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

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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 60F254 aluminium plates using mixture of Toluene- Ethyl acetate-Formic acid- Methanol (3 : 6 : 1.6: 0.4 v/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 antidiysenteric 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°C 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 60F254 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

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.

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

![Fig.3: Chromatogram of EECT (2µl).](image-url)
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)
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 strengthening the capillaries of blood vessels, which are the results of its high free radical scavenging and antioxidant capacity. In addition hypolipidaemic, cytoprotective, antiallerge\[^{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.\textsuperscript{[5]}\textsuperscript{[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 \textit{Clitoria ternatea} root contain major polyphenolics, rutin and gallic acid which provide a scientific rationale for its multiple biological effects.

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REFERENCES


