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Ethnopharmacognostical Screening of *Clitoria ternatea* Linn.

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Abstract: *Clitoria ternatea* L. var. *pilosula* Wall. from ancient times has a place in the heart of Hindus due to its sacred value for the lord Shiva. This plant have dual significance firstly, they are promising future food secondly, these medicinal plants can have some active constituents for future pharmaceutical study. They are used in the treatment of a number of ailments including body-aches, infections, urinogenital disorders and as antihelmintic and antidote to animal stings. Establishing the standard is an essential step to identify the quality and purity of the drug. This can be achieved through the pharmacognostical and phytochemical analysis. In present study investigation of macroscopic, microscopic characters were studied along with powder behaviour, fluorescence studies and phytochemical screening etc. Fluorescence analysis showed the plant sample is free of any foreign matter and adulternants. The reaction of the leaves powder with different chemical indicated the presence of the compound such as phenol, coumarins, alkaloids, tannin, xanthoprotein and reducing sugar. The pharmacognostical study helps to reveal the purity of the sample. The study establishes the pharmacognostical and physico-chemical standards of the crude drug and helps to differentiate the plant sample from the adulterants.
Keywords: Pharmacognosy, leaf, *Clitoria ternatea* L., Phytochemical constituents.

Introduction:

In developing countries, about 80% of the population really depends on traditional medicine for their healthcare. So there arises a need to screen medicinal plants for active chemical compounds as a base for further pharmacological studies. The medicinal plants, which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies, this has been brought about by the acknowledgement of the value of medicinal plants as potential sources of new compounds of therapeutic value and as a source of lead compounds in drug development[1]. There is now growing evidence that indicates a strong relationship between ethnic knowledge and sustainable use of biodiversity. In order to fully tap the natural resources of our country, it is very important to put the comprehensive utilization and process of natural resources. Medicinal plants are an important therapeutically aid for various ailments. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. Mostly secondary metabolites are responsible for medicinal activity of plants. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [2].

Clitoria ternatea L. var. *pilosula* Wall. (Fabaceae) is commonly known as 'Shankhapushpi' found all over India. It is vigorous, strongly, persistent, herbaceous perennial legume. Almost all parts of this plant are reported to have medicinal properties. Flowers of this plant has been using in a religious purposes since the

ancient times. The plant has been using traditionally to treat infertility, worm infestation, skin diseases, tonsillitis, appetizer, digestant, vermicide, cough, asthma etc.[3,4]. Many of the medicinal values are evaluated by many workers such as nootropic, anxiolytic, antidepressant, anticonvulsant, sedative, antipyretic, anti-inflammatory and analgesic activities[5-7]. To the best of our knowledge, there are few pharmacognostical studies on *Clitoria ternatea* has been reported in the literature. Therefore, the present investigation was planned to study the pharmacognostical standardization has been performed for leaf of the plant.

2. Materials and Methods:

Collection and identification of plant material: *C. ternatea* (white variety) is a green climber, growing all over the Kolhapur district. Plant was collected at their flowering stage. The identification of the plant was done by using relevant literature. Fresh healthy leaves of *C. ternatea* (white variety) were collected during the month July-August 2013. The leaves were washed and air dried under shade.

Organoleptic characters: The selected leaf material was subjected to organoleptic (colour, odour and taste) were recorded[8].

Physico-chemical studies: The percentage of total ash, acid insoluble ash, moisture content, dry weight were determined according to the method described in WHO guidelines on quality control methods for medicinal plants material[9].

Macroscopic characters: The morphological and taxonomical observations were made and the characters were described in technical terms[10].

Microscopic study: Transverse section (T.S.) of petiole and leaf taken through midrib were taken, cleared in chloral hydrate, mounted with glycerin and observe under microscope for its anatomical details [11]. Qualitative leaf microscopies to determine trichome, stomatal number, stomatal index, vein islet number were carried out [10].

Stomatal index: Stomatal index is the percentage which the number of stomata forms to the total number of epidermal cell, each stomata being counted as one cell. Stomata index can be calculated by using following equation[12].

$$I = \frac{S}{E + S}$$

I= Stomatal index

S= No. of stomata per unit area

E= No. of epidermal cells in the same unit area.

Powder microscopy: Powdered crude drugs consist of the fragments of cells in the form of recognizable tissues and the study of surface constants like fibers, lignified vessel, epidermal cells, calcium oxalate crystals, starch grains etc.

Powder behavior and Fluorescence study: Powder behavior and Fluorescence study of the leaf with different chemical reagents were determined under natural light and fluorescent UV light[13, 14].

Extraction and Phytochemical screening: Then leaf powder used for powder behavior, fluorescence and phytochemical analysis. Leaf powder was extracted using pet ether, methanol, chloroform, acetone, ethanol and aqueous as a solvent. 20 gram of dried leaves powder was weighed and put in a cheese cloth and subjected to extract successively with 200 ml ethanol in Soxhlet extractor until the extract was clear. All the extracts were condensed and preserved in refrigerator in air tight bottles. The percentage yield of extract, preliminary phytochemical tests of the extract were performed using specific reagents by methods[15-19].

Results:

Today, natural products derived from plants are being tested for the presence of new drugs with new modes of pharmacological action, utilizing the special feature of higher plants to produce a large number of secondary metabolites[20]. The studies such as physical evaluation, preliminary phytochemical test and Fluorescence analysis add to the quality control and quality assurance for proper identification of the plant[21].

Macroscopic Characters:

Drug generally occurs in the form of leaves and leaflets. The leaves are pinnate with 5-7 leaflets. Leaflet with small petiolule, ovate or elliptic oblong, obtuse, entire, and glabrous or with a few short appressed hairs, base obtuse or acute. Leaflets are 3 to 5 cm long, 2 to 3 cm wide in dark green colour.

Microscopic Characters:

The transverse section of leaf shows a dorsiventral structure. The both upper and lower epidermis of leaf consists of single layered cells, covered with thick cuticle. Some of epidermal cells of both surfaces elongated outwards and forming bicellular trichomes with one basal cells smaller than terminal cells. Below the upper epidermis columnar, elongated, compactly arranged single rows of palisade cells. Spongy parenchyma is found throughout tissue and is composed of 4-5 layered loosely arranged spherical parenchyma cells. The vascular bundle crescent shaped consists of xylem and phloem. Pericycle present in the form of broken ring. Rest of the tissues between epidermis and pericycle composed of oval to polygonal, thin walled, 3-5 layered, parenchymatous cells. It shows few prismatic crystals of calcium oxalate which is transperant spot. On lower side paracytic stomata are present.

Stomatal index:

Stomata present on both the surface of leaf. The type of stomata is Paracytic. The stomatal index is 60 on lower surface and vein islet number 23 per sq.mm.

Powder microscopy:

The powder is olive green in colour. It contains epidermal cells with paracytic stomata, fragments of trichomes with warty cuticle and wavy thin walled. It also shows groups of spongy parenchyma and palisade cells, fibers, veins and epidermal cells.

Fig.1 Habit

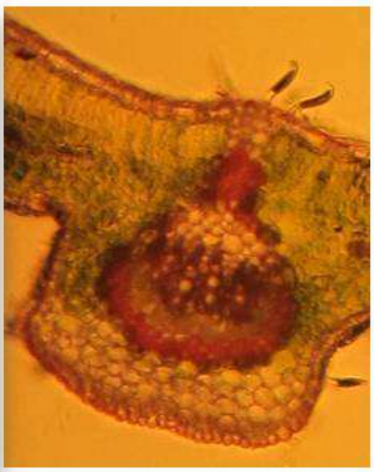

Fig.2 T.S. Of Leaf

Fig.3 Paracytic stomata

Organoleptic character and physical constants:

The fresh leaves were soft and dark green when fresh. Dried leaves were olive green in colour with characheristic odour. The physico-chemical parameters are helpful in judging the purity and quality of the drug [22]. The percentage of active principles in the plant is determined only in the dry condition. Hence, the moisture lost percentage is very important to decide about the condition of the crude drug. The moisture should be kept minimum to prevent the drug from various kinds of decomposition[23]. The percentage of ash was 9.73% and acid insoluble ash was 0.45%. The total ash and the acid insoluble ash indicate the presence of any foreign matter, inorganic composition and purity of the drug. The low value in the leaves powder showed that the sample is free of any foreign matter.

Table 1: Organoleptic characters of leaves *Clitoria ternatea* (White variety)

Sr. No.	Characters	Observations		
		When fresh	After drying	Powder
1.	Colour	Dark green	Olive green	Olive green
2.	Odour	Characteristic	Characteristic	Characteristic
3.	Texture	Soft	fibrous	Coarse powder
4.	Taste	Bitter	Bitter	Bitter

Table 2: Physical constants of powdered leaves of *Clitoria ternatea* (White variety)

Sr. No.	Parameters	Results
1.	Total Ash	9.5±0.23%
2.	Acid insoluble ash	0.45%
3.	Moisture content	12.63±0.15%
5.	Stomatal index (Upper surface)	Very few
6.	Stomatal index (Lower surface)	60
7.	Vein islet number	23/sq.mm

Powder behavior & Fluorescence study:

The examination of powder showed the colour developed for the respective compounds such as pistachio for the flavonoids, dark olive green for the tannin, forest green for cystiene, white precipitate for alkaloids and rufous for xanthoprotein (Table 3). The fluorescence analysis is useful for the proper identification and adulteration of crude drugs and specific compounds. The leaf powders are treated with various chemicals revealed various colours in the Short and Long wavelength under UV light. For example when the powder was treated with aqueous 1 N HCl the leaf powder exhibited varied office green and hunter green colour under 254nm & 366nm wavelength respectively in UV light likewise all the results are depicted in Table 4.

Phytochemical analysis:

The Phytochemical tests are useful for the detection of various chemical constituents. The phyto chemical test carried out on the various extract like pet ether, methanol, chloroform, acetone, ethanol, and Aqueous. The preliminary phytochemical screening revealed the presence of Tannin, Phenol, Alkaloids, Courmarins, reducing sugar, flavones. Kamilla et al (2009)[24] carried out qualitative phytochemical screening of secondary metabolites from various parts of *Clitoria ternatea*. The preliminary phytochemical screening observed that the leaf methanolic extract contains moderate level of tannin, cardiac glycosides and steroids and mild level of alkaloids. Jayachitra et al (2012) [25] was carried out qualitative analysis of petroleum ether extract of leaves of both variety of *Clitoria ternatea*. They revealed the presence of alkaloids, phenolics, flavonoids, sugars in two varieties of *Clitoria ternatea*.

Table 3: Powder behavior with different chemical reagent.

Sr. No.	Reagent	Colour/Behaviour	Inference
1.	Powder as such	Dark spring green	----------
2.	Powder + 1N NaOH (aq.)	Pistachio	Flavonoids present
3.	Powder + 5% Iodine	No change	----------
4.	Powder + 40% NaOH + Lead acetate	Forest green	Cystiene present
5.	Powder + Mayers reagent	White ppt.	Alkaloids present
6.	Powder + Conc. H_2SO_4	Dark green	----------
7.	Powder + 5% $FeCl_3$	Dark olive green	Tannin present
8.	Powder + 1% $AgNO_3$	Office green	----------
9.	Powder + 5 % KOH (aq.)	No change	----------
10.	Powder + Conc. HNO_3 + Ammonia	Rufous	Xanthoprotein present

Table 4: Fluorescence study of powder with different chemical reagent in visible and UV light.

S.N.	Powder with chemical reagent	Visible light	Short wavelength	Long wavelength
1.	Powder as such	Dark spring green	Hunter green	Pakistan green
2.	Powder + Distilled water	Mantis	India green	Office green
3.	Powder + 1N NaOH (Aq.)	Pigment green	Hunter green	Dartmouth green
4.	Powder + 1N NaOH (Alco.)	Pistachio	Dark olive green	Fern green
5.	Powder + HCl (Conc.)	Pistachio	Office green	Hunter green
6.	Powder + H_2SO_4 (Conc.)	India green	Sea green	Pakistan green
7.	Powder + HNO_3 (Conc.)	Mikado yellow	Dimgray	Darkslate gray
8.	Powder + 10% HCl	Lime green	Dartmouth green	Hunter green
9.	Powder + Acetone	Islamic green	Pakistan green	Darkspring green
10.	Powder + 5 % KOH	Apple green	Darkolive green	Hunter green

Table 5: Preliminary phytochemical screening of leaf.

S. N.	Chemical compound	Extracts					
		Pet. Ether	Methanol	Chloroform	Acetone	Ethanol	Aqueous
1.	Phenol	+++	++	-	+++	++	-
2.	Anthraquinones	-	-	-	-	-	+
3.	Flavones	+++	+++	++	+	+++	+++
4.	Tannins	-	++	+	++	+	++
5.	Coumarin	++	-	-	-	-	-
6.	Saponins	-	+	-	+	+	+
7.	Alkaloids	-	-	-	+	+++	-
8.	Reducing Sugar	-	-	-	+	-	+++
9.	Amino acid	-	+	-	-	++	+
10.	Glyosides	-	-	-	-	+	-
11.	Oil.	-	-	-	-	-	-

[+++ High, ++ Moderate, + Slight, Pet. Ether= Petroleum ether]

Conclusion:

The study of pharmacognostical and phytochemical characters helps to establishing the standards that is an essential step to identify the quality and purity of the drug. The study undertaken with the *Clitoria ternatea* (White variety) leaves revealed different parameters that will be useful in scientific evaluation, identification and authentication of the drugs.

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