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FLAVONOID PROFILE OF *Clitoria ternatea* Linn

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ABSTRACT

The present study was aimed to reveal the flavonoid profile of Clitoria ternatea L leaves, stem and seeds using the HPTLC analysis. The powdered leaves, stem and seed samples were extracted with 150 mL of methanol for 8-12 h by using the Soxhlet apparatus. The Ethyl acetate-butanone-formic acid-water (5:3:1:1) was employed as mobile phase for flavonoids. The developed plate was sprayed with 1% ethanolic aluminium chloride as spray reagent and dried at 100°C in hot air oven for 2min. The plate was photo-documented at UV 366 nm and daylight using Photo-documentation chamber.The methanolic extract of stem, leaves and seeds of Clitoria ternatea showed the presence of 24 bands with 18 different Rf values with range 0.01 to 0.96. Out of 24 bands, 10 bands with seven Rf values viz., 0.04, 0.10, 0.28, 0.37, 0.49, 0.65 and 0.85 were idenified as flavonoids, the other bands were noted as unknown metabolites. Developed HPTLC chromatogram of Clitoria ternatea methanolic extracts of vegetative and reproductive parts could be used efficiently for identification, and quality assessment of the plant in the pharmaceutical industries. These profiles may used as chemical marker to solve plant systematic problems.

Keywords: Clitoria ternatea; HPTLC; Chromatography; Flavonoids.

INTRODUCTION

Flavonoids are present everywhere in nature and are classified into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. Flavonoids are water soluble polyphenolic molecules include 15 carbon atoms. Globally, about 4,000 flavonoids have been identified from plants (URL, 2010). The flavonoids have provoked extensive attention recently because of their medicinal properties and probable beneficial effects on human health. Flavonoids are becoming very popular because their biopotentials. A number of studies proved its biopotentials as antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor and antioxidant activities (URL, 2010). Recently, epidemiological studies have shown that flavonoid intake is inversely related to mortality from coronary heart disease and to the incidence of heart attacks. Flavonoids may help provide protection against neurodegenerative diseases such as Parkinson's and Alzheimer's by contributing, along with antioxidant vitamins and enzymes, to the total antioxidant defense system of the human body (URL, 2010). Studies have revealed that flavonoids prevent the oxidation of low-density lipoprotein thereby tumbling the risk for the development of atherosclerosis.

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In pharmacognosy, the phytochemical assessment is one of the important and vital tool for quality assessment, which includes preliminary phytochemical screening, chemoprofiling and marker compound analysis using modern analytical techniques such as fluorescence, UV-VIS, FT-IR, HPLC, HPTLC and GC-MS. Several pharmacopoeia including monographs of the plant materials illustrate only the physico-chemical characters. The modern methods portraying the classification and quantification of active constituents in the plant material may be helpful for proper standardization of herbs and its formulations (WHO, 1998; Hari Prasad and Ramakrishnan, 2012). Currently HPTLC is regularly used as an option to HPLC for the quantification of plant products because of its simplicity, accuracy, cost-effectiveness and rapidity (Wasim Aktar *et al.,* 2008; Yamunadevi *et al.,* 2012; Karthikeyan *et al.,* 2013). The standardized chromatographic profile is not only an alternative analytical tool for validation, but also an approach to express the differnt prototype of chemical constituents dispersed in the herbal drugs. In pharmacognosy, the HPTLC can operate as a tool to differntiate the herbal drug from its adulterants (Toma´s-Barbera´n *et al.,* 2001; Ram Mauji *et al.,* 2011).

Clitoria ternatea L. (Fabaceae) is an herbaceous perennial legume treasured for its medicinal importance. Due to its multiple

pharmaceutical applications the plant has been adopted in the traditional Indian system of medicine (folk medicine). The plant flavonoid posseses antibacterial, anxiolytic, anti-depressant, anticonvulsant, analges antipyretic activities, anti-inflammatory, and anti-stress properties and is believed to promote memory and intelligence (Parimaladevi *et al.,* 2003). The whole plants and seed extract are employed against stomaitis piles, sterility in female, hematemesis, insomnia, epilepsy, psychosis, leucorrhea and polyurea. The seeds are purgative, cathartic, and useful in visceralgia (Mhaskar *et al.,* 2010). Kumar et al. (2008) validated taraxerol in Clitoria ternatea using HPTLC. But there is no report on the flavonoids profile of Clitoria ternatea L. To fulfill the lacuna the present study was aimed for the identification of characteristic flavonoid HPTLC profiles of selected, medicinally important plant Clitoria ternatea leaves, stem and seeds. This work will provide a pave to identify possible phytochemical markers, to distinguish the medicinally important plant from its adulterants.

METHODOLOGY

Clitoria ternatea L. was collected from natural habitats, Coimbatore District, Tamil Nadu, India, and authenticated by Dr. E.G. Wesely, Department of Botany, AA College, Namakkal, Tamil Nadu and India. The fresh materials of *Clitoria ternatea* stem, leaves and seeds were separated shade dried and powdered using the electric homogenizer. The powdered samples were extracted with 150 mL of methanol for 8-12 h by using the Soxhlet apparatus. Preliminary phytochemical screening was done by following the standard method described by Harborne (1998). HPTLC studies were carried out following Wagner *et al.* (1996). For the present study CAMAG HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software were used. The Ethyl acetate-butanone-formic acid-water (5:3:1:1) was employed as mobile phase for flavonoids. The developed plate was sprayed with 1% ethanolic aluminium chloride as spray reagent and dried at 100°C in hot air oven for 2 min. The plate was photo-documented at UV 366 nm and daylight using Photo-documentation (CAMAG REPROSTAR 3) chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR3) and captured the images under White light, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag). Yellow fluorescence bands in the HPTLC plate confirmed the flavonoid presence in the given sample.

RESULTS AND DISCUSSION

The results of preliminary phytochemical analysis validated the existence of flavonoids in the methanolic extracts of *Clitoria ternatea* stem, leaves and seeds. For HPTLC analysis and separation, various compositions of the mobile phase were exaimned in order to obtain high resolution and reproducible peaks. The mobile phase Ethyl acetate-butanone-formic acid-water with the ratio 5:3:1:1 produced high resolution and reproducible peaks in the HPTLC system (Table 1 - 4; Fig. 1. A – J). The methanolic extract of stem, leaves and seeds of *Clitoria ternatea* showed the presence of 24 bands with 18 different Rf values with range 0.01 to 0.96 (Table – 1 to 4). Out of 24 bands, 10 bands with seven Rf values viz., 0.04, 0.10, 0.28, 0.37, 0.49, 0.65 and 0.85 were idenified as flavonoids, the other bands were noted as unknown metabolites. In general more degree of flavonoids diversity has been observed in leaves and stem when compared to the reproductive part (Seed). Maximum number (6) of flavonoids has been observed in leaves followed by stems (3) and seed (1). The flavonoid HPTLC system of seeds showed seven bands, of which only one band with Rf value 0.65 is idenitified as flavonoids. Out of seven bands, five bands were present only in seeds of *Clitoria ternatea* viz., 0.01, 0.11, 0.32, 0.38 and 0.8. The stems of *Clitoria ternatea* showed nine bands in the TLC system, of which four bands with Rf values 0.05, 0.58, 0.74 and 0.83 are unique to stem only (Table III). Eight bands were observed in the leaves of *Clitoria ternatea*. The bands with Rf values 0.04, 0.49, 0.85 and 0.95 are distinctive to the leaves and they failed to show their presence in other vegetative and reproductive parts of the plant. The band with the Rf value 0.65 is present commonly in seeds, leaves and stem of the plant. The bands with the Rf values 0.1, 0.28 and 0.37 are showed their jointly presence in stem and leaves of *Clitoria ternatea*. The flavonoids with the Rf values 0.96 was shared by seed and stem of *Clitoria ternatea*.

Flavonoids are omnipresent in photosynthesising cells and therefore distributed commonly in the plant kingdom (Deshmukh *et al.,* 2008). Flavonoids showed its presence in fruit, vegetables, nuts, seeds, stems and flowers and represent a common essential component of the human diet.

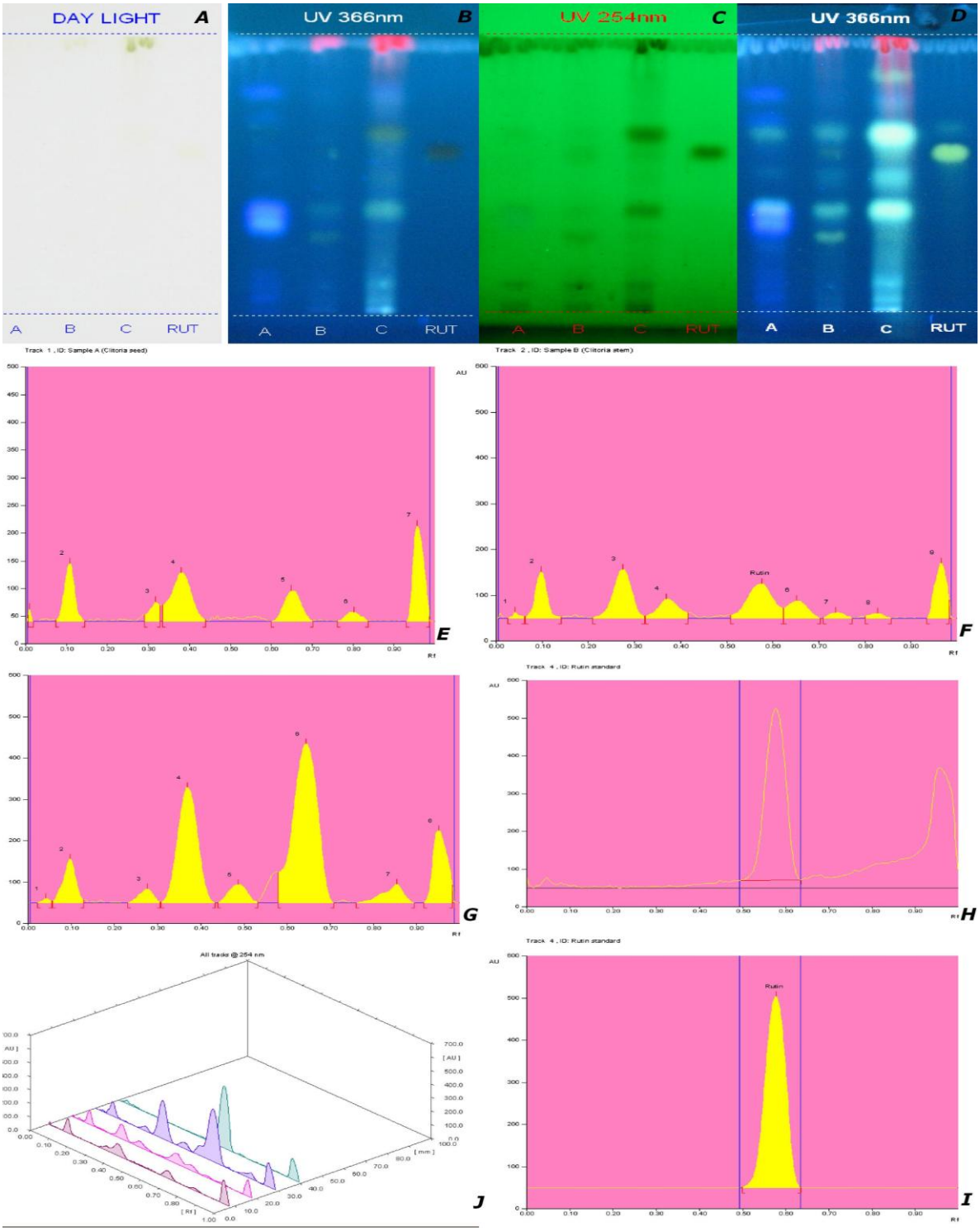

Figure 1. HPTLC Profile of *Clitoria ternatea* L. **A** before derivation – Day light; B. before derivation - under UV 366; C. before derivation - under UV 254; D. after derivation - under UV 366; E. seed - Peak densitogram display - Scanned at 254 nm; F. stem - Peak densitogram display - Scanned at 254 nm; G.leaves - Peak densitogram display - Scanned at 254 nm; H. Rutin– Base line display - Scanned at 254 nm; I. Rutin - Peak densitogram display - Scanned at 254 nm; J. 3D display of HPTLC Chromatogram of *Clitoria ternatea* – seed, stem and leaves methanolic extracts

Table I. HPTLC Flavanoid Profile of *Clitoria ternatea* methanolic extract

MW-Rf	Seed	Stem	Leaves
0.01	+		
0.04			+
0.05		+	
0.10		+	+
0.11	+		
0.28		+	+
0.32	+		
0.37		+	+
0.38	+		
0.49			+
0.58		+	
0.65	+	+	+
0.74		+	
0.80	+		
0.83		+	
0.85			+
0.95			+
0.96	+	+	

Table II. HPTLC Flavanoid Profile of *Clitoria ternatea* Seed

Peak	Rf	Height	Area	Assigned substance
1	0.01	22.7	123.8	Unknown
2	0.11	104.8	2427.5	Unknown
3	0.32	35.0	783.8	Unknown
4	0.38	88.1	4038.3	Unknown
5	0.65	55.4	2147.2	Flavonoid 1
6	0.80	16.8	583.1	Unknown
7	0.96	172.7	4211.2	Unknown
RUTIN	0.58	457.4	20373.5	Rutin standard

Table III. HPTLC Flavanoid Profile of *Clitoria ternatea* Stem

Peak	Rf	Height	Area	Assigned substance
1	0.05	13.6	172.8	Unknown
2	0.10	101.5	2373.6	Unknown
3	0.28	107.1	3992.5	Flavonoid 1
4	0.37	42.1	1780.9	Flavonoid 2
5	0.58	75.7	3917.9	Unknown
6	0.65	38.7	1537.8	Flavonoid 3
7	0.74	12.1	386.0	Unknown
8	0.83	10.7	312.2	Unknown
9	0.96	120.5	2810.4	Unknown
RUTIN	0.58	457.4	20373.5	Rutin standard

The results of the present study also confirm the flavonoids presence in the methanolic extract of stem, leaves and seeds of *Clitoria ternatea* and supplemented the previous observations. Increasingly, flavonoids are becoming the subject of medical research. A number of research indicated various useful properties such as antimicrobial activity, antioxidant activity, antiallergic activity, anti-inflammatory activity, oestrogenic activity and enzyme inhibition (Shirwaikar *et al.,* 2004; Manokaran *et al.,* 2008; Deshmukh *et al.,* 2008; Appia Krishnan *et al.,* 2009). In traditional medicines, medicinal plants have played incredibly to the traditional and western medicines in the drug discovery.

Table IV. HPTLC Flavanoid Profile of *Clitoria ternatea* Leaves

Peak	Rf	Height	Area	Assigned substance
1	0.04	11.4	173.1	Flavonoid 1
2	0.10	107.1	2874.5	Flavonoid 2
3	0.28	34.3	1031.9	Unknown
4	0.37	279.8	11961.3	Flavonoid 3
5	0.49	45.0	1775.4	Flavonoid 4
6	0.65	385.1	20287.4	Flavonoid 5
7	0.85	45.4	1923.6	Flavonoid 6
8	0.95	175.7	5368.9	Unknown
RUTIN	0.58	457.4	20373.5	Rutin standard

Chromatographic profile of metabolites can be employed for the evaluation of quality uniformity and stability of herbal extracts or products by visible observation and comparison of the standardized profile (Rajkumar *et al.,* 2010). In the present study we established the HPTLC profile for the vegetative and reproductive parts of *Clitoria ternatea* to identify and differentiate the *Clitoria ternatea* from the other crude drugs and its adulterants. The HPTLC chromatogram of *Clitoria ternatea* revealed the flavonoid occurrence in the seed (0.65), stems (0.28, 0.37 and 0.65) and leaves (0.04, 0.10, 0.37, 0.49, 0.65 and 0.85). The results of presdent study confirmed the flavonoids occurrence in the vegetative and reproductive parts and supported the multiple pharmaceutical applications. The HPTLC method developed for the identification of *Clitoria ternatea* is simple, precise, specific, accurate, rapid and cost effective. Developed HPTLC chromatogram of *Clitoria ternatea* methanolic extracts of vegetative and reproductive parts could be used efficiently for identification, and quality assessment of the plant.

CONCLUSION

The developed HPTLC fingerprints will help the manufacturer for quality control and standardization of herbal formulations. Such chemoprofiles are useful in differentiating the species from the adulterant and act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

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