WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 10, 766-775. Research Article ISSN 2277– 7105

HPTLC METHOD FOR ESTIMATION OF FLAVONOIDS AND PHENOLIC ACID IN *CLITORIA TERNATEA* ROOT EXTRACT

Saravanan J.[1], Swathi K. P.[1] and R. Arivukkarasu[2]

[1]Department of Pharmacology, KMCH College of Pharmacy Coimbatore, Tamil Nadu.
[2]Department of Pharmacognosy, KMCH College of Pharmacy, Coimbatore, Tamil Nadu.

Article Received on
11 August 2016,
Revised on 31 August 2016,
Accepted on 20 Sapt. 2016
DOI: 10.20959/wjpr201610-7095

***Corresponding Author'**
Swathi K. P.
Department of Pharmacology, KMCH College of Pharmacy Coimbatore, Tamil Nadu.

ABSTRACT

Clitoria ternatea is a well known bioactive plant used in traditional system of medicine. The aim of the present study is to evaluate the ethanolic extract of white flowered variety of *Clitoria ternatea* roots (EECT) with standard flavonoid markers such as rutin, quercetin, gallic acid by HPTLC techniques. The chromatographic separation was carried out on precoated silica gel $60F_{254}$ aluminium plates using mixture of Toluene- Ethyl acetate-Formic acid- Methanol (3 : 6 : 1.6: 0.4v/v) as mobile phase and densitometric evaluation of spots was carried out at 254 nm using Camag TLC Scanner-3 with win CAT 1.3.4 version software. The identity of the constituents in the ethanol extract of *Clitoria ternatea* were confirmed by overlaying their UV absorption spectra with the standard markers rutin, quercetin and gallic acid at 254 nm. The percentage of active constituents present in the extract was calculated from the peak area. From the result of HPTLC analysis it was evident that EECT contains phytoconstituents like gallic acid (0.8%) and rutin (0.06%).

KEYWORDS: HPTLC, *Clitoria ternatea*, rutin, gallic acid.

INTRODUCTION

Clitoria ternatea (Family: Fabaceae) is a well known plant in traditional medicine. It has two varieties blue flowered and white flowered. This has been used for centuries as a memory enhancer, antistress, anxiolytic, anticonvulsant, tranquilizing, antidepressant and sedative agent. It has antitubercular and antidysenteric properties and is an antidote to snake poison and scorpion poison.[1] A wide range of secondary metabolites such as flavonol glycosides,

triterpenoids, steroids and anthocyanins have been identified and isolated from *Clitoria ternatea*.[2] The extract of *Clitoria ternatea* possesses a wide range of pharmacological activities including anti inflammatory, analgesic, diuretic, antidiabetic, local anesthetic, antimicrobial, insecticidal, antipyretic, blood platelet aggregation-inhibiting and smooth muscle relaxing properties.[3] Earlier studies have proved that the roots of white flowered variety show good antioxidant activity as compared to blue flowered variety.[4] The antioxidant activities represent a key parameter to evaluate the biological property of the plant. Thus, it is essential to characterize and quantify the important compounds present in the plant.[5]

Nowadays the quality of medicinal plant products is assured by using modern analytical techniques and applying suitable standards. HPTLC has come out as a significant tool for the qualitative, and semi quantitative quantitative phytochemical analysis of herbal drugs. HPTLC has excellent resolution which permits simultaneous identification of several samples in a single run, using a small quantity of mobile phase. It is a high output, time saving and a rapid low cost analytical method.[6]

The aim of the present study is to evaluate the ethanolic extract of white flowered variety of *Clitoria ternatea* roots (EECT) with standard flavonoid markers such as Rutin, Quercetin, Gallic acid by HPTLC techniques

MATERIALS AND METHOD

Instrumentation

Analysis was performed on a Camag HPTLC system equipped with a sample applicator Linomat V, twin trough development chamber (10x10) size, TLC Scanner III, Wincats integration software was used.

Sample collection and extract preparation

The roots of white flowered variety of *Clitoria ternatea* were collected from Thrissur district of Kerala and authenticated from the Botanical survey of India (BSI) Coimbatore, Tamil Nadu (No.BSI/SRC/5/23/2015/Tech/2551). The coarse powder of *Clitoria ternatea* roots were extracted with ethanol in Soxhlet extractor. Extract was concentrated to dryness in rotary evaporator under reduced pressure to yield a dark brown mass of ethanol extract of *Clitoria ternatea* roots (EECT).

Extract preparation for HPTLC analysis

Accurately weighed 500mg of EECT was dissolved in 5ml ethanol. The sample was then filtered by using Whatmann filter paper (No.1).

Preparation of standard solution

Accurately weighed 10mg of standards (quercetin, rutin, and gallic acid) were taken separately in to 10ml volumetric flask, and the solution was made up to 10ml with ethanol to obtain a concentration 1mg/ml.

Samples Loading

The plates were pre-washed with methanol and activated at 60^0C for 10 min prior to chromatography. 2, 5, 10µl of EECT and 5µl of standard solutions such as Rutin, Gallic acid(GA) and Quercetin (QUER)were loaded as 6mm band length in the 10 x 10cm Silica gel $60F_{254}$ TLC plate using Camag microlite syringe and CAMAG LINOMAT V instrument.

Mobile phase

The organic solvents such as Toluene- Ethyl acetate- Formic acid- Methanol (3: 6: 1.6: 0.4 v/v) was used as a mobile phase. The twin trough developing chamber was saturated with the mobile phase for 10 minutes before the development of samples loaded TLC plate.

Spot development

The samples loaded plate was kept in TLC twin trough developing chamber with respective mobile phase and developed up to 85mm.

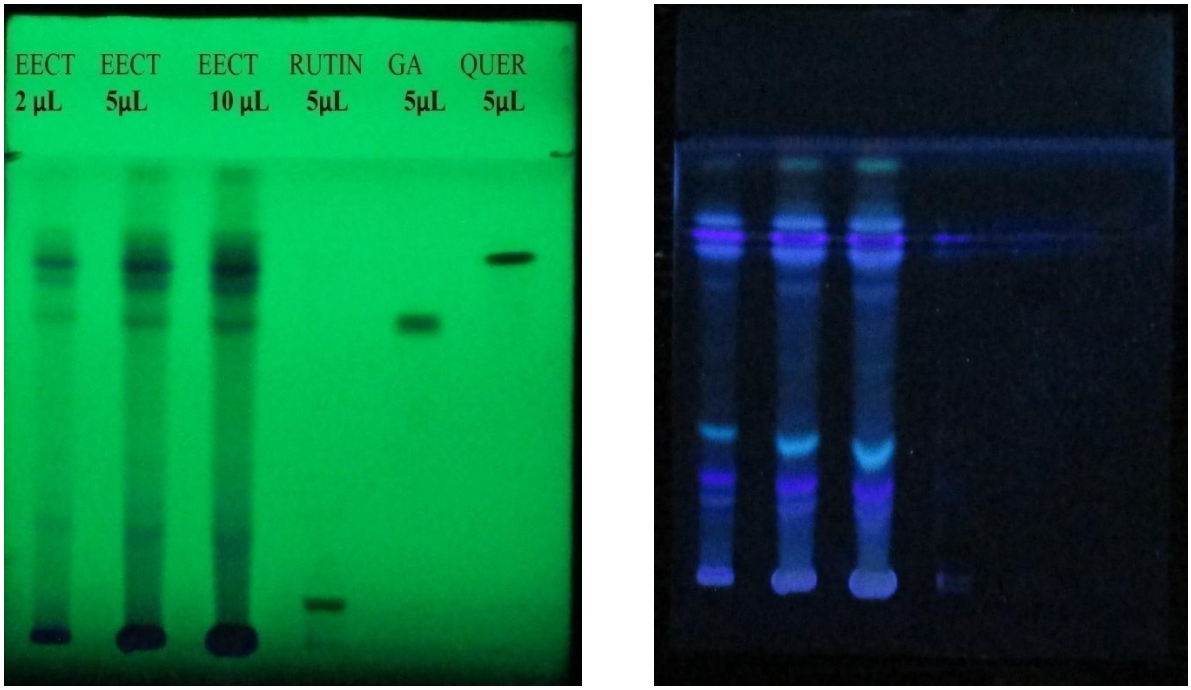
Photo-documentation

The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at UV 254 and 366nm.

Scanning

The plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at UV366 and 254nm.The peak table, peak display and peak densitogram were noted. The percentage of active constituents present in the extract was compared with that of standard.

RESULTS



A **B**

Fig.1: HPTLC Chromatogram of ethanol extract of *Clitoria ternatea* and standard markers. A-Photo documentation at 254nm, B-Photo documentation at 366nm

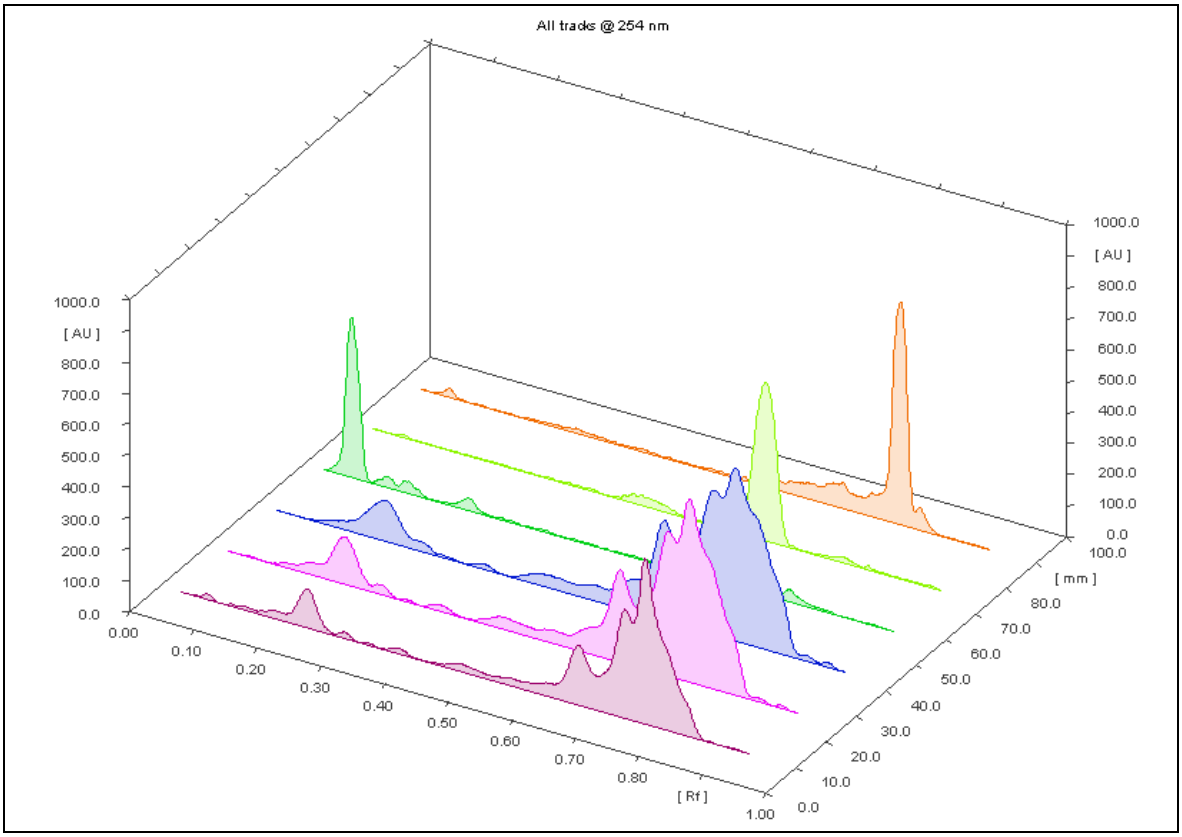


Fig. 2: 3D display of Standards and EECT at 254nm

Table 1: HPTLC analysis on *Clitoria ternatea* root extract correlate with standard flavonoid and phenolic acid marker compounds such as Rutin, Quercetin, Gallic acid.

Track No	Sample	Number of peaks	Rf	Standard present
1	EECT 2µl	10	**0.07**, 0.18, 0.23, 0.29, 0.38, 0.47, 0.55, **0.66**, 0.73 and 0.76	Rutin and Gallic acid
2	EECT 5 µl	09	0.21,0.27,0.36,0.47,0.54,**0.65**,0.73,0.76, and 0.87	Gallic acid
3	EECT 10 µl	10	0.20,0.26,0.36,0.46,0.54,**0.64**,0.72,0.76,0.87 and 0.90	Gallic acid
4	Std Rutin 5 µl	1	**0.07**	Rutin
5	Std Gallic acid 5 µl	1	**0.65**	Gallic acid
6	Std Quercetin 5µl	1	**0.79**	Quercetin

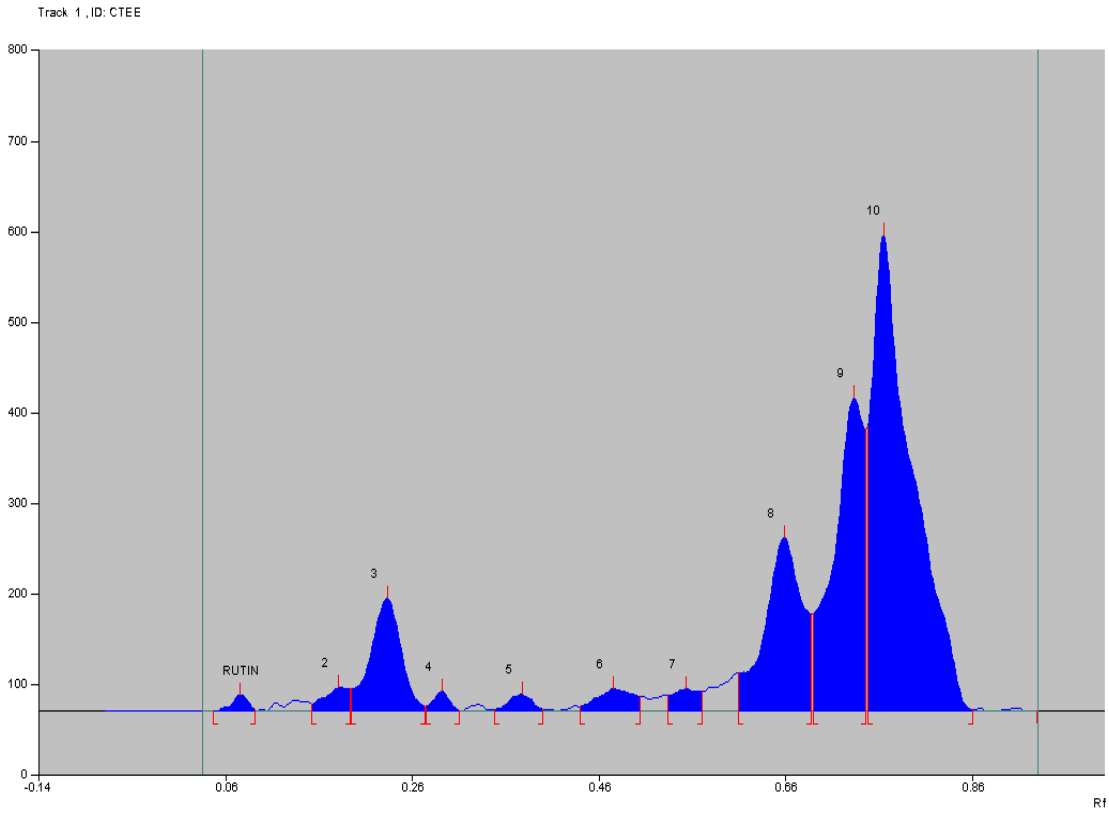


Fig.3: Chromatogram of EECT (2µl).

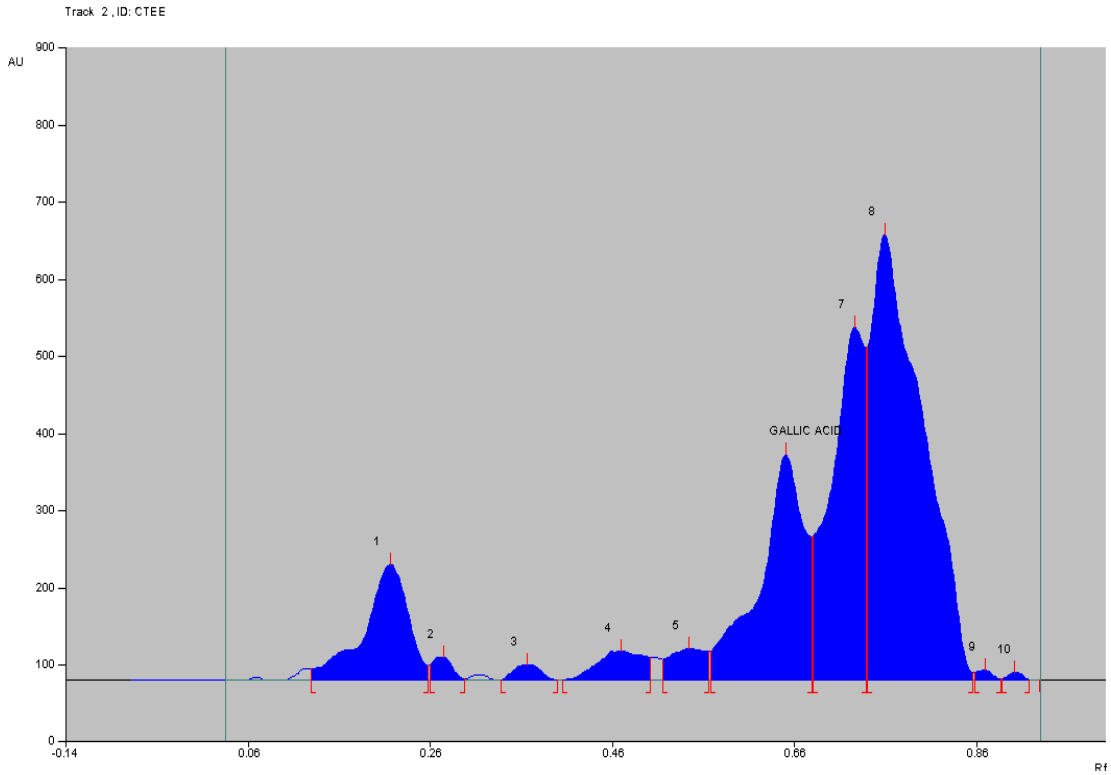


Fig.4: Chromatogram of EECT (5µl)

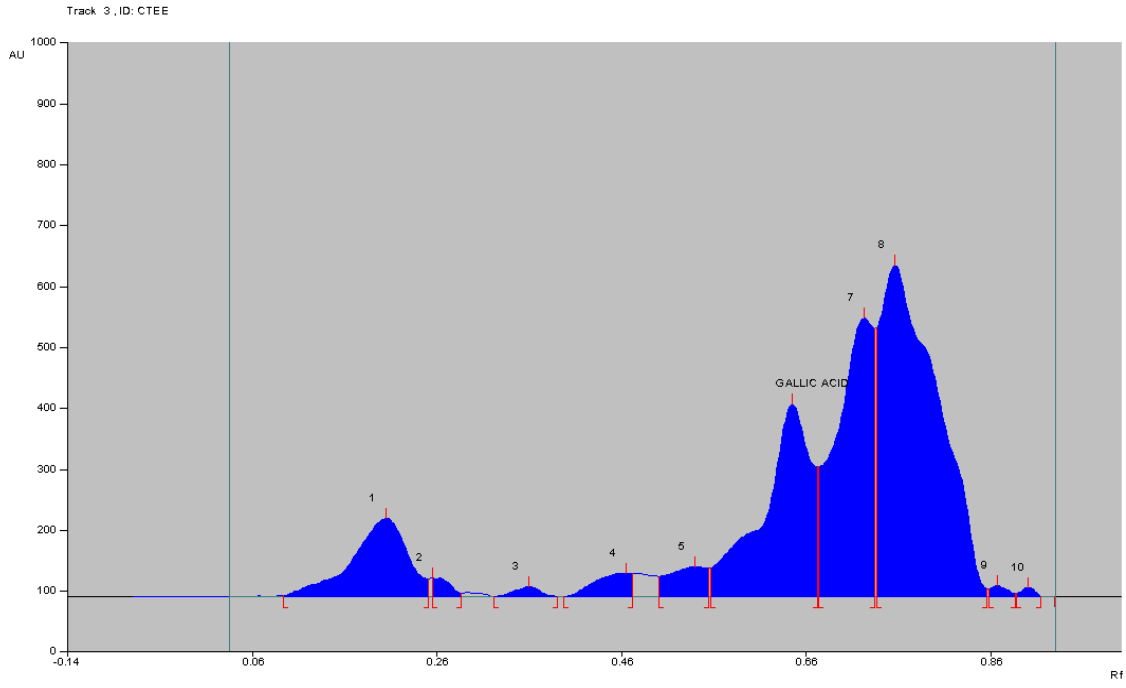


Fig.5: Chromatogram of EECT (10µl)

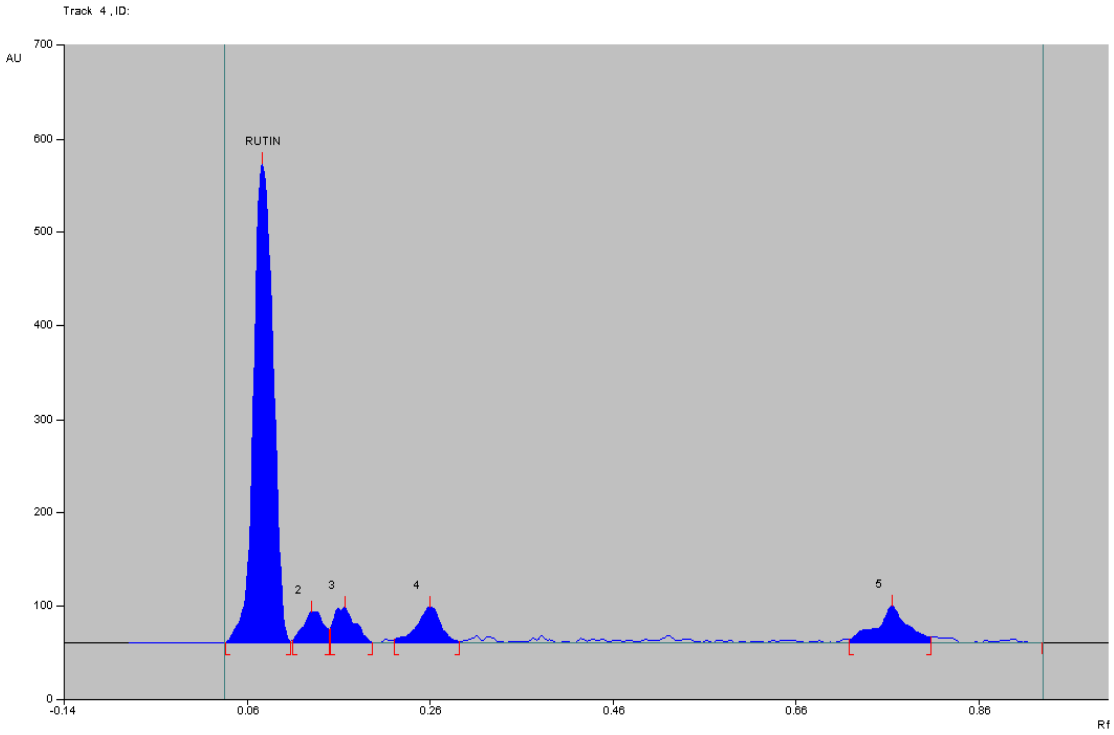


Fig.6: Chromatogram of standard rutin (5µl)

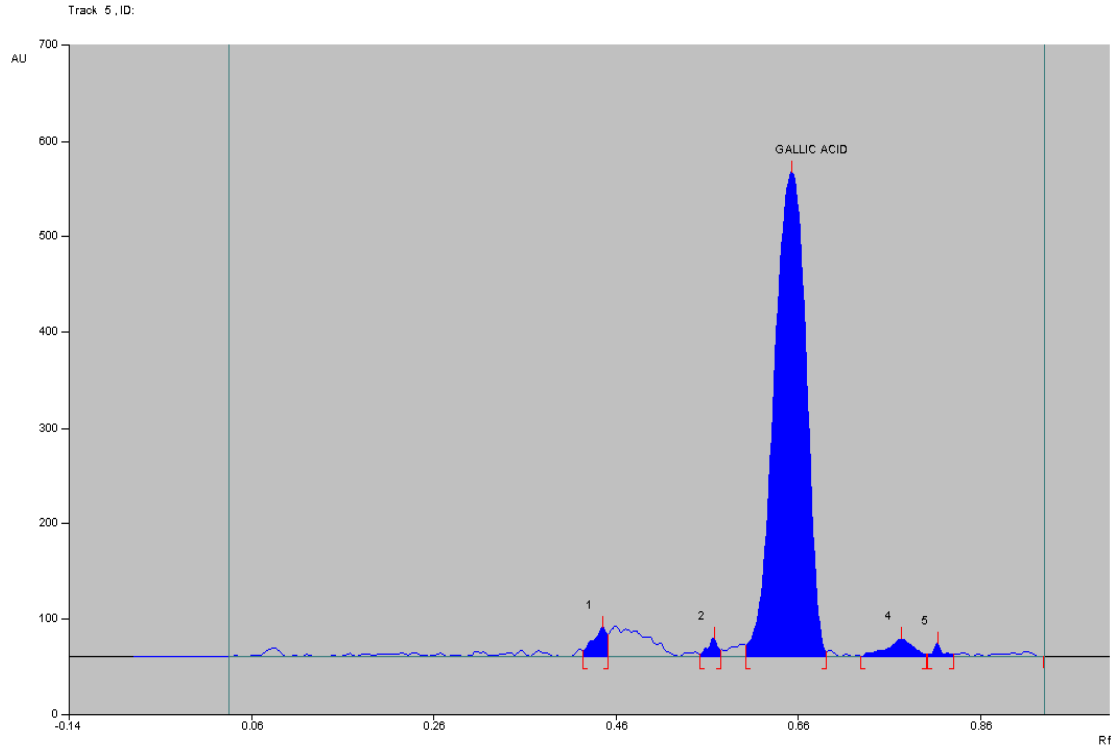


Fig.7: Chromatogram of standard gallic acid (5µl)

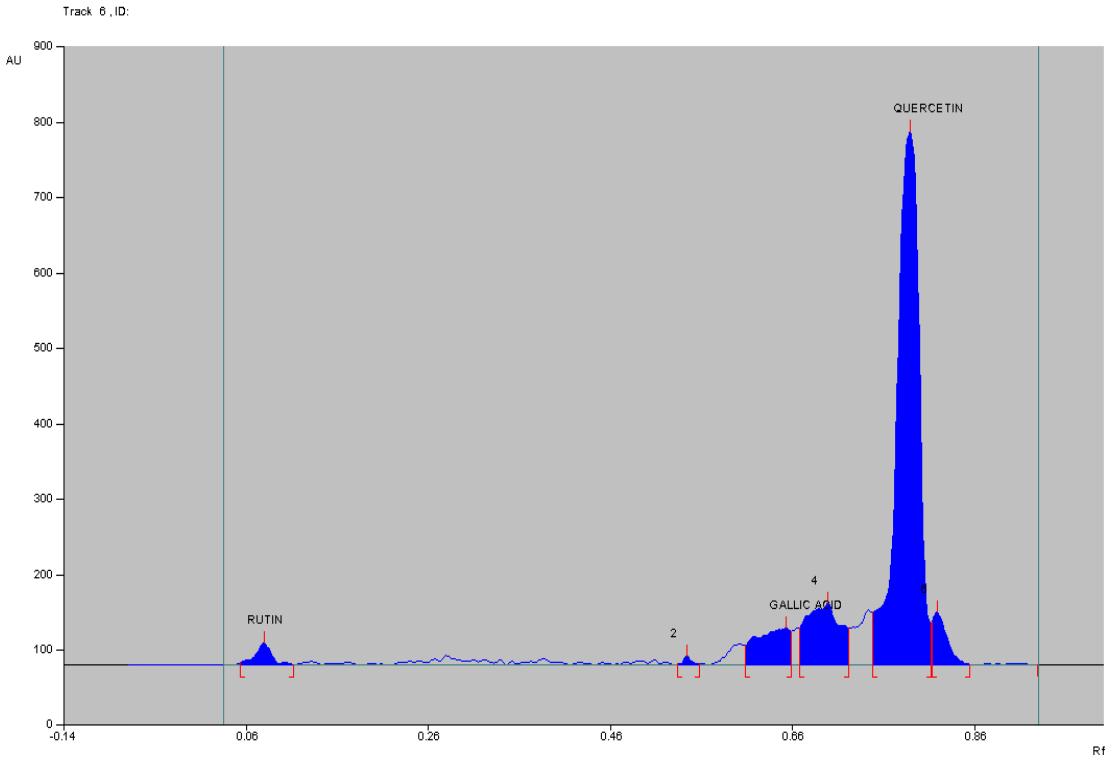


Fig 8: Chromatogram of standard quercetin (5µl)

DISCUSSION

Several studies have reported that the polyphenolic compounds like flavonoids, phenolic acids in the plant *Clitoria ternatea* may be the reason behind its multiple biological effects like antioxidant activity[4], anti inflammatory and analgesic activity[7][8], antiasthmatic activity[9], antidiabetic activity[10] etc. In order to justify and quantify the presence of these polyphenolic compounds the extract was subjected to HPTLC screening against marker compounds such as gallic acid, quercetin and rutin. The phytoconstituents in the three concentrations of sample were identified and confirmed by comparing the chromatogram obtained from the reference markers and quantified from the corresponding peak area. From the result of HPTLC analysis it was evident that EECT contains gallic acid and rutin.

Rutin is a flavonoid glycoside well-known as Vitamin P and has antiplatelet, antihypertensive and antiviral properties, as well as strengthing the capillaries of blood vessels, which are the results of its high free radical scavenging and antioxidant capacity. In addition hypolipidaemic, cytoprotective, antiallergic[11], anti-inflammatory, antibacterial and antiprotozoal properties[12], antispasmodic and anticarcinogenic[13] activities have also been reported.

Gallic acid is phenyl propanoid, chemically it is 3, 4, 5- Trihydroxybenzoic acid, possesses astringent activity and plays a potential protective role against different kinds of oxidative damaged diseases.[5] [14]

The phytoconstituents were quantified from the corresponding peak areas and it was found to contain 0.06% of rutin and 0.8% of gallic acid per 100mg of extract.

CONCLUSION

The present study revealed that the ethanol extract of white flowered variety of *Clitoria ternatea* root contain major polyphenolics, rutin and gallic acid which provide a scientific rationale for its multiple biological effects.

ACKNOWLEDGEMENT

The authors owe deep gratitude to Dr. A. Rajasekaran, Principal and Staff members in KMCH college of Pharmacy Coimbatore, for providing facilities to carry out the research work.

REFERENCES

1. A.K. Gupta. Reviews on Indian medicinal plants., 2008; 7th ed: 172-180.
2. R. Kavitha, V. Premalakshmi. Phytochemical analysis of ethanolic extract of leaves of *Clitoria ternatea* L, Int J Pharm Bio Sci., 2013 Oct; 4(4): 236–242.
3. Pulok K. Mukherjee, Venkatesan Kumara, N. Satheesh Kumara, Micheal Heinrich, The Ayurvedic medicine *Clitoria ternatea*—From traditional use to scientific Assessment, Journal of Ethnopharmacology., 2008 Sep; 120: 291–30.
4. A.P Patil, V.R.Patil. Comparative evaluation of antioxidant activity of root of blue and white flowered variety of *Clitoria ternatea* Linn, International Journal of Pharmacology., 2011; 1-7.
5. Tapan Seal. Quantitative HPLC analysis of phenolic acids, flavonoids and ascorbic acid in four different solvent extracts of two wild edible leaves, *Sonchus arvensis* and *Oenanthe linearis* of North-Eastern region in India. Journal of Applied Pharmaceutical Science., 2016 February; 6(02): 157-166.
6. P. Deepa, S. Murugesh *et al.* HPTLC analysis of *Dolichandrone atrovirens* (sprague) plant bark, world journal of pharmaceutical research., 2013 Dec; 3(1): 1019-1029.
7. Patil Amol P, Patil Vijay R. Anti-inflammatory and analgesic activity of leaves of white flowered variety of *Clitoria ternatea* linn. *Acta Biomedica Scientia.*, 2015; 2(1): 1-4.

8. B. Parimala Devi, R.Boominathan, Subhash C.Mandal. Anti inflammatory, analgesic and antipyretic properties of *Clitoria ternatea* root. Fitoterapia., 2003 Feb; 74: 345–349.
9. Dnyaneshwar J. Taur, Ravindra Y. Patil, Evaluation of antiasthmatic activity of *Clitoria ternatea* L. roots. Journal of Ethnopharmacology., 2011 Apr; 136: 374–376.
10. Prashant R. Verma, Prakash R. Itankar, Sumit K. Arora, Evaluation of antidiabetic, antihyperlipidemic and pancreatic regeneration, potential of aerial parts of *Clitoria ternatea* , Rev Bras Farmacogn., 2013 Oct; 23: 819-829.
11. Rosane, W. I, Oliveira, Z. D, Fernandes, S. C, Vieira, I. C. Development of a biosensor based on gilo peroxidase immobilized on chitosan chemically crosslinked with epichlorohydrin for determination of rutin. Journal of Pharmaceutical and Biomedical Analysis., 2006; 41: 366–372.
12. Casa, C. L, Villegas, I, Alarco´ n de la Lastra, C, Motilva, V, Martı´n Calero, M. J. Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. Journal of Ethnopharmacology., 2000; 71: 45–53.
13. Webster, R. P, Gawde, M. D, Bhattacharya, R. K. Protective effect of rutin, a flavonol glycoside, on the carcinogen-induced DNA damage and repair enzymes in rats. Cancer Letters., 1996; 109: 185–191.
14. Rajasekaran.A, Arivukkarasu.R, Archana.D. HPTLC Method for estimation of Gallic acid and Rutin in Haritaki -An Ayurvedic Formulation. International Journal of PharmTech Research., 2011 April; 3(2): 986-999.