhi, India; 1956. p. 247.
6. Chatterjee A, Prakashi SC. The treatise on indian medicinal plants. Vol. 4. National institution of science and communication and information resources; 2003. p. 216-7.
7. Khandelwal KR. Practical pharmacognosy techniques and experiments. Edn. 2. Nirali Prakashan, New Delhi; 2000. p. 51, 13-8.
8. Anonymous. Indian pharmacopoeia. Vol. 2. Ministry of Health and Family Welfare, Govt. of India, Controller of Publication: New Delhi; 1996. p. 53-4.
9. Mukharji PK. Quality control of herbal drugs. Edn 1. Business Horizons Pharmaceutical Publishers, New Delhi; 2002. p. 186-95.
10. Gupta AK. Quality standard of indian medicinal plant. Vol. 1. Indian Council of Medical Research, New Delhi; 2003. p. 236-7.
11. Kokate CK. Practical pharmacognosy. Edn 4. Vallabh Prakashan, Delhi; 1994. p. 148.
12. Sim Sk. Medicinal plant alkaloids. University of Toronto Press; 1969. p. 9.
13. Evans and Trease. Pharmacognosy. Edn 15. W. B. Saunders company Ltd; 2002. p. 193.
14. Gupta AK, Tandan N, Sharma M. Quality standard of indian medicinal plants. Vol. 3. Indian Council of Medical Research, New Delhi; 2005. p. 79-83.
15. Harborne JB. Phytochemical methods. Edn 2. Chapan and Hall Ltd, London; 1973. p. 42.
16. Feigle F. Identification of individual organic compound. In: Spot tests in organic analysis. Edn 4. Elsevier publishing company, London; 1956. p. 419-21.
17. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. Edn. 12. Nirali prakashan; 1999. p. 145-55.
18. Geissman A. Modern methods of plant analysis. Springer-Verlag Berlin Heidelberg. New York; 1955. p. 471.
19. Wagner H, Bladt S. Plant drug analysis, a thin layer chromatography atlas. Edn. 2. Springer-Verlag Berlin Heidelberg, New York; 1996. p. 342.
20. Stahl E. Thin layer chromatography,a laboratory hand book. Edn 2. Springer-Verlag Berlin Heidelberg, New York; 1969. p. 114.