PHARMACOGNOSTICAL STANDARDIZATION AND PHYSICOCHEMICAL EVALUATION ON THE LEAF OF *CLITORIA TERNATEA* LINN.(FABACEAE) - RESEARCH ARTICLE

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ABSTRACT

Clitoria ternatea Linn (Fabaceae) a very common garden flowering plant is found all over the india. The folklore claims the use of the wholeplant for curing various ailments.The aim of the present work to study the Pharmacognostic and physicochemical evaluation undertaken on the leaves of *Clitoria ternatea* Linn.The Pharmacognostical study involves the following parameters like macroscopic,microscopic studies ,histochemical analysis,powder microscopy and physicochemical analysis.The present study also investigates the physicochemical parameters by using standard methods .In this study these observations will help in the Pharmacognostical identificational and standardisation of the drug in the crude form and also to distinguish the drug from its adulteration.

KEYWORDS : *Clitoria ternatea*,Pharmacognostical,Physicochemical,Standardization

INTRODUCTION

Clitoria ternatea L (Fabaceae) a indigenous herb ,commonly known as Aparajita and Butterfly pea.It is a twinning herb used for curing various diseases and symptoms.Traditional use for this plant are longstanding.Numerous pharmacological properties were demonstrated by this plant, includingthosethatwereantibacterial,antioxidant,anticancer,hypolipidemic,cardiovascular,neurological,antiinflammatory,analgesic,antipyreticandimmunesystem.Pharmacognostic evaluation is the first and foremost step to determine identity and to evaluate the quality and purity of the crude drug.Physico chemical evaluation is the ability to elicit a pharmacological or therapeutic effect is related to the influence of various physical and chemical properties of the chemical substance on the biomolecule that it interacts.So the objective of present study was to perform Pharmacognostic investigation and Physicochemical evaluation on *Clitoria ternatea* leaves.

MATERIALS AND METHOD

1.1. PROCUREMENT OF MATERIALS

The mature leaves of *Clitoria ternatea* Linn were collected from Orchard botanical garden,pattambi,kerala. .The plant specimen was authenticated by Dr Ranjusha A P, Department of Botany,NSS College ,ottapalam .The fresh leaflets were used for macroscopic and microscopic studies.The leaflets were separated and dried in shade .The dried leaflets were made in to moderately coarse powder for further analysis and stored in airtight container

1.2. PHARMACOGNOSTICAL STUDIES

1.2.1. *Macroscopic evaluation*

Organoleptic evaluation can be done by means of organs of sense. This refers to the evaluation of crude drug by color, odour, size, shape, taste; special features including touch, texture etc and leaf structure like margin, apex, base surface etc. The macroscopic study is the morphological description of the plant parts which are seen by naked eye or magnifying lens.

1.2.2. *Histochemical analysis*

The section of the fresh leaves were taken and stained with respective reagents to localize components ,viz, tannins,starch,aleurone grains,lignified cells and oil gobules.

Stains and Reagents

1) *Ferric chloride*

For examining the presence of **tannin**.Dissolve 5 g of ferric chloride in 100 ml of water

2) *Iodine solution*

For identifying **starch** and **aleurone grains** in the section or powder.Solution is made by dissolving 2.6 g of Iodine and 3 g of potassium iodide in 100 ml water

3) *Hydrochloric acid (Hcl)*

Used for moistening the section or powder with phloroglucinol.It is examined **lignified cells**

4) *Sudan Red*

For examining the presence of **oil globules**.It is prepared by dissolving 0.5 g of Sudan Red in 100 ml glacial acetic acid AR.

1.2.3. *Microscopic evaluation*

Stains and Reagents

Saffranin : Dissolve 1 gm Saffranin in 100 ml distilled water

Glycerol: Mix equal amount of glycerol and distilled water

Microscope

Leica DM 1000 LED.Trinocular 'Leica' microscope attached with 'Leica DFC 295' digital camera connected to the computer and Leica Application software LAS Version 3.6.1

Microtome

Plant Microtome,Automatic MT3

Take microtome sections of the materials. Select thin sections, stain with Phloroglucinol, mount in glycerine, observe through microscope and transfer the images to computer.

1.2.4. *Powder microscopy*

A small quantity of the powder was treated with staining solution mounted in glycerine and observed under microscope, transferred the images of powder characters to the computer using the computer controlled microscopic system and camera.

1.2.5. *Determination of Physico-chemical constants*

1.2.5.1. *Moisture content*

5g of the powdered leaves was placed in tarred evaporating dish. Drying was carried out at 105˚C for five hours. The drying was continued with intermittent weighing at half an hour interval until difference between two successive weighing was not more than 0.01gm difference.

$$\mathbf{Moisturecontent} = \frac{freshweight - dryweight}{freshweight} \times \mathbf{100}$$

1.2.5.2. ***Ash value***

The in-organic content remaining after incinerating a crude drug is called as ash content. An ash value implies the naturally inherent inorganic salts or those imparted from external sources. Official ash values are chiefly used for quality confirmation of powdered drugs. Determination of ash value helps in knowing absence of mineral matter that is accidentally introduced from earth, sand, floor sweepings, absence of other parts of plant, absence of adulterated and exhausted drug, and absence of materials that possess with stone cells, starch which modify the value. Total ash value is used for ensuring quality of drugs which possess with little calcium oxalate. Acid-insoluble ash is referred if calcium oxalate present is high. Treatment of ash with hydrochloric acid leaves only silica which is imparted from soil and hence acid-insoluble ash is preferred. Water soluble ash is the difference between total ash and water insoluble residue. Sulphated ash is usually used for un-organized drugs to control non-volatile inorganic impurities. In the test, sulphuric acid is used to decompose and oxidize organic matter resulting only with sulphate salt of cations.

a. Total ash

Two grams of ground air dried material were accurately weighed out in a crucible previously ignited for 30 minutes. The material was spread in an even layer and ignited at a temperature more than 450˚C until it was indicating the absence of carbon, cooled in the desiccators and weighed. Calculated the content of total ash per gram of air dried material.

b. Acid insoluble ash

The total ash obtained was boiled with 25 ml of 2 M HCl for 5 minutes. The insoluble matter was collected on an ash less filter paper (Whatman- 41), washed with hot water. Transferred the filter paper containing insoluble matter to the original crucible, ignited, weighed and calculated the percentage acid-insoluble ash with reference to the air-dried drug.

c. Water soluble ash

To the crucible containing the total ash, 25 ml each of water was added and boiled for 5 minutes. The insoluble matter was collected in sintered glass crucibles. Washed with hot water and ignited in a crucible for minutes at a temperature not exceeding 450˚C. The weight of that

residue in mg was subtracted from the weight of total ash. The content of water soluble ash was calculated per gram of air dried material.

d. Sulphated ash

To the crucible containing the total ash, a few drops of sulphuric acid was added again and ignited as before, cooled and weighed to get a constant weight. Then calculated the percentage of sulphated ash with reference with reference to the air dried drug.

1.2.5.3. *Extractive values*

Extractive values are used for evaluation of crude drugs when they cannot be estimated by any other method. Extractive values by different solvents are used to assess quality, purity and to detect adulteration due to exhausted and incorrectly processed drugs. And gives an idea about the nature of the chemical constituents present in the crude drug. Useful for the estimation of constituents extracted with the solvent used for extraction. Employed for material for which as yet no suitable chemical or biological assay exists.

a. Petroleum ether soluble extractive value

Macerated 5 grams of coarsely powdered air dried plant with 100 ml of pet. ether in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

b. n- Hexane soluble extractive value

Macerated 5 grams of coarsely powdered air dried plant with 100 ml of n-Hexane in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

c. Benzene soluble extractive value

Macerated 5 grams of coarsely powdered air dried plant with 100 ml of benzene in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

d. Chloroform soluble extractive value

Macerated 5 grams of coarsely powdered air dried plant with 100 ml of chloroform in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

e. Ethyl acetate extractive value

Macerated 5 grams of coarsely powdered air dried plant with 100 ml of ethyl acetate in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

f. Acetone extractive value

Macerated 5 grams of coarsely powdered air dried plant with 100 ml of acetone in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

g. Water soluble extractive value

Macerated 5 grams of coarsely powdered air dried plant with 100 ml of water in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand

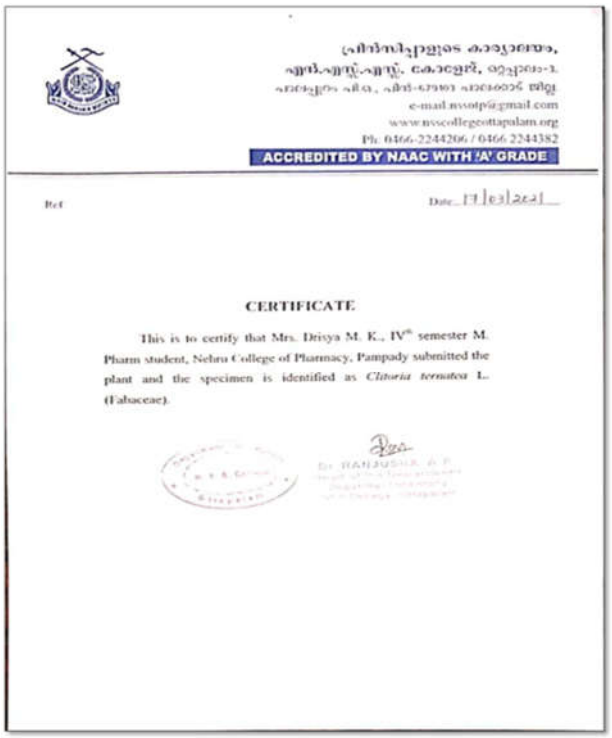
undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

h. Ethanol soluble extractive value

Macerated 5 grams of coarsely powdered air dried plant with 100 ml of ethanol in a stoppered flask for 24 hours, with occasional shaking during the first 6 hours and allowed to stand undisturbed for another 18 hours, filtered rapidly, by taking precautions against loss of solvents. The 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105˚C and weighed. Calculated W/W soluble extractive with reference to air dried material.

RESULT AND DISCUSSION

1.1. AUTHENTIFICATION &IDENTIFICATION



ACCREDITED BY NAAC WITH 'A' GRADE

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Date 11/03/2021

CERTIFICATE

This is to certify that Mrs. Divya M. K., IV[th] semester M. Pharm student, Nehru College of Pharmacy, Pampady submitted the plant and the specimen is identified as *Clitoria ternatea* L. (Fabaceae).

FIGURE NO: 01

1.2. PHARMACOGNOSTICAL STUDIES

1.2.1. *Macroscopic evaluation*

Features	Observations
Colour	Green
Size	About 4cm (1.6 inch) long3cm (1.2 inch) in wide
Odour	Characteristic
Taste	Bitter
Texture	Smooth
Shape	Elliptic and obtuse
Petioles	2-2.5 cm long
Apex	Acuminate or emarginate
Margin	Entire
Base	Symmetrical
Venation	Reticulate

Table No 01: Macroscopic evaluation of *Clitoria ternatea* Linn leaves

1.2.2. *Histochemical analysis (leaf) of clitoria ternatea*

Sl No	Plant Constituents test	Observation
1	Test for Tannins	++
2	Test for Starch	+
3	Test for aleurone grains	+
4	Test for lignified cells	+
5	Test for oil globules	+

++-High concentration ,+ Normal concentration

Table No 02: Histochemical analysis (leaf) of *Clitoria ternatea*

1.2.3. *Microscopic evaluation - Transverse section*

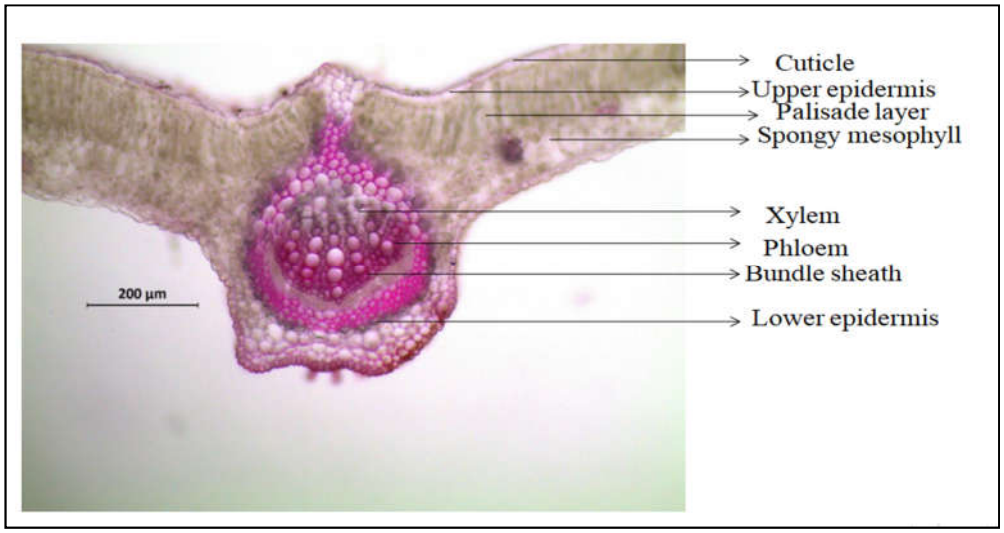

Fig 02: Transverse section of *Clitoria ternatea* leaf

1.2.4. Powder microscopy

Powder microscopic examination of the leaf of *Clitoria ternatea* L showed following

Fig 03: Vessel fragments

Fig 04: Fibres from vessels from lamina portion

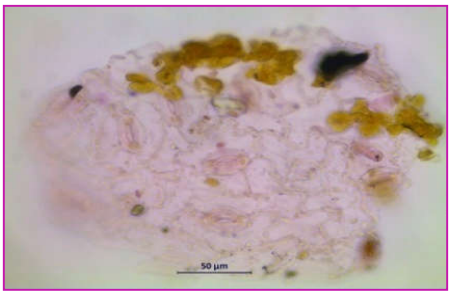

Fig 05: Lower epidermis of leaf with stomata

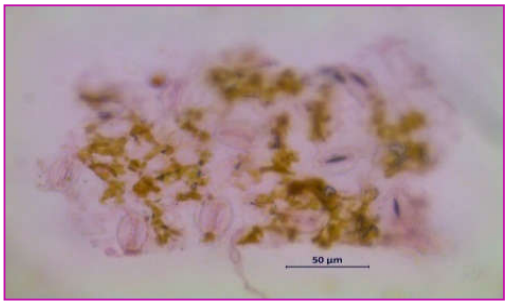

Fig 06: Lower epidermis in surface view with underlying parenchyma cells

Fig 07: Lignified cells from midrib

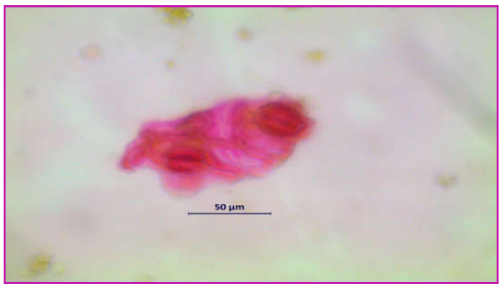

Fig 08: Epidermal cells with stomata

Fig 09: Trichome

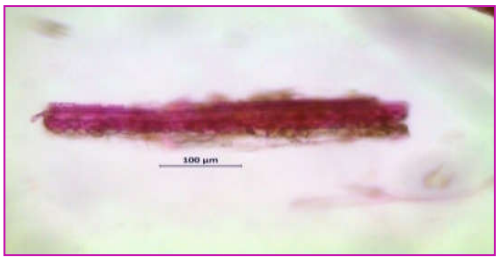

Fig 10: Fibre crystal

1.2.5. Physico-chemical constants

SI.No	Physico-chemical constants	Result(%w/w)
I	**Moisture content**	11.43
II	**Ash value**	
	a) Total ash	4.21
	b) Acid insoluble ash	1.10
	c) Water soluble ash	1.34
	d) Sulphated ash value	1.71
III	**Extractive value**	
	a) Petroleum Ether Soluble Extractive	1.76
	b) n Hexane- Soluble Extractive	5.19
	c) Benzene Soluble extractive	1.3
	d) Chloroform Soluble Extractive	2.24
	e) Ethyl Acetate Soluble Extractive	**10.1**
	f) Acetone Soluble Extractive	13.1
	g) Ethanol Soluble Extractive	13.23
	i) Water Soluble Extractive	17.69

Table No 03: Physico-chemical analysis

CONCLUSION

The present investigation pertains the detailed Pharmacognostical and physicochemical studies of *Clitoria ternatea*.L. Pharmacognostical evaluation of the leaf of *Clitoria ternatea* was carried out in order to establish the identity and to standardize the plant.The physicochemical characterization intends to find out their quality and purity of the drug.Moisture content provides information on loss on drying and presence of excess water in the crude material.The extractive value gives an idea about the unit yield of active constituents from the leaf of the plant extracted with particular solvent chemical constitution of the drug.The ash value determines the earthy matter and other impurities present along with the drug..These studies also suggest that the observed Pharmacognostic,,physicochemical parameters are of great value in the quality control and formulation development.The current study may be useful to supplement the information with regard to its standardization,identification and carrying out further research and its use in Ayurvedic system of medicine.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

REFERENCE

1. Taur D J,Patil R Y ,Khalate A H.Phytochemical investigation and evaluation of Clitoria tewrnatea seeds on clonidine induced catalepsy in mice.Pharmacology online,2009;3:215-330
2. C Karthika,S Mannivannan.Pharmacognostic,physicochemical analysis and phytochemical screening of the leaves of W.trilobata L.International journal of ChemTech Research;2018:11(2) :124-131
3. Jain R A,Shukls S H .Pharmacognostic Evaluation and Phytochemical studies on stem of Clitoria ternatea linn.Pharmacognosy Journal;2011:3(24) :62-66

4. T Regupathi,K Chithra .Physicochemical Analysis of Medicinal Herbs,Eclipta alba(L) Hassk and Lippia nodiflora(Linn).International Journal of Pharmaceutical and Phytopharmacological Research (eIJPPR);2015:4(4):249-251
5. Patnia S ,Saha A N ,et al.Physicochemical ,phytochemical and elemental analysis of stem bark and roots of Berberis asiatica .Adv in App.Sci.Res;2012;3(6);3624-3628
6. Joseph L,George M.Pharmacognostical profiling of Geranium ocellatum leaves.Int J.Med.Arom.Plants;2011:1(3):351-354
7. Gite V N,Pokharkar R D,et al .Evaluation of physicochemical standardization parameters of Enicostemmaaxillare.J Biosci.Tech;2010:1(4):187-190
8. Sangita Shukala,Rashmikapatel et al.Study of Phytochemical and diuretic potential of methanoland aqueous extracts of aerial parts of Phyla nodiflora linn.Internatiional J.Pharma and pharmaceutical Sci;2009:1(1):85-91
9. Sabiha Sumbul,M Aftab Ahmad,M Asif,Mohd Akhtar,Ibne Saud.Physicochemical and phytochemical standardization of berries of Myrtus communis Linn.J Pharma Bioallied Sci;2012:4(4):322-326
10. Muhammad Ezzudin R,Rabeta M S.A Potential of Telang tree(Clitpria ternatea) in human health.Food Research;2018;2(5).415-420
11. Sangekar S N.Morphological,anatomical and Pharmacological studies in Clitoria ternatea L.International Journal of Life Sciences;2020;A 14;17-22
12. Dibya Devismita,Dr Sunitha Bhatnagar.Comparative Analysis of Clitoria ternatea blue and white varieties.World Journal of Pharmaceutical Research;2023;12(4);1967-1972
13. Sethiya N K,Trivedi A,Patel M B,Mishra S H.Comparative Pharmacognostical investigation on four ethnobotanicals traditionally used as Shankhpushpi in India .J Adv Pharm Technol Res;2010;1(4)388-395.
14. Johansen D A 1940.Plant Microtechnique.McGraw- Hill,New York .USA
15. Tandon N Sharma P.Quality Standards of Indian Medcinal Plants Volume 16.Medicinal Plants Division ,Indian Council of Medicinal Research ,New Delhi;2018;293-294