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PHYTOCHEMICAL EVALUATION AND HPTLC FINGERPRINT PROFILE OF *CLITORIA TERNATEA* L. ROOT [WHITE FLOWERING VARIETY] GROWN IN UDUPI DISTRICT, KARNATAKA, INDIA

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Abstract

To study the phytochemical constituents of *Clitoria ternatea* L. (white flowering variety) grown in udupi district. Physicochemical parameters, qualitative estimation of carbohydrates, alkaloids, terpenes, saponins, tannins, steroids, flavonoid quinines, carboxylic acid and amino acids and finger print analysis by HPTLC method. Present study showed, extraction yields of water extracts was 7.83%, 17.2% of total ash, 5.7% is acid insoluble ash and 7.9% is water soluble ash. The moisture content found to be 14.5%. Presence of various phytoconstituents like alkaloids, flavonoids, steroid, carbohydrates coumarins, resin. HPTLC, plates observed in absorbance mode at both 254nm, 366 nm and visible light range 620nm best results were shown at 620 nm. Densitometry records of HPTLC finger print scanned at wavelength 620 nm for alcoholic extract of *Clitoria ternatea root* revealed the presence of 9 phytoconstituents and Rf values ranged from 0.04 to 0.83. The component with *R*f values 0.06, 0.67 were found to be more predominant as the percentage area is more with 24.71% and 35.53% respectively.

Present study provides the scientific data functional ability and establishment of standards for the white variety of *Clitoria ternatea*.

Key words: *Clitoria ternatea* root, phytoconstituents, HPTLC.

Introduction

Therapeutic usage of medicinal plants for the treatment of various ailments and for cosmetic purposes has been growing all over the world (Mazid Khan and Mohammad, 2012); (Laxminarayana Bairy *et al.*, 2011). In recent years herbs are gaining importance due to the presence of active components and their nutritive values (Jain *et al.*, 2010). In India, approximately 80% of population depends on herbal therapies to battle certain ailments (Bodeker, 2010). *Clitoria ternatea* Linn (Family Fabaceae) is one such herb which was commonly used since ancient Indian system of medicine (Ayurveda) for the preparations of rasayanas (rejuvenating tonics). It is commonly known as ‘‘Butterfly pea.’’ or Aparajitha or shankha pushpa. It is an evergreen climber, available throughout the year, grows approximately up to 3 m (9feet) high. Its flowers leaves, fruits, root and stem, are used in medicinal preparation in Ayurveda. The leaves are compound made of three to nine oval or elliptical leaflets. The flowers are 2-4 cm long and in various shades of blue with a yellow throat or pure white, big standard petal with light yellow throat, resembles the shape of conch-shell hence the name Shankha Pushpa. Flowers of blue variety is used in the preparation of health drinks dyes and eyeliners. This herb is available in different parts of India. Fruits are small pods, approximately 10cm long. Roots of this plant are having unique fragrance, bitter in taste and tertiary roots contains small nodules.

In Ayurveda Shanka Pushapa is used as, laxative,

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memory boosting, diuretic, antihelminthic, general tonic and in treatment of infertility, dementia, burning sensation, leprosy, inflammation, leukoderma, bronchitis, asthma, pulmonary tuberculosis, ascites and fever (Mukherjee *et al.*, 2008); (Nirmal *et al.*, 2008). *Clitoria ternatea* is commonly used in the preparations by using this herb are of rejuvenating supplements such as 'Chyavanaprash' and Shankapushpi syraps, tablet Mentat are used mainly as a memory boosters, antiepileptic treatments.

To validate traditional uses of *Clitoria ternatea*, various studies have undertaken. The root parts were found to be more effective. Oral supplementation of aqueous root extract of *Clitoria ternatea* (100mg/kgbw) (CTR) has enhanced memory in passive avoidance test and spatial learning task using T-maze (Rai *et al.*, 2001), in rats. Alcoholic extracts of aerial and root parts of *Clitoria ternatea*, used at various dosages to attenuate electroshock-induced amnesia in rats and also has more efficacy in memory retention was found in higher dosages (Mehla *et al.*, 2013). It had shown to increase the ACh content and acetylcholinesterase activity in the rat hippocampus (Rai *et al.*, 2002); (Taranalli and Cheeramkuzhy, 2000) and these effects were similar to the standard cerebroprotective drug pyritinol. *Clitoria ternatea* was found to increase the dendritic arborisation of CA3 pyramidal neurons, dendritic intersection and branching points both in apical and basal dendrites of the hippocampus of the rat and improved the learning and memory in rats (Rai *et al.*, 2005). It is also proved to have anxiolytic, anti-stress and anticonvulsant, antioxidant properties (Patil and Patil, 2011); (Jain *et al.*, 2003); (Kamkaen and Wilkinson, 2009); (Almeida *et al.*, 2014). Studies shows that root of white flowering variety of *Clitoria ternatea* has more antioxidant property than the blue flowering variety (Patil and Patil, 2011); (Almeida *et al.*, 2018). Alcoholic and aqueous root extracts of *Clitoria ternatea* were found to have antimicrobial activity (Rao *et al.*, 2017). As per our knowledge, there are no studies available to identify the active components present in the root of White flowering variety of *Clitoria ternatea*.

Aim of the present study was to study the Physiochemical parameters, to identify the bioactive compound in aqueous root extract (*viz*., carbohydrates, alkaloids, terpenes, saponins, tannins, steroids, phenols, flavonoid quinines, carboxylic acid and amino acids) and to develop the HPTLC finger printing of alcoholic root of extract *Clitoria ternatea* root.

Materials and Methods

Sample collection

Locally grown 2-3 year old plants of *Clitoria ternatea* (white flowering variety) were identified and confirmed as *Clitoria ternatea* in the department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal and by a botanist from Mahathma Gandhi Memorial College, (Government of India aided college) where the voucher specimen of the plant was deposited in the college herbarium (MGM College Herbarium. No.9/ 2016). Roots were collected, dried in the shade for 15days and were powdered.

Water soluble extractive procedure

4 g of the sample was weigh accurately in a glass stoppered flask. 100 ml of distilled water was add and the flask was shaken occasionally for 6 hours. The mixture was allowed to stand for 18 hours. Then the mixture was filtered rapidly taking care not to lose any solvent. Pipetted out 25ml of the filtrate in a pre-weighed 100 ml beaker. Evaporated to dryness on a water bath. The sample was kept in an air oven at 105°C for 6 hours and then cooled in a desiccator and weighed. The experiment was repeat twice. Average value of the sample was take.

Chemicals

Chemicals used in the study were collected from Himedia chemicals

Preliminary qualitative phytochemical test

Preliminary phytochemical screening was carried out for alkaloids, steroids, triterpenoids, carbohydrates, flavonoids, tannins, coumarine raisin, phenols quinines and carboxylic acids and amino acids were carried out as described below give table.

Tests for alkaloids

Tests	Colour if positive	Alcoholic extract of *clitoria ternatea*
Alkaloids		
Dragendroff's test	Orange red precipitate	Orange red precipitate
Wagners test	Reddish brown precipitate	Reddish brown precipitate
Mayers test	Dull white precipitate	Dull white precipitate
Hagers test	Yellow precipitate	Yellow precipitate
Steroids		
Liebermann- buchard test	Bluish green colour	Bluish green colour

Table Continue ...

Table Continue ...

Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer
Carbohydrate		
Molish test	Violet ring	Violet ring
Fehlings test	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate
Tannin		
With $FeCl_3$	Dark blue or green or brown	Buff color
Flavanoids		
Shinoda's test	Red or pink	Red color
Saponins		
With $NaHCO_3$	Stable froth	No stable froth
Triterpenoids		
Tin and thionyl chloride test	Pink	Brown color
Coumarins		
With 2 N NaOH	Yellow	Yellow color
Phenols		
With alcoholic ferric chloride	Blue to blue black	Bluish black color
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No effervescence
Amino acid		
With ninhydrine reagent	Purple colour	Yellow color
Resin		
With aqueous acetone	Turbidity	Turbidity
Quinone		
Conc. sulphuric acid	Pink/purple/red	Brown color

Fingerprint analysis by HPTLC

1g of *Clitoria ternatea* whole plant powder was extracted with 10 ml of *alcohol*. 4, 8 and 12µl of the above extract was applied on a pre-coated silica gel F254 on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene: Ethyl acetate (7.0: 1.0). The developed plates were visualized in short UV, long UV and then derivatised with vanillin sulphuric acid further scanned under 254nm, 366nm and 620nm. R_f, colour of the spots were recorded.

Results

The results of the present study shows, extraction yields water extracts of was 7.83%.

Physicochemical parameter observed that 17.2% of total ash, 5.7% is acid insoluble ash and 7.9% i water soluble ash (Table 1).The moisture content found to be 14.5%. Phytochemical Screening tests of Aqueous extracts of *clitoria ternatea* root showed the presence of various phytoconstituents like alkaloids, flavonoids, steroid, carbohydrates coumarins, resin (Table 2).

HPTLC study revealed that *Clitoria ternatea* root alcoholic extract has best results in Toluene: Ethyl Acetate solvent system. After scanning and visualizing the plates in absorbance mode at both 254nm, 366 nm and visible

Table 1: Physicochemical parameter *Clitoria terntaea* root.

Total yield	7.83gms
Total ash	17.2%
Acid insoluble ash	5.7%
Water soluble ash	7.9 %
The moisture content	14.5%

Table 2: Preliminary phytochemical screening of *Clitoria ternatea* root.

Test	Inference
Alkaloid	+
Steroid	+
Carbohydrate	+
Tannin	-
Flavanoids	+
Saponins	-
Terpenoid	-
Coumarins	+
Phenols	-
Carboxylic acid	-
Amino acids	-
Resin	+
Quinone	-
(+) - present; (-) - negative	

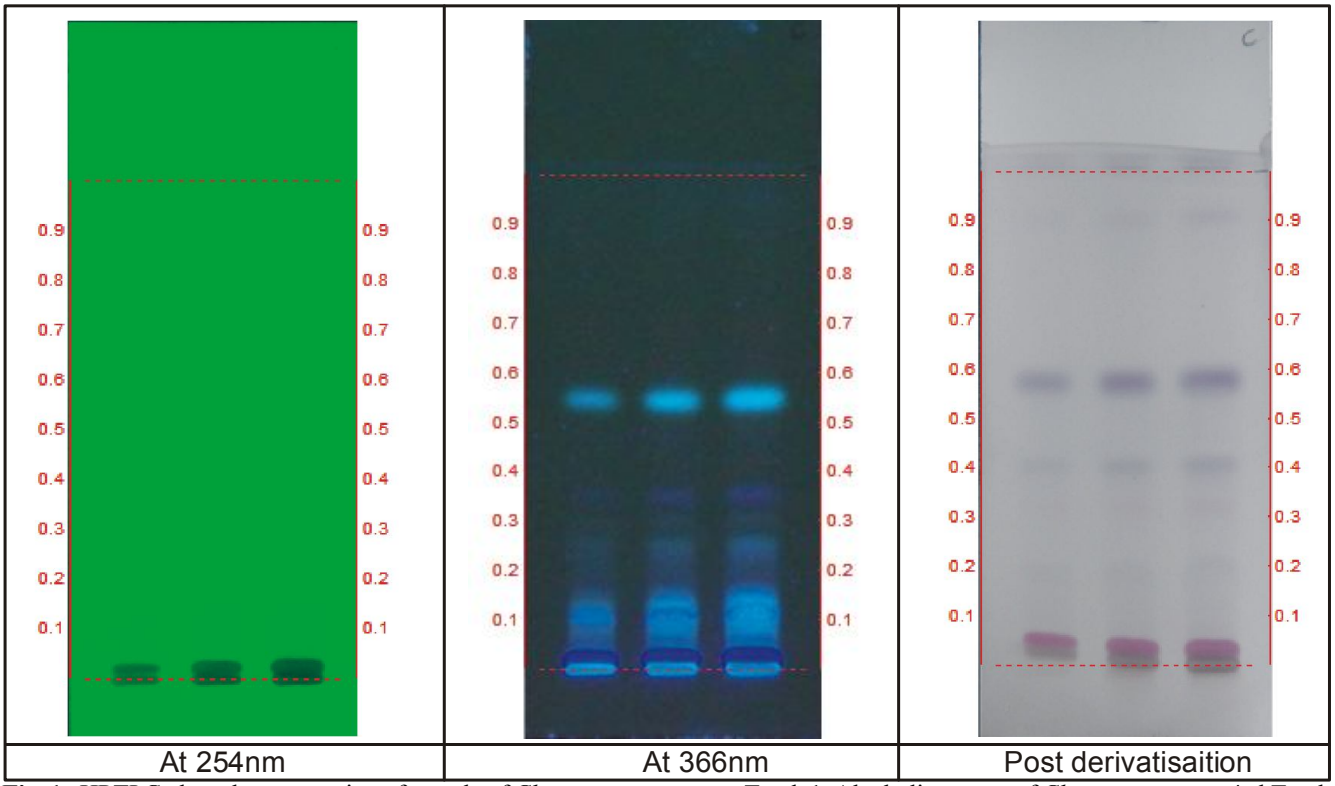
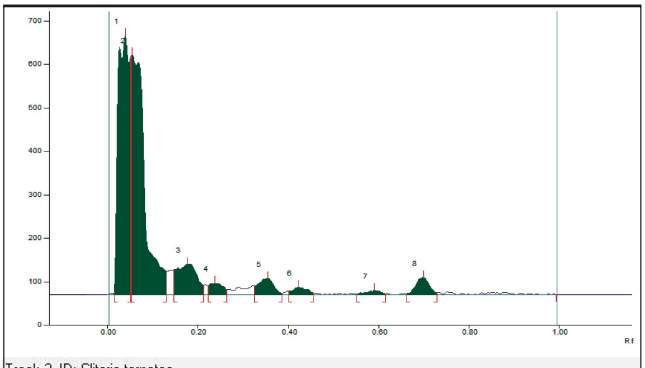


Fig. 1: HPTLC photodocumentation of sample of *Clitoria ternatea* root ,Track 1: Alcoholic extract of *Clitoria ternatea*- 4µl,Track 2: Alcoholic extract of *Clitoria ternatea*- 8µl,Track 3: Alcoholic extract of *Clitoria ternatea*- 12µl,Solvent system- Toluene: Ethyl acetate (7.0: 1.0).

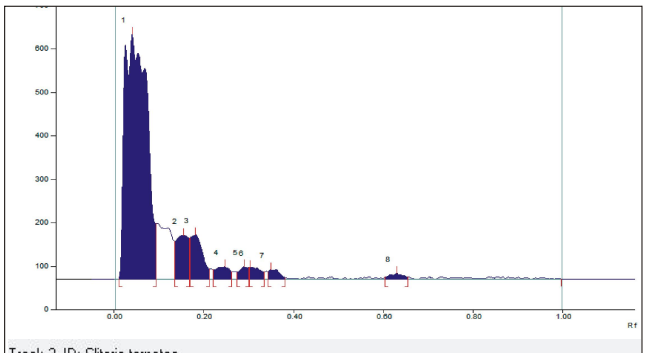
light range 620nm best results were shown at 620 nm. The HPTLC images shown in fig. 1 indicate that all sample constituents were clearly separated without any tailing and diffuseness.

Densitometry records of HPTLC finger print scanned at wavelength 620 nm for alcoholic extract of Clitoria *ternate* root (Fig. 2) revealed the presence of 9 phytoconstituents. The Rf values ranged from 0.04 to 0.83. It is also clear from (Fig. 2) the densitometry graph as shown in fig. 2 shows that out of 9 components, the component with *R*f values 0.06, 0.67 were found to be more predominant as the percentage area is more with 24.71% and 35.53% respectively



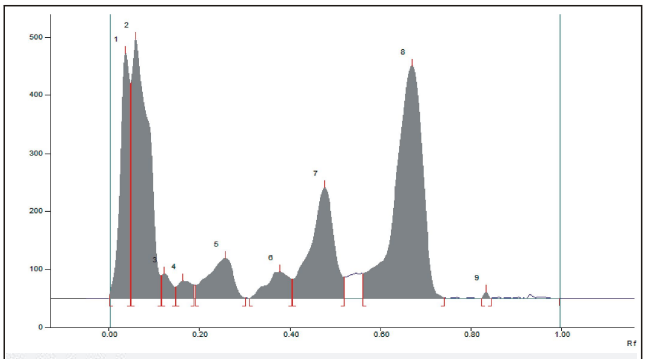
Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	7.3 AU	0.04 Rf	596.3 AU	44.09 %	0.05 Rf	45.5 AU	10757.6 AU	38.05 %
2	0.05 Rf	551.1 AU	0.05 Rf	552.8 AU	40.87 %	0.13 Rf	53.3 AU	12161.1 AU	43.02 %
3	0.15 Rf	57.3 AU	0.18 Rf	70.7 AU	5.22 %	0.21 Rf	22.3 AU	2284.1 AU	8.08 %
4	0.22 Rf	21.0 AU	0.24 Rf	26.5 AU	1.96 %	0.26 Rf	11.8 AU	584.3 AU	2.07 %
5	0.33 Rf	20.2 AU	0.36 Rf	38.3 AU	2.83 %	0.39 Rf	3.8 AU	954.3 AU	3.38 %
6	0.40 Rf	8.2 AU	0.42 Rf	17.3 AU	1.28 %	0.46 Rf	4.7 AU	416.0 AU	1.47 %
7	0.55 Rf	0.9 AU	0.59 Rf	10.9 AU	0.80 %	0.62 Rf	1.3 AU	232.3 AU	0.82 %
8	0.66 Rf	2.5 AU	0.70 Rf	39.7 AU	2.93 %	0.73 Rf	4.8 AU	881.1 AU	3.12 %

Fig. 2A: Densitometric scan of the sample of *Clitoria ternatea* root (at 254nm).



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	1.7 AU	0.04 Rf	564.8 AU	63.46 %	0.10 Rf	27.9 AU	20093.6 AU	76.23 %
2	0.14 Rf	87.0 AU	0.16 Rf	101.6 AU	11.42 %	0.17 Rf	94.6 AU	2028.8 AU	7.70 %
3	0.17 Rf	95.3 AU	0.18 Rf	102.3 AU	11.49 %	0.21 Rf	24.1 AU	1988.5 AU	7.54 %
4	0.22 Rf	21.9 AU	0.25 Rf	28.8 AU	3.24 %	0.26 Rf	17.5 AU	647.9 AU	2.46 %
5	0.28 Rf	16.1 AU	0.29 Rf	28.7 AU	3.23 %	0.30 Rf	27.6 AU	408.7 AU	1.55 %
6	0.30 Rf	27.6 AU	0.30 Rf	28.1 AU	3.16 %	0.34 Rf	17.2 AU	496.5 AU	1.88 %
7	0.34 Rf	16.8 AU	0.35 Rf	22.1 AU	2.49 %	0.38 Rf	4.0 AU	400.6 AU	1.52 %
8	0.60 Rf	4.4 AU	0.63 Rf	13.5 AU	1.52 %	0.66 Rf	4.6 AU	295.0 AU	1.12 %

Fig. 2B: Densitometric scan of the sample of *Clitoria ternatea* root (at 366nm).



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.00 Rf	4.8 AU	0.04 Rf	421.8 AU	25.43 %	0.05 Rf	69.7 AU	6217.5 AU	13.00 %
2	0.05 Rf	372.4 AU	0.06 Rf	445.6 AU	26.86 %	0.12 Rf	39.3 AU	11813.4 AU	24.71 %
3	0.12 Rf	40.1 AU	0.12 Rf	42.9 AU	2.59 %	0.15 Rf	19.6 AU	661.6 AU	1.38 %
4	0.15 Rf	20.1 AU	0.16 Rf	30.3 AU	1.83 %	0.19 Rf	23.6 AU	667.4 AU	1.40 %
5	0.19 Rf	23.3 AU	0.26 Rf	69.4 AU	4.18 %	0.30 Rf	1.2 AU	2728.1 AU	5.71 %
6	0.31 Rf	0.1 AU	0.38 Rf	46.0 AU	2.77 %	0.40 Rf	33.4 AU	1634.7 AU	3.42 %
7	0.41 Rf	33.4 AU	0.48 Rf	191.1 AU	11.52 %	0.52 Rf	36.8 AU	7030.4 AU	14.70 %
8	0.56 Rf	42.7 AU	0.67 Rf	400.5 AU	24.15 %	0.74 Rf	1.6 AU	16987.1 AU	35.53 %
9	0.82 Rf	0.4 AU	0.83 Rf	11.3 AU	0.68 %	0.85 Rf	0.3 AU	73.9 AU	0.15 %

Fig. 2C: Densitometric scan of the sample of *Clitoria ternatea* root (at 620nm).

Discussions

Phytochemicals are chemical compounds that are synthesized during the various metabolic processes having variety of pharmacological activities. They function as plant defence mechanisms against pathogenic organisms and consumption of specific herbs have found to develop the defence mechanisms by improving endogenous antioxidant status and immune systems in humans and animals. These compounds are classified as phenols, quinines, flavonoids, tannins, alkaloids, glycosides and polysaccharides. Phytochemical analysis of *Clitoria ternatea* root, grown in Udupi district has revealed the presence of alkaloids, steroids, flavanoids in aqueous root extract could be the reasons for its effect on CNS (Rai, 2010); (Almeida *et al.*, 2014).

HPTLC fingerprint studies confirmed the results of phytochemical screening by the presence of various coloured bands at different wavelengths with specific solvent systems, symbolizing the presence of 9 phytocompounds. According to Ayurveda literature properties of plant vary according to the region of growth and season of collection of the herbs so as the phytoconstituents.

Previous studies shows that roots of *Clitoria ternatea* forms the nodules, which contain higher amount of plant growth substance such as indole acetic acid, kinetin and gibberellic acid, Tryptophan, precursor of indole acetic acid (Ahmad, Uddin and McLaughlin, 1984). Studies investigated the presence of free amino acids and amides in the root nodules of *Clitoria ternatea*. Other studies reported the isolation and identification of pentacyclic triterpenoids, taraxerol and taraxerone from the root (Formatting Citation). Petroleum ether extract from root part of different varieties of *Clitoria ternate* grown in Gujarath have shown rich in taraxerol and β-sitosterol (Makasana *et al.*, 2016).

In present study densitometric HPTLC fingerprint profile could be used as marker for quality evaluation and standardization of the drug as HPTLC fingerprint analysis used for the authentication of the plant extracts and its constituents and to detect the quality of herbal formulations, thus enabling an assessment of plant extract quality, also a diagnostic tool for the correct identification of the plant a and ensure therapeutic efficacy. Thus, HPTLC fingerprint profile along with their Rf values were recorded, which would serve as a reference standard for the scientist engaged in research on the medicinal properties of plant. Further quantitative analysis of phytochemicals in *Clitoria ternata* root (white variety) is essential to confirm its therapeutic benefits. Though further work to characterize the other chemical constituents and perform quantitative estimation with marker compounds is has to be done. It can be a good estimator of genetic variability in plant population

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