Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 1107-1109
Received: 14-11-2017
Accepted: 15-12-2017

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A study on the different pigments in *Clitoria ternatea* L. Varities (Fabaceae)

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Abstract
Medicinal Plants are rich source of therapeutic compounds that have tremendous applications in pharmaceutical industry. *Clitoria* has three varieties *Clitoria ternatea* L. Var. *ternatea* Hook. F. (white), *Clitoria ternatea* L. Var. *ternatea* Hook. F. (Blue) and *Clitoria ternatea* L. Var. *Pleniflora* Fantz. (Blue var), *Clitoria* is an attractive perennial climber with conspicuous blue or white flowers. Plant leaves contain a number of light absorbing pigments, many of which are associated with photosynthesis. Chlorophyll a, b, xanthophylls and Carotene are the primary photosynthetic pigments responsible for capturing light energy for use in photochemical reactions. The present study aimed to calculate the Rf value of identified pigments of Clitoria varieties using Thin Layer chromatography.

Keywords: *Clitoria* varieties, Light absorbing pigments, Chlorophyll a, Chlorophyll b, Xanthophyll, Carotene

1. Introduction
India is rich in biological diversity, which has about 40,000 species of plants. Among this 15,000 species are angiosperms (WCMC, 1992) [5]. In kerala there is about 5094 species under 1537 genera and 221 families of angiosperms (Sasidharan, 2012) [3].
Fabaceae family comprises about 670 genera and nearly 20,000 species of trees, shrubs, vines and herbs and are distributed worldwide; found throughout India in tropical areas. Fabaceae is the third largest family of angiosperms. The distinguishing characters of the family fabaceae are compound leaves and the production of fruits known as legumes. Legumes help increase soil nitrogen and provide rich sources of vegetable protein for humans, livestock, and wild animals. The flowers of the legume family are diverse but uniformly bilaterally symmetric. Indeed, fifty-million-year-old fossil legume flowers provide the first instance of bilaterally symmetrical flowers in the fossil record of flowering plants. *Clitoria ternatea* L. belongs to fabaceae family known as Butterfly pea and Shankapushpi; a perennial twining herb, found in tropical equatorial areas. The plants are seen much adaptive to various ranges of temperatures and humidity. They are tolerant to frost and dry conditions (Anonymous 1998) [1]. *Clitoria ternatea* is a vigorous, strongly persistent, herbaceous perennial legume; stem fine twining, sparsely pubescent, sub erect at base, and 0.5-3 m long. Leaves pinnate with 5 or 7 leaflets; petioles 1.5-3 cm long; stipules persistent, narrowly triangular,1-6 mm long, subulate, prominently 3 nerved; rachis 1-7 cm long; stipules filiform, 2mm long; leaflets elliptic. *Clitoria* has three varieties *Clitoria ternatea* L. Var. ternatea Hook. F. (white), *Clitoria ternatea* L. Var. *ternatea* Hook. F. (Blue) and *Clitoria ternatea* L. Var. *Pleniflora* Fantz. (Blue var), *Clitoria* is an attractive perennial climber with conspicuous blue or white flowers. It is traditionally used to treat various ailments (Sivarajan and Balachandran 1994) [4]. The roots and seeds are used as thenerves tonic and laxative. The leaves and roots are used in the treatment of urinogenital disorders, anthelmintic and antidote to animal stings (Anonymous, 1995).]. Flowers of butterfly pea contain anthocyanins. Anthocyanins are plant pigment, which are responsible for red violet-blue color in plant flowers (Harborne, 1988) [2]. Many of the research had been done for extracting the anthocyanin present in different varieties of Butterfly pea compared to other flowers. This study was aimed to identify the major pigments present in the leaves of *Clitoria ternatea* varieties using thin layer chromatography.

Materials and Methods
Thin Layer Chromatography (TLC)
The stationary phase is applied onto the plates uniformly and then allowed to dry and stabilize. These days, however, ready- made plates are preferred. With a pencil, a thin mark is made at the bottom of the plate to apply the sample spots. Then, samples solutions are applied on the spots marked on the line in equal distances.

The mobile phase is poured into the TLC chamber to a leveled few centimeters above the chamber bottom. A moistened filter paper in mobile phase is placed on the inner wall of the chamber to maintain equal humidity. Now, the plate prepared with sample spotting is placed in TLC chamber so that the side of the plate with the sample line is facing the mobile phase. Then the chamber is closed with a lid. The plate is then immersed such that the sample spots are well above the level of mobile phase for development. Allow sufficient time for the development of spots. Then remove the plates and allow them to dry. The sample sopots can be seen in a suitable UV light chamber.

$$R_f = \frac{\text{Distance pigment traveled (mm)}}{\text{Distance solvent traveled (mm)}}$$

Determination of Chlorophyll

The estimation of total chlorophyll was carried out using Arnon formula. The first step involved in this process is chopping of green leaves in to small pieces. From this 2g of tissue were weighed out. It is then homogenized in 20ml of 80% Acetone using mortar and pestle under cold conditions. The green solution was filtered through a four folded muslin cloth. Then centrifuged at 1000rpm for 5minutes.The supernatant was collected and its volume is measured. From that 1ml was pipette out and diluted it to 10times by adding 9ml of acetone solution. Reading the absorbency at 645m and 663nm using spectrophotometer.

Calculation

Mg chlorophyll a/g tissue= $12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$

Mg chlorophyll b/g tissue = $22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W}$

Mg total chlorophyll/g tissue = $20.2(A_{645}) + 8.02(A_{663}) \times \frac{V}{1000 \times W}$

Where A=absorbance at specific wavelength
V=final volume of chlorophyll extract in 80% acetone
W=fresh weight of tissue extracted.

Result and Discussions

Determination of Chlorophyll

Chlorophyll is a pigment present in all plants which give green colour to them. *Clitoria ternatea* L. varieties leaves have high chlorophyll content of 6.58 mg in *Clitoria ternatea* L. Var. *pleniflora* (blue var.) as shown in Table: 1. Thus they can be used as a relatively cheap and very easily available sources of chlorophyll instead of chlorophyll supplements for availing the health benefits of chlorophyll compounds.

Table 1:

Sl No	Taxa studied	Chlorophyll a (mg)	Chlorophyll b (mg)	Total chlorophyll (mg)
1.	*Clitoria ternatea* L. (Blue)	0.971	3.532	4.504
2.	*Clitoria ternatea* L. (White)	0.204	3.377	3.581
3.	*Clitoria ternatea* L. Var. pleniflora (Blue var)	1.138	5.442	6.58

Thin Layer Chromatography

To separate and identify pigments present in *Clitoria ternatea* L. varieties and also calculated the R_f value of different pigments present in each variety.

Table 2: Rf value and pigments identified in *Clitoria ternatea* L. (Blue) Distance that solvent moved: 3cm

Pigment number	Band colour	Distance Travelled	R_f value	Pigment identification
1	Yellow	1.7	0.56	Xanthophyll
2	Light green	1.8	0.6	Xanthophyll
3	Blue green	1.9	0.63	Chlorophyll a
4	Dull yellow	2	0.66	Chlorophyll b
5	Orange	2.6	0.86	Carotene

Table 3: Rf value and pigments identified in *Clitoria ternatea* L. (White) Distance that solvent moved: 3 cm

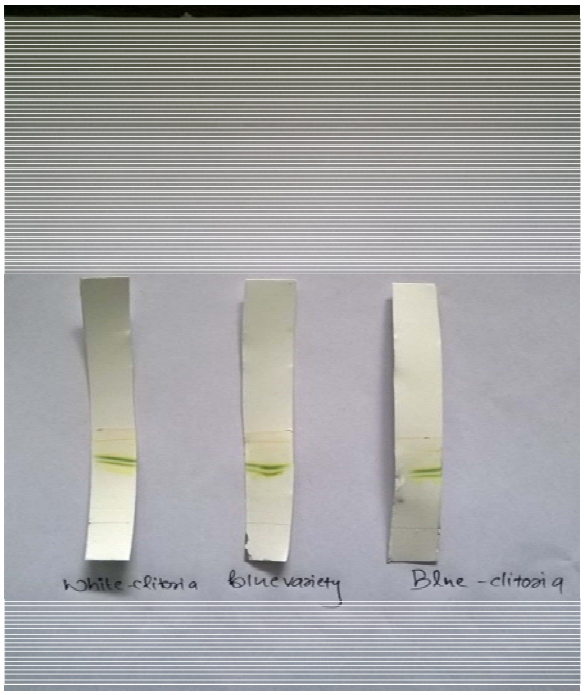
Pigment number	Band colour	Distance Travelled	R_f value	Pigment identification
1	Yellow	1.7	0.56	Xanthophyll
2	Light green	1.7	0.56	Chlorophyll b
3	Dark green	1.9	0.63	Chlorophyll a
4	Yellow shade	2	0.66	Xanthophyll
5	Orange	2.5	0.83	Xanthophyll

Table 4: Rf value and pigment identified in *Clitoria ternatea* L. Var. *pleniflora* (blue var.) Distance that solvent moved: 3 cm

Pigment number	Band colour	Distance Travelled	R_f value	Pigment identification
1	Yellow	1.6	0.53	Xanthophyll
2	Light green	1.7	0.56	Chlorophyll b
3	Yellow shade	1.8	0.6	Xanthophyll
4	Dark green	1.9	0.63	Chlorophyll a
5	Orange	2.6	0.86	Carotene

Plant leaves contain a number of light absorbing pigments, many of which are associated with photosynthesis. Chlorophyll a and b are the primary photosynthetic pigments responsible for capturing light energy for use in photochemical reactions. The present study aimed to calculate the Rf value of identified pigment of Clitoria varieties using Thin Layer chromatography.

The hypothesis of the green pigment chlorophyll being the greater contributor in *Clitoria ternatea* L. Var. *Pleniflora* (blue var) in White *Clitoria* xanthophylls being the greater and in clitoria blue Xanthophyll, chlorophyll and carotene being same. The Rf value of xanthophyllsis (0.56); Chlorophyll a (0.63), chlorophyll b (.66) and carotene is the lowest pigment it shows (0.86) in *Clitoria ternatea* L. (white) and *Clitoria ternatea* L. Var. Pleniflora (blue var). If leaves from the same plant were used, the result might shows a more linear relationship, because while they are certainly related, leaf colour and pigment are not always accurate and predictors of photosynthetic activity (Rouhani 1977). An experiment exploring the balance of sunlight and temperature could help find the best environment for photosynthetic efficiency, leading to an increased yield of photosynthesis products.



Thin layer chromatogram pattern in *Clitoria ternatea* varities

Conclusion

Plants leaves contain a number of light absorbing pigments, many of which are associated with photosynthesis. Chlorophyll a and b are the primary photosynthetic pigments responsible for capturing light energy for use in photochemical reactions. In this experiment, the different pigments present in the leaves of *Clitoria ternatea* varieties are separated using the technique of thin layer chromatography. Most leaves are green due to chlorophyll. Leaves contain different pigments also, which give them their respective color. Green chlorophyll is the most common type of pigment, but there are also carotenoids and xanthophylls. Chlorophyll, which is essential for photosynthesis, usually hides the other pigments.

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