INTERNATIONAL JOURNAL IN PHARMACEUTICAL SCIENCES

Journal Homepage: https://www.ijpsjournal.com

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Research Article

Pharmacognostical, Phytochemical Characterisation and Formulative Study of *Trichodesma indicum* R. Br

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ARTICLE INFO

Received: 06 Jul 2023
Accepted: 07 Jul 2023
Published: 12 Sept 2023

Keywords:
Trichodesma indicum, pharmacognostical studies, formulation

DOI:
10.5281/zenodo.8337516

ABSTRACT

The aim of the proposed study was to collect the useful information of the traditional medicinal plant Trichodesma indicum and upgrade the knowledge about the plant. It belongs to family Boraginaceae. Pharmacognostic studies of plant drug are carried out for the evaluation of drug and to detect the adulteration. It includes dermal characters like stomata, trichomes and anatomical features. Phytochemical studies of this plant shows presence of steroids, triterpenoids, tannins, flavonoids, saponins etc. In the formulative studies, cream has been prepared and shows with good washability, solubility and smooth greasy texture. Ethanobotanical studies shows various uses such as anti-inflammatory, antimitotic, antioxidant, anti-diabetic etc.

INTRODUCTION

The plant kingdom is the most important treasure of the nature. Mother earth has given vast

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Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

resources of flora and fauna both terrestrial and marine. The Indian subcontinent is enriched by a variety of aromatic and medicinal plants. This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional system of medicine.[1]

PHARMACOGNOSY

Pharmacognosy is defined as the study of crude drugs obtained from plants, animals and mineral kingdom and their constituents. The word Pharmacognosy is derived from two Latin words *pharmakon*, 'a drug' and *gignoso*, 'to acquire knowledge of'. It means 'knowledge or science of drugs. The word 'pharmacognosy' were probably first coined by Johann Adam Schmidt (1759-1809) in his manuscript *Materia Medica*, which was published in Vienna in 1811. It was C.A. Seydler, a medical student at Germany, in 1815 that created the term Pharmacognosy in his doctoral thesis *Analectia Pharmacognostica.*

In India medicinal properties of plants are described in Rigveda and in Atharvaveda (3500-1500B.C). The basic medicinal texts in this world region are Charaka Samhita, Susruta Samhita, Ashtanga Hrdayam Samhita.[2]

SCOPE

During the first half of the nineteenth century apothecaries stocked the crude drugs for the preparation of herbal tea mixtures, all kinds of tinctures, extracts and juices which in turn were employed in preparing medicinal drops, syrups, infusions, ointments and liniments. Then medicinal plants became one of its major objects of interest and in time.

The second half of the nineteenth century witnessed a galloping growth in the field of medicinal plants where by the Phytochemist's gainfully succeeded in isolation and characterization of the pure active constituents. Eventually, these active constituents replaced the crude drugs, with the development of semisynthetic and synthetic medicine. Thus, the fate of herbal drugs became more prominent and brighter gradually.Even up to the beginning of twentieth century pharmacognosy was a descriptive subject mainly to botanical science, now disciplines like organic chemistry, biochemistry, biosynthesis, pharmacology and modern methods are incorporated.

Today applied science of pharmacognosy has a better knowledge of the active constituents and the prominent therapeutic activity on the human beings. Researchers are exploiting not only the classical plants but also related species all over the world that may contain similar type of constituents. Just like terrestrial germplasm, investigators had also diverted their attention to marine flora and fauna, and wonderful execution of genetic engineering aspects and tissue culture which provides a step towards a genetically modified products and bio transformed natural products. Population rises, inadequate supply of drugs, prohibitive cost of treatments, side effects of several allopathic drugs and development of resistance to currently used drugs for infectious diseases have led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments.[3,4]

Lastly, crude drugs and their products are profitable commercial products, it is estimated that world market for plant drugs may account for about 2,00,000 crores. When these are collected from wild sources, the amount collected could only be small, the price commanded was exorbitantly high. Drug plants, standardised extracts and therapeutically active pure constituents have become a significant market commodity in the international trade. In the light of these glorious facts, scope of pharmacognosy seems to be enormous in the field of medicine, bulk drugs, food supplements, pharmaceutical aids, pesticides, dyes, tissue culture, biotechnology, engineering and so on.[4]

DRUG DISCOVERY FROM PLANTS

Green plants synthesis and preserve a variety of biochemical products, many of which are extractable and used as chemical feedstocks or as raw material or various scientific investigations leading to the discovery of drugs from plant. Current research in drug discovery from natural source includes numerous interdisciplinary fields and various methods of analysis including botanical, phytochemical, biological and molecular techniques.

Ethnobotanical approaches are the base for selecting the appropriate model for investigation. After that botanical identification and stabilization is done which is further subjected to extraction and isolation of constituents. Purity of isolated is obtained using modern chromatographic techniques, then it is subjected to characterization and confirmation of compounds. Bioassays are performed to find its therapeutic potential and toxicities, then subjected to development of formulations. Prepared formulations are standardized and subjected to various clinical and non- clinical examinations.[5]

FUTURE

Today, herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The blind dependence on synthesis is over and people are returning to the naturals with hope of safety and security. Plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered; now the large numbers of plants are constantly being screened for their possible pharmacological value. The use of modern isolation techniques and pharmacological testing procedures may find their way into medicine as purified substances rather than in the form of galenical preparations. An integrated approach for the cultivation, conservation and preservation of important plant species through plant molecular biology, plant tissue culture; research on the rationale and methodology of ayurvedic medical practice; isolation of active constituents and their development into new therapeutics; standardization and validation of known herbal medicines and other related aspects needed to be focused upon.

The future development of herbal drug industry and pharmacognosy would be largely dependent upon the reliable methodologies for identification of marker compounds and also upon the standardization and quality control. The extend of development will be based on pharmacognosists and Phyto chemists that is how they explore the wonder drug molecules from nature.[6,7]

PLANT INTRODUCTION

The Genus Trichodesma

Trichodesma R. Br. is a genus of about 45 species known from tropical and subtropical regions of Africa, Asia and Australia. Brown described *Trichodesma* in 1810. It belongs to the family Boraginaceae established by Jussieu. The group comprises predominantly perennial herbs, the flowers characterized by anthers with prolonged connectives, often twisting above the thecae, and a prominent accrescent calyx, the absence of fomices, anthers usually with fairly long, soft hairs on the back. The name *Trichodesma* is derived from the Greek words, *thrix* or *trikhos* (hair), *desme* (band or bundle). *Trichodesma* plants have been used in traditional medicines throughout the world to treat common diseases such as ear pain, intestinal worms, wounds, cough, fever, dysentery, and rheumatism.8, 9

Trichodesma indicum R. Br

Trichodesma indicum R. Br is generally known as Indian Borage and belongs to Boraginaceae family which is a major group of angiosperms. The plant is found as a weed throughout the greater parts of India and stony dry wastelands of Pakistan. Later it has been distributed in many south Asian region such as Bhutan and Burma. The plant is acrid and bitter in taste. It is an erect, spreading, branched

and annual herb, about 50 centimetres in height with hairs springing from tubercles.10,11

Fig no 1: *Trichodesma indicum*

VERNACULAR NAMES [12, 13]

Hindi	-	Chhotakalpa
Gujarati	-	Undhanphuli
Kannada	-	Kattetumesoppu
Tamil	-	Kalluthaithumbi
Telugu	-	Guvvagutti
Marati	-	Chotakalpa
Sanskrit	-	Adhapushpi
English	-	Indian borage

SYNONYMS

- *Boraginella indica* (*L.*) Kuntze.
- *Borago indica* L.
- *Borago spinulosa* Roxb.
- *Borraginoides sagittate* Moench.
- *Pollichia indica* (L.) Medic.
- *Trichodesma amplexicaule* Roth
- *Trichodesma hirsutum* Edgew.
- *Trichodesma perfoliatum* Wall.

SCIENTIFIC CLASSIFICATION [10]

Kingdom	-	Plantae
Phylum	-	Tracheophyta
Class	-	Magnoliopsida
Order	-	Boraginales
Family	-	Boraginaceae
Genus	-	*Trichodesma*
Species	-	*Trichodesma indicum*

BOTANICAL DESCRIPTION OF TRICHODESMA INDICUM[10,14]

Habitat	Altitude – 1500m Found throughout India on roadside and stony dry wasteland.
Habit	Erect, spreading, branched and annual herb.
Height	50 cm
Leaves	Stalkless, opposite, lanceolate, 2- 8cm long pointed at the tip and heart-shaped at the base.
Flowers	Flowers occurs singly in the axils of the leaves and usually violet, light blue or purple in colour. The calyx is green, hairy and 1- 1.3cm long with pointed sepals. The corolla is pale blue with limb about 1.5cm in diameter and the petals are pointed.
Fruits	Fruit is ellipsoid and is enclosed by the calyx. The nutlets are about 5mm long and rough on the inner surface.
Seeds	4 Nutlets
Fruiting season	Throughout the year
Flowering season	September – November and January – March

Table no 1: Botanical description of *Trichodesma indicum*

Fig no 2: Flower of *Trichodesma indicum*

Fig no 3: Flower bud of *Trichodesma indicum*

Fig no 4: Root of *Trichodesma indicum*

Fig no 5: Leaf of *Trichodesma indicum*

USAGE OF *TRICHODESMA INDICUM* PLANTS IN FOLK MEDICINE[11]

S.No	Plant part used	Uses	Place of use
1.	Roots	To reduce swelling. To cure body ache and anasarca.	Chota Nagpur, India. Kandhamal district, Orissa.
2.	Leaves	Healing of cuts, wounds, and bleeding.	Kandhamal district, Orissa, and various places in Tamil Nadu, India.
3.	Fresh leaves	To cure stomach upset and dysentery to children.	Tiruchirappalli, Tamil Nadu, India.
4.	Leaves and roots	To cure tumor, snake bite and urinary diseases.	Chattisgarh, India.

Table no 2: Usage of *Trichodesma indicum* in folk medicine

CHEMICAL CONSTITUENTS[11, 15]

The major chemical constituents of *Trichodesma indicum* are steroids, triterpinoidal saponins and flavonoids. Phytochemical investigation revealed the presence of phytosterols, tannins, sugars, flavonoids, protein, saponins and free amino acids.

S.No	Category of phytocompounds	Constituents present
1.	Terpenoids	Alpha-amyrin, lupeol
2.	Fatty acids and esters	Oleic acid, linoleic acid, palmitic acid, stearic acid, linolenic acid, n-decyl laurate, n-tetradecanyl laurate, n-nonacosanyl palmitate, stigmast-5-en-3β-ol-21(24)-olide, and lanast-5-en-3β-D-glucopyranosyl-21(24)-olide(18,19)
3.	Alkaloids	Monocrotalline, supinine (13-15)
4.	Aliphatic hydrocarbons and ketones	Hexacosane, n-pentacos-9-one, n-dotriacont-9-one-13-ene
5.	Steroidal compounds	Stigmast-5-en-3β-ol-23-one

Table no 3: Chemical constituents of *Trichodesma indicum*

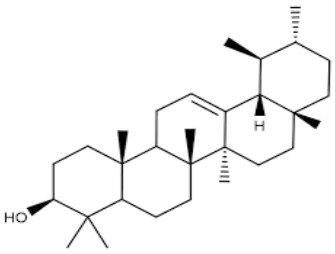


Fig no 6: Structure of α – amyrin

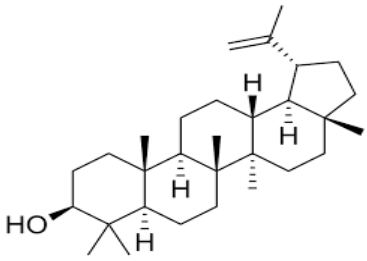
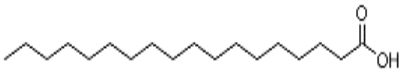


Fig no 7: Structure of Lupeol



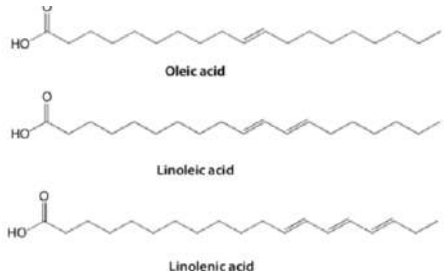


Fig no 8: Structure of stearic acid **Fig no 9: Structure of Oleic acid, Linoleic acid, Linolenic acid**

REVIEW OF LITERATURE

K. Srikanth*; et al*; in 2002 observed that the methanol extract of whole plants of *Trichodesma indicum* R.Br. has significant inhibition in frequency of sulphur dioxide induced cough reflux in Swiss albino mice. It is effective in all the tested doses when compared with untreated control group.

James B. Perianayagam; *et al;* in 2006 studied that the chloroform extract of *Trichodesma indicum* has been evaluated for anti- inflammatory activity against oedema produced carrageenan, dextran, histamine and serotonin, and against formation of granulation tissues by cotton pellet in rats. The effect was compared with the activity of indomethacin, cyperoheptadine and dexamethasone against different types of inflammation.

K. Ravi Shankar; *et al*; in 2008 subjected various extract of the plant *Trichodesma indicum* to preliminary phytochemical screening and it was shown that flavonoids, triterpenes, tannins, saponins were present. Flavonoids and triterpenes were present in alcoholic extract, tannins and saponins in aqueous extract, steroids and saponins in petroleum ether and chloroform extract and these were confirmed by thin layer chromatographic study.

Neelambra Verma; *et al;* in 2008 found that *Trichodesma indicum* of family- Boraginaceae is a cross pollinated species. Its complete regeneration was accomplished through in vitro techniques. The zygotic embryos placed on Murashige and Skoog medium fortified either with kinetin, n- benzyl aminopurine or alpha naphthalene acetic acid produced callus and adventitious shoots, whereas those placed on medium supplemented with 2,4 dichlorophenoxy acetic acid formed callus. On subculture, the nodal pieces produced axillary shoots and that were suitable for further proliferation.

Shweta S. Saboo; *et al;* in 2009 found that *Trichodesma indicum* has been used for its therapeutic effect in folk medicine that include anti-inflammatory, analgesic and anticancer properties. In this work the shoot extract of Trichodesma indicum are screened for their antimitotic and antiproliferative activities.

James B. Perianayagam; *et al;* in 2011 evaluated the ethanol extract of *Trichodesma indicum* for possible analgesic and antipyretic potential using several experimental models and the ethanol extract at doses of 100, 200 and 400 mg/kg exhibited in significant inhibition of acetic acid induced abdominal constrictions in mouse. The extract also produce a dose related fall in rectal temperature in rat for up to 4 hrs after its administration in a dose dependent manner and the efficacy was similar to that of aspirin.

Sudharshan Reddy Dachani; *et al;* in 2012 done study to provide *in-vitro* evidences for antioxidant and antidiabetic potential of *Trichodesma indicum* and to generate a stronger biochemical rationale for further investigation animal models and support traditional claim.

Vanitha A; *et al;* in 2015 studied the phytochemical and anatomical structure of *Trichodesma indicum R.Br.* In this study the macroscopic and microscopic characters were used to establish botanical identity of the herbal drug. The methanolic extract showed the presence of secondary metabolites like flavonoids, alkaloids, steroidal compounds, saponins, tannins and phenolic compounds. Aqueous extract showed the presence of flavonoids, alkaloids, steroids, saponins and tannins.

K. Narendra; *et al*; in 2015 screened four extracts (HETI, ACTI, METI and AQTI) of *Trichodesma indicum* and invitro anti-inflammatory enzymatic assay showed significant inhibition against lipoxygenase. *In-vivo* and inflammatory activity was determined by carrageenan induced rat paw oedema method in experimental rats. The findings of studies demonstrated both *in-vitro* and *in-vivo* anti-inflammatory activity of the leaves of *Trichodesma indicum.*

P. L. Rajagopal; *et al;* in 2016 studied the phytochemical screening and *in-vitro* anti-inflammatory activity of the alcoholic and aqueous extracts of the flowers of *Trichodesma indicum.* The phytochemical screening revealed the presence of flavonoids, terpenoids and steroids.

K. Narendra; *et al;* in 2016 investigated the antidiabetic activity of *Trichodesma indicum* in both *in-vitro* amylase assay and *in-vivo* streptozotocinnicotinamide induced type 2 diabetic rats. *Trichodesma indicum* leaves where extracted with four solvents hexane, acetone, methanol and aqueous. The results showed that methanolic leaf extract has moderate α- amylase inhibitory activity when compared to acarbose. The antidiabetic activity of four extracts prominently reduces blood glucose level in streptozotocinnicotinamide induced diabetic rats. Methanol extract has shown estimable decrease of blood glucose level along with glibenclamide. These findings suggest that antidiabetic property of *Trichodesma indicum* methanol extract in type 2 diabetic mellitus is potential.

AIM AND PLAN OF WORK

AIM: We have selected the plant *Trichodesma indicum* and we are planned to study the pharmacognostical, phytochemical characterisation and along with this we have planned to conduct formulative study on *Trichodesma indicum*.

PLAN OF WORK

- Pharmacognostical study
  - ★ Morphological evaluation of fresh leaf
  - ★ Microscopical studies of fresh leaf
  - ★ Macroscopical studies of dry powder
- Phytochemical characterization
  - ★ Extraction
  - ★ Preliminary phytochemical screening
  - ★ Thin layer chromatography
- Formulation and evaluation of *Trichodesma indicum* cream
  - ★ Formulation of cream
  - ★ Evaluation of cream

MATERIALS AND METHODS

I. PHARMACOGNOSTICAL STUDY

★ Morphological evaluation of fresh leaf [16,17]

Collection and authentication of specimen

The plant specimen for the study was collected from Coimbatore, Tamil Nadu. The collected plant was examined and authenticated by Dr. S. Sukumaran, Ph.D, Assistant Professor Department of Botany and Research Centre and Dr. N. Maybel Starlin M.sc, Ph.D, Associate Professor, Head, PG Department of Botany, Nesamony Memorial Christian College, Marthandam, Tamil Nadu.

Morphological characters

The morphological characters like colour, odour, taste, size, shape, extra features of the leaves were studied by using sensory characters.

★ Microscopical studies of fresh leaves[17,18,19]

The sections of leaf specimens were taken using sharp blade and stained with various staining

reagent like phloroglucinol- HCl and observed under microscope.

★ **Macroscopical studies of dry powder[20]**

Preparation of powder

The collected leaves of *Trichodesma indicum* were washed with running tap water. Then the leaves were dried under shade. The leaves were pulverized into coarse powder. Coarse powder (passed through sieve no. 18 and retained on sieve no.60) was used for phytochemical studies.

II. PHYTOCHEMICAL CHARACTERIZATION

★ **Extraction**

The process of separating active principles from powdered crude drugs by using suitable solvents is called extraction. The choice of solvent depends upon the characteristics of secondary metabolites like polarity, pH and thermal stability.

Preparation of extracts[18,19,20]

In the present study 100g of coarsely powdered plant of *Trichodesma indicum* was weighed and boiled with 2000ml water. Then the marc is pressed and dried at a temperature not exceeding 50ºC. The filtrate was weighed and calculated the percentage yield in terms of air-dried material.

Fig no 10: Extraction process of *Trichodesma indicum*

★ **PRELIMINARY PHYTOCHEMICAL SCREENING [17,18,19]**

Preliminary phytochemical screening was done to identify different constituents present in extracts i.e., carbohydrates, proteins, lipids, flavonoid, tannins, glycosides, alkaloids, essential oils etc. Aqueous extracts of *Trichodesma indicum* leaves subjected to preliminary phytochemical screening.

i. Detection of alkaloids

a) Mayer's test: 2 ml of the extract was treated with 2 ml of Mayer's reagent.
b) Dragendorff's test: 2ml of the extract was treated with 2 ml of Dragendorff's reagent.
c) Hager's test: 2 ml of the filtrate was treated with 1-2 ml of Hager's reagent.
d) Wagner's test: 2ml of the filtrate was treated with 1-2 ml of Wagner's reagent.
e) Tannic acid test: 2ml of the extract was treated with 2 ml of tannic acid solution.

ii. Detection of carbohydrates

a) Molisch's test: 1ml of the test solution was fixed with 2 ml of Molisch reagent, shaken the mixture and added 1 ml of concentrated sulphuric acid along with the sides of the test tube.
b) Benedict's test: Mixed 2 ml of the Benedict's reagent with 2 ml of the test solution. Boiled in a water bath.
c) Fehling's test: Boiled 1 ml of the test solution with 1 ml Fehling's solution A and 1ml of Fehling's solution B on a water bath.
d) Barfoed's test: Mix 2 ml of the Barfoed's reagent with 1 ml of the test solution and boiled in a water bath.
e) Iodine test: Mix 0.5 ml of iodine solution with 1ml of test solution.
f) Seliwanoff's test: Boil 2 ml of seliwanoff's reagent with 1 ml of test solution.

iii. Detection of proteins and amino acids

a) Biuret's test: About 2ml of extract was mixed with 2 ml Biuret reagent.
b) Millon's test: 2 ml of the extract was mixed with 2 ml of Millon's reagent and boiled.
c) Xanthoprotein test: 2 ml of the extract was treated with 1 ml of concentrated Nitric acid and Sulphuric acid. Cooled the solution and made alkaline with 10% sodium hydroxide.

d) Ninhydrin test: boiled 2 ml of the extract with 1 ml of 5% ninhydrin solution in a water bath for 5 minutes.

iv. Detection of glycosides

a) Borntrager's test: To a little quantity of sample solution add Sulphuric acid and Carbon tetrachloride. Separate the organic layer and shake with dilute Ammonia.

b) Modified borntrager's test (modified anthraquinone test for C- glycosides): To little quantity of sample solution add Ferric chloride solution, Hydrochloric acid and Carbon tetrachloride. Separate the organic layer and shake with dilute ammonia.

c) Legal test: Mix 1 ml of the test solution with 2 ml of Pyridine and Sodium nitroprusside.

v. Detection of tannins and phenolic compounds

a) Ferric chloride test: Mix 2 ml of the test solution with few ml of 5% Ferric chloride solution.

b) Lead acetate test: Mix 2 ml of test solution with 1 ml of Lead acetate solution.

c) Dilute iodine test: Mix 2 ml of the test solution with dilute iodine solution.

d) Potassium dichromate test: Mix 2 ml of the test solution with potassium dichromate solution.

e) Dilute nitric acid test: Mix 2 ml of test solution with dilute nitric acid.

vi. Detection of flavonoids

a) Shinoda test: To 2 ml of the sample solution add magnesium powder or zinc powder and few drops of concentrated hydrochloric acid or sulphuric acid.

b) Sulphuric acid test: Add few drops of concentrated sulphuric acid to few ml of sample solution.

c) Lead acetate test: Mix 2 ml of the test solution with lead acetate solution.

d) Alkali test: Treated the test solution with increasing amount of sodium hydroxide.

vii. Detection of steroids and triterpenoids

a) Liebermann test: Mix 2 ml of the test solution 2 ml of acetic anhydride and boiled. Then add 0.5 ml of concentrated sulphuric acid.

b) Liebermann- Buchard test: Mix 2 ml of the test solution with 1 ml of Chloroform and 1 ml acetic anhydride, then add 1 drop of concentrated sulphuric acid.

c) Salkowski test: Dissolve 1- 2 mg of sample in 1 ml of chloroform and add 1 ml of concentrated sulphuric acid.

viii. Detection of saponins

a) Foam test: Shaken few mg of extract with 20 ml of distilled water.

ix. Detection of mucilage

a) Ruthenium red test: Treated the powder with ruthenium red.

b) Swelling test: Dissolved the powder in water

★ THIN LAYER CHROMATOGRAPHY[17,21,22,23]

Thin layer chromatography is a solid- liquid technique which works on the principle that, different compounds have different affinities and the compounds will separate based on their affinities towards stationary phase and mobile phase. Plant extracts contains various types of bioactive compounds having different polarities. Their separation can be easily done using Thin Layer Chromatography (TLC). In the present investigation, the qualitative TLC parameters for aqueous extract of *Trichodesma indicum* are studied.

Basic steps involved in TLC

- Adsorbent selection and plate preparation
- Solvent selection
- Saturation of chromatographic chamber with solvent
- Sample application as spots or bands over the chromatographic plate.
- Running the chromatogram
- Detection

- Qualitative and quantitative analysis

Procedure

TLC plates were prepared by using silica gel G and the coated plates were allowed to dry in air and activated by heating in hot air oven at 105ºC for 1 hour. The extracts were dissolved in respective solvents and in TLC chamber previously saturated with different solvent systems. By trial-and-error method appropriate solvent system were developed and different spots are developed. The coloured substances were visual on the chromatogram. Colourless components were detected using visualizing agent, UV light and spray reagent and R_f values were calculated.

R_f value= $\frac{Distane\ travelled\ by\ the\ solute}{Distance\ travelled\ by\ solvent}$

FORMULATION AND EVALUATION OF *TRICHODESMA INDICUM* CREAM[24,25]

Procedure

★ Take the liquid paraffin and beeswax in a chinadish and heat at 70°C and maintain that heating temperatures (oil phase).

★ In other beaker, dissolve borax and distilled water by maintaining temperatures 70°C with water bath. Then immediately add *Trichodesma indicum* extract into it with continuous mixing.

★ Then gently add heated aqueous phase in heated oily phase with continue stirring using glass rod until it forms a smooth cream. When cream is formed, add perfume (Rose oil) as fragrance.

LIST OF INGREDIENTS FOR THE FORMULATION OF CREA

S.No.	Ingredients	Quantity Required
1.	*Trichodesma indicum* extract	0.5/ 1/ 2g
2.	Beeswax	5g
3.	Liquid Paraffin	15ml
4.	Borax	0.25g
5.	Perfume	q. s

Table no 4: List of ingredient for the formulation of cream

EVALUATION OF CREAM

1) Morphological evaluation

- Physical properties[26]: The cream was observed for the colour, odour, appearance, foreign particles and grittiness.

2) Physicochemical evaluation

- Washability[27]: The ease of removal of the cream applied was examined by washing the applied part with tap water and the ease with which the washing of the cream was observed.
- pH of the cream[28]: The pH meter should be calibrated using standard buffer solution. About 0.5g of the cream was taken and dissolved in 50.0ml of distilled water then pH was measured using pH meter.
- Irritancy test[29,30]: An area (1 sq. cm) on the left hand dorsal surface was used for this purpose. The cream was applied to the specified area and time was noted. Irritancy, erythema, edema, was checked if any for regular intervals up to 24hr.
- Phase Separation[31,32]: The prepared cream was transferred in a suitable wide mouth container. Set aside for storage the oil phase and aqueous phase separation were visualizing after 24 hours.

RESULT

QUALITY CONTROL

I. MORPHOLOGICAL EVALUATION OF FRESH LEAF.

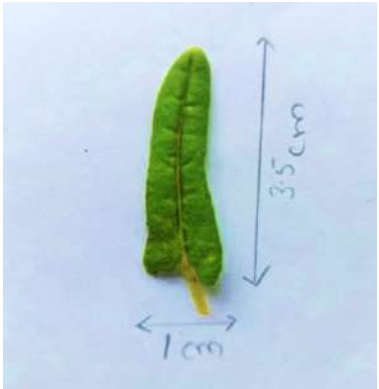


Fig no 11: Leaf of *Trichodesma indicum*

SI. No	FEATURES	OBSERVATION
1	Colour	Green
2	Odour	Specific
3	Taste	Bitter
4	Shape	Oblong- lanceolate
5	Apex	Acute
6	Base	Acute- auriculate
7	Venation	Parallel
8	Margin	Entire

Table no 5: Morphological features of fresh leaf

II. MICROSCOPICAL STUDIES OF FRESH LEAVES.

The transverse section of the leaf was taken through the midrib and it was mounted on a slide stained with phloroglucinol and HCl then it was observed under 10X objective microscope then the following tissues were observed.

- The T. S of leaf show upper and lower epidermis with thin cuticle. On both the epidermis trichomes and stomata are present.
- Mesophyll is differentiated into palisade parenchyma and spongy parenchyma.
- The palisade parenchyma are two layered, and cells are compactly arranged and elongated.
- Spongy parenchyma is two to three layered, cells are loosely arranged with large intercellular spaces.
- In the middle region arc shaped vascular bundle.
- The stomata of both the surfaces are anisocytic, the guard cells are surrounded by three subsidiaries. Number of stomata is more on lower surface of leaf.
- The trichomes are present on upper and lower leaf surface. The trichomes on upper surface are more than lower. The trichomes on upper surface is unicellular filliform and on lower surface unicellular and conical. The trichomes are longer on the upper surface.

Fig no 12: T.S of *Trichodesma indicum* leaf

Fig no 13: T.S of trichome

III. Macroscopical studies of dry powder.

The dry powder was characterized by its morphological features like green colour, presence of specific odour and bitter taste.

Fig no 14: Dry powder of *Trichodesma indicum*

- **Extraction**

SI.No	Extract	Colour	Nature	Percentage yield
1.	Aqueous	Brown	Powder	92%

Table no 6: Colour, nature, and percentage yield of aqueous extract of *Trichodesma indicum*

- **Preliminary photochemical screening**

SI. No	Qualitative tests	Result
1.	**Test for alkaloids**	
	Mayer's test	-
	Dragendorff's test	-
	Hager's test	-
	Wagner's test	-
	Tannic acid test	-
2.	**Test for carbohydrates**	
	Molisch's test	-
	Fehling's test	-
	Benedict's test	-
	Barfoed's test	-
	Iodine test	-
	Seliwanoff's test	-
3.	**Test for protein and amino acids**	
	Biuret test	-
	Millon's test	-
	Ninhydrin test	-
	Xanthoprotein test	-
4.	**Tests for steroids and triterpenoids**	
	Liebermann test	+
	Libermann- Burchard	+
	Salkowski test	+
5.	**Tests for steroids**	
	Borntrager's test	-
	Modified Borntrager's test	-
	Legal test	-
	Baljet test	-
6.	**Test for Tannins**	
	Ferric chloride test Leas acetate test	+
	Potassium dichromate test	+
	Dil. HNO_3 test	+
	Dil. Iodine test	+
	Bromine water test	+
7.	**Tests for flavonoids**	
	Shinoda test	+
	Sulphuric acid test	+
	Lead acetate test	+
	Alkali test	+
8.	**Test for saponins**	
	Foam test	+
9.	**Test for mucilage**	
	Ruthenium red test	+
	Swelling test	+
10.	**Test for fixed oil and fats**	
	Filter paper test	-

Table no 7: Phytochemical screening test

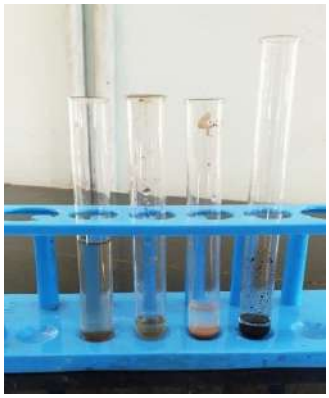


Fig no 15: Phytochemical tests of aqueous extract

- **Thin layer chromatography**

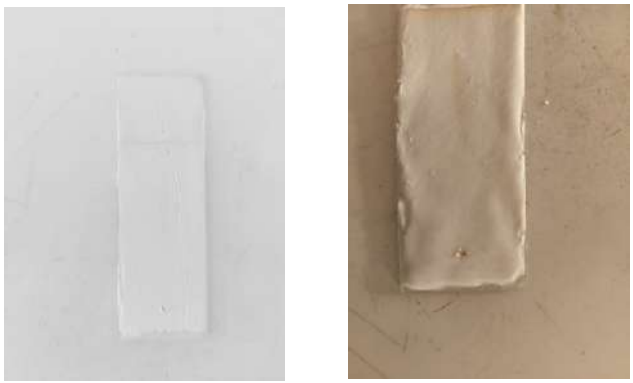


Fig no 16: TLC plates of aqueous extract of *Trichodesma indicum*

SI. No	Extract	Solvent system	No. Of spots	Colour of spots	Rf value
1.	Aqueous	Ethyl acetate: Glacial acetic acid: Formic acid: Water (6.6: 0.8: 0.8: 1.8)	2	Brown	0.75
				Brown	0.87

Table no 8: Thin Layer Chromatography of aqueous extract of *Tricodesma indicum*

- **Formulation study**

- **Formulation of medicated cream using aqueous extract of *Trichodesma indicum* at various concentration**

0.5% cream **1% cream** **2% cream**

Fig no 17: Cream of aqueous extract of *Trichodesma indicum* at various concentration

- **Evaluation of medicated cream using aqueous extract of *Trichodesma indicum* at various concentrations.**

Parameters	Observation		
	Creams of aqueous extract of *Trichodesma indicum*		
	0.5% cream	1% cream	2% cream
Colour	White	Light brown	Dark brown
Odour	Characteristic odour for all		
Consistency	Smooth, homogenous, non- greasy		
Foreign particles	Free from foreign particles and grittiness		
pH	6.7± 0.0003	6.71± 0.0003	6.8± 0.0003
	Good for skin pH		
Solubility	Soluble in boiling water		
Washability	Good		
Skin irritation	No skin irritation, redness, dryness		

Table no 9: Evaluation of Physiochemical parameters

DISCUSSION

Macroscopic evaluation

The leaves of *Trichodesma indicum* were observed to be fresh young leaves, simple green with specific odour and bitter taste. It has a Oblong-lanceolate and parallel venation.

Phytochemical screening

The results of phytochemical screening of aqueous extract of leaves mainly reveal the presence of tannins, flavonoids, triterpenoids and saponins.

Thin layer chromatography

The Thin layer chromatographic studies proved the presence of flavanoids, tannins and saponins. The Rf value was calculated to determine its identity, purity and strength.

Formulative study

The cream formulated using *Trichodesma indicum* has known to exhibit good solubility, washability and having no skin irritation and also shows without any foreign particles. A smooth greasy cream has been formulated with various concentrations (0.5%, 1%, 2%).

CONCLUSION

The study of *Trichodesma indicum* plant revealed that this plant is used traditionally in the treatment of various diseases. The presence of certain phytochemicals shows medicinal properties such as alkaloids, glycosides, flavonoids, saponins, steroid and cardiac glycosides. Ethanobotanical studies shows that various parts of this plant are useful for anti-inflammatory, anti-pyretic, anti-diabetic, antioxidant, antimitotic, analgesic activity. Monocrotalline, supinine (13-15) are some alkaloids and Alpha-amyrin, lupeol are some terpenoids that are mostly found in this plant.The morphological studies of fresh leaves shows green colour, bitter taste, oblong shape, specific odour. The microscopical studies of fresh leaves shows that the trichomes re present in the upper and lower leaf surface, stomata of both sides are anisocytic, spongy parenchyma is two to three layered. The macroscopical studies of dry powder shows green colour, specific odour and bitter taste.

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HOW TO CITE: Abish Mobin M., Deepthi Dileep, S. Jasbin Nisha*, Thomas R., Daniel Xavier Prasad, Surabhi G. S., Prasobh G. R., Pharmacognostical, Phytochemical Characterisation and Formulative Study of *Trichodesma indicum* R. Br, Int. J. in Pharm. Sci., 2023, Vol 1, Issue 9, 241-256. https://doi.org/10.5281/zenodo.8337516