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QUALITATIVE AND QUANTITATIVE STUDY OF PHYTO-COMPOUND PRESENT IN ROOTS OF *CLITORIA TERNATEA* USING SPECTROSCOPY AND CHROMATOGRAPHIC TECHNIQUES

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Abstract

The present study was undertaken to evaluate the phytochemical profile, thin-layer chromatographic (TLC) characteristics, and total phenolic and flavonoid content of root extracts of *Clitoria ternatea*. Successive extraction was carried out using solvents of increasing polarity (hexane, chloroform, ethyl acetate, ethanol, and distilled water). The extractive values indicated maximum yield with aqueous (7.25% w/w) and ethanolic (6.67% w/w) solvents, suggesting a predominance of polar constituents. Preliminary phytochemical screening revealed the presence of flavonoids, phenols, proteins, carbohydrates, saponins, and diterpenes in different extracts, with flavonoids being consistently detected in ethyl acetate, ethanol, and aqueous extracts. TLC analysis confirmed the presence of multiple flavonoid components, with ethanol and ethyl acetate extracts showing the highest diversity and quercetin-like compounds (Rf ≈ 0.63). Quantitative estimation demonstrated that the ethanolic extract contained the highest phenolic (1.04 mg/100 mg) and flavonoid (1.65 mg/100 mg) content, followed by the aqueous extract (flavonoids: 1.41 mg/100 mg). These findings suggest that ethanol is the most efficient solvent for extracting phenolic and flavonoid constituents from *Clitoria ternatea* roots, highlighting its potential for further pharmacological and therapeutic investigations.

Introduction

Medicinal plants are a rich source of bioactive compounds that play a pivotal role in traditional medicine and modern drug discovery. Among these, *Clitoria ternatea* L. (family Fabaceae), commonly known as butterfly pea, is a perennial climbing legume widely distributed in tropical and subtropical regions of Asia and Africa. Traditionally, different parts of this plant, including roots, leaves, and flowers, have been utilized in Ayurvedic and folk medicine for their anti-inflammatory, anxiolytic, nootropic, antidiabetic, and nephroprotective activities

(Mukherjee et al., 2008; Jain et al., 2011). The root extracts, in particular, are reported to possess significant therapeutic potential owing to their rich phytochemical composition.

Phytochemical screening serves as a primary tool to identify secondary metabolites such as alkaloids, flavonoids, tannins, phenols, saponins, and glycosides, which are responsible for a variety of pharmacological activities (Harborne, 1998). Qualitative phytochemical tests provide preliminary insights, while quantitative estimations such as total phenolic content (TPC) and total flavonoid content (TFC) offer a measure of the concentration of these bioactive constituents. Phenolic compounds are known for their antioxidant and free radical scavenging properties, while flavonoids exhibit diverse biological activities including anti-inflammatory, cardioprotective, and neuroprotective effects (Pietta, 2000; Panche et al., 2016).

Spectrophotometric methods, particularly the Folin–Ciocalteu assay for TPC and aluminum chloride colorimetric assay for TFC, are widely employed for such quantitative analysis owing to their simplicity, sensitivity, and reproducibility (Singleton & Rossi, 1965; Chang et al., 2002). In addition, chromatographic techniques such as thin layer chromatography (TLC) provide valuable qualitative and semi-quantitative information on the phytochemical profile of plant extracts. TLC not only confirms the presence of phytoconstituents but also aids in the preliminary identification of compounds through retention factor (Rf) values when compared with standards (Wagner & Bladt, 1996).

Given the pharmacological relevance of *Clitoria ternatea*, systematic evaluation of its root extracts using spectrophotometric and chromatographic approaches is essential to establish a scientific basis for its therapeutic applications. The present study focuses on the qualitative and quantitative assessment of phytocompounds in the roots of *Clitoria ternatea* through preliminary phytochemical screening, determination of TPC and TFC, and confirmation by TLC profiling. This integrated approach provides comprehensive insights into the phytochemical richness of the roots, highlighting their potential as a source of natural therapeutic agents.

Material and Methods

Material

The chemicals and reagents used in the study were procured from reputed suppliers to ensure analytical-grade quality and reliability of results. Aluminum trichloride, Fehling's A & B solutions, Folin Ciocalteu reagent,

potassium dichromate, potassium iodide, sodium bicarbonate, and lead acetate were obtained from Loba Chemie Pvt. Ltd., Mumbai, while chloroform, ethanol, and methanol were supplied by Qualigens Fine Chemicals, Mumbai. Dichloromethane, disodium hydrogen phosphate, sodium chloride, and sulfuric acid were purchased from S. D. Fine Chem. Ltd., Mumbai. Ferric chloride and sodium nitroprusside were sourced from Remedy Labs, Ahmedabad. Gelatin and nitric acid were procured from Thomas Bekar Chemicals Pvt. Ltd., Mumbai, whereas picric acid was obtained from Evans Chem India Pvt. Ltd., Mumbai. Additionally, zinc chloride and sodium hydroxide were supplied by Merck India Ltd., Mumbai. These chemicals were used for qualitative and quantitative phytochemical analyses, including total phenolic content (TPC), total flavonoid content (TFC), and thin-layer chromatography (TLC).

Methods

Collection of *Clitoria ternatea*

Roots of *Clitoria ternatea* were collected from Vindhya Herbals Bhopal. After the plant was collected they have been processed for cleaning in order to prevent the deterioration of phytochemicals present in plant. After collection, excess soil is gently removed from the roots without washing them to prevent loss of water-soluble compounds. The roots are then spread evenly on drying trays in a well-ventilated, shaded area to air dry naturally.

Successive extraction with different solvents by maceration method

The shade dried roots of *Clitoria ternatea* (50gm) were extraction with Hexane using maceration method. The extraction was continued till the defatting of the material had taken place.

Defatted roots powdered of *Clitoria ternatea* were exhaustively extracted with different solvent (Chloroform, ethyl acetate, ethanol and distilled water) by maceration process. The extracts were evaporated above their boiling points. Finally, measured the percentage yield of the dried extracts. The recovered extracts were then reduced in a rotary evaporator and finally stored in airtight containers at 4°C for further use.

Determination of extractive value (% yield)

The % yield of yield of each extract was calculated by using formula:

$$\textbf{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Phytochemical screening

Plants produce a wide variety of chemical

compounds that are not directly involved in their primary metabolic processes such as growth, development, and reproduction. These compounds are known as secondary metabolites or natural products.

Secondary metabolites have been of great interest to researchers because of their diverse biological activities, such as serving as defense mechanisms against herbivores, pathogens, and environmental stresses, as well as playing important roles in plant signaling and communication.

Secondary metabolites can be classified into several categories based on their chemical structures and biosynthetic pathways. Some of the major classes of secondary metabolites in plants include alkaloids, flavonoids, terpenoids, phenolics, and glucosinolates. These compounds can have a range of pharmacological properties and have been used in traditional medicine for thousands of years.

The qualitative chemical experiments were carried out with some modifications for different extracts according to the standard methods (Trease and Evans; 1978).

Separation and Identification of phytoconstituents in *Clitoria ternatea* extract by thin layer chromatography

Each solvent extract was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Glass capillaries were used to spot the sample for TLC applied sample volume 1-micro litre by using capillary at distance of 1 cm at 5 tracks. In the twin trough chamber with different solvent system toluene: ethyl acetate: formic acid (5:4:1) for flavonoids used (Anil et al., 2023). After pre-saturation with mobile phase for 20 min for development were used. The movement of the active compound was expressed by its retention factor (Rf), values were calculated for different samples. The developed thin layer chromatographic plates were visualized in normal light, short UV light (254nm), and long UV light (365nm) using TLC cabinet (Electronic India).

Once chromatogram was developed the R_f Value of spot was calculated using formula:

$$\mathbf{Rf} = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

amount of gallic acid per gram or milliliter of the extract. The determination of TPC is typically carried out using spectrophotometric

Quantitative estimation of bioactive compound

Estimation of total phenol content

The total phenolic content of the extract was determined by the modified folin-ciocalteu method (Parkhe et al., 2019). 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 5 - 25µg/ml was prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Two ml (1mg/ml) of this solution was used for the estimation of phenol. 2 ml of each extract or standard was mixed with 1 ml of folin-ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 15 min for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Estimation of total flavonoids content

Determination of total flavonoids content was based on aluminium chloride method (Meda et al., 2005). 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5-25µg/ml were prepared in methanol. 10mg of dried extracts of were dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this solution was used for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; absorbance was measured at 420 nm.

Results and Discussion

The extractive values of *Clitoria ternatea* root (Table 1) revealed that the highest yield was obtained with distilled water (7.25% w/w), followed by ethanol (6.67% w/w), ethyl acetate (4.32% w/w), chloroform (0.75% w/w), and hexane (0.42% w/w). This indicates that the root constituents are predominantly polar in nature, as aqueous and ethanolic solvents extracted higher quantities compared to non-polar solvents such as hexane and chloroform.

Phytochemical screening (Table 2) demonstrated that flavonoids were consistently present in ethyl acetate, ethanol, and aqueous extracts, suggesting their strong polarity and affinity towards medium-to-high polarity solvents. Phenolic compounds were detected in chloroform and ethanol extracts, while proteins were observed in ethanol and aqueous extracts. Carbohydrates were detected only in the ethyl acetate extract, while saponins were confirmed in aqueous extract. Interestingly, diterpenes were only present in the chloroform extract, while sterols and tannins were absent in all tested extracts. These results confirm that the phytochemical profile of *Clitoria ternatea* roots is highly solvent-dependent.

Thin layer chromatography (TLC) analysis (Table 3, Figure 1) further supported the presence of flavonoids. Ethanol and ethyl acetate extracts exhibited the highest number of spots under UV light, suggesting a diverse array of flavonoid compounds. The ethanol extract showed 6 spots under short UV and 10 spots under long UV, indicating rich chemical complexity and higher flavonoid diversity compared to other extracts. The Rf values of certain spots were comparable to the standard quercetin (Rf = 0.63), confirming the presence of quercetin-like flavonoids in these extracts. The aqueous extract, however, did not exhibit any detectable spots under UV light, despite showing positive results for flavonoids in preliminary screening, which may be attributed to the low concentration of flavonoids or limitations of the solvent system used.

Quantitative analysis (Table 4) revealed that the ethanol extract contained the highest total phenolic content (1.04 mg/100 mg) and total flavonoid content (1.65 mg/100 mg), followed by the aqueous extract with significant flavonoid content (1.41 mg/100 mg) but negligible phenolic content. Ethyl acetate extract also showed moderate phenolic (0.50 mg/100 mg) and flavonoid (0.71 mg/100 mg) content, while chloroform extract contained the least amount. These results suggest that ethanol is the most efficient solvent for extracting phenolic and flavonoid compounds from *Clitoria ternatea* roots.

The findings confirm that *Clitoria ternatea* root is a rich source of flavonoids and phenolic compounds, which are known for their antioxidant, anti-inflammatory, and neuroprotective properties. The ethanol extract, in particular, demonstrated superior phytochemical richness and diversity, making it a potential candidate for further pharmacological investigations. The variation in extractive yield and phytochemical profile across solvents emphasizes the importance of solvent selection in phytochemical studies.

Table 1: Extractive values of root extracts of *Clitoria ternatea*

Sr. No	Extracts	% Yield (w/w)
1	Hexane	0.42

2	Chloroform	0.75
3	Ethyl acetate	4.32
4	Ethanol	6.67
5	Distilled water	7.25

Table 2: Result of phytochemical screening of Hexane extract of *Clitoria ternatea*

S. No.	Constituents	Hexane extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	**Alkaloids**					
	Hager's Test:	-ve	-ve	-ve	-ve	-ve
	Wagner Test:	-ve	-ve	-ve	-ve	-ve
2.	**Glycosides**					
	Conc. H_2SO_4 Test:	-ve	-ve	-ve	-ve	-ve
3.	**Flavonoids**					
	Lead acetate Test:	-ve	+ve	+ve	+ve	+ve
	Alkaline Reagent Test:	-ve	+ve	+ve	+ve	+ve
4.	**Diterpenes**					
	Copper acetate Test:	-ve	+ve	-ve	-ve	-ve
5.	**Phenol**					
	Ferric Chloride Test:	-ve	-ve	-ve	-ve	-ve
	Folin Ciocalteu Test:	-ve	+ve	+ve	+ve	-ve
6.	**Proteins**					
	Xanthoproteic Test:	-ve	-ve	-ve	+ve	+ve
7.	**Carbohydrate**					
	Fehling's Test:	-ve	-ve	+ve	-ve	-ve
8.	**Saponins**					
	Froth Test:	-ve	-ve	-ve	-ve	+ve

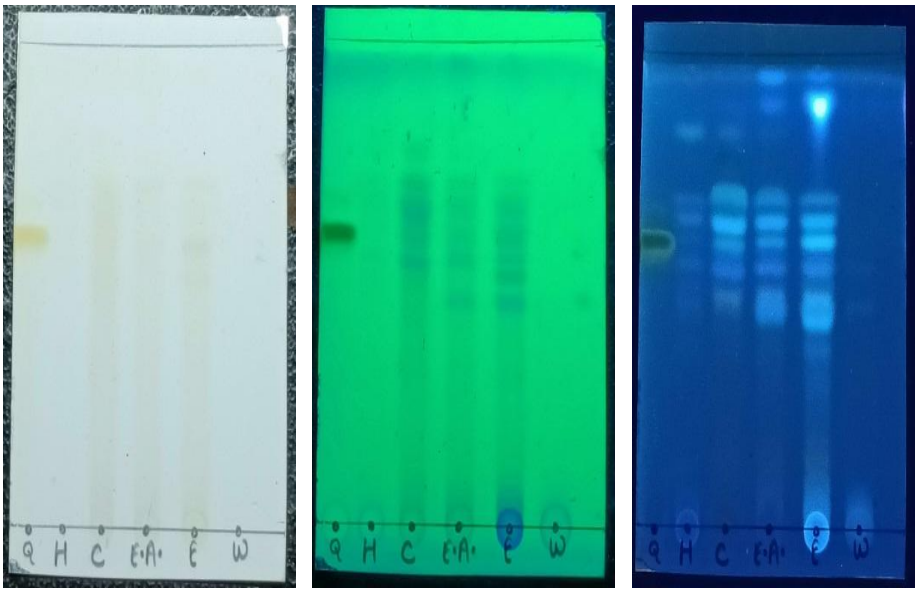
9.	**Tannins** Gelatin Test:	-ve	-ve	-ve	-ve	-ve
10.	**Sterols** Salkowski's Test:	-ve	-ve	-ve	-ve	-ve

+Ve = Positive, -Ve= Negative

Table 3: TLC of *Clitoria ternatea*

TLC of *Clitoria ternatea* (Flavonoids)			
S. No.	**Mobile phase** Toluene: Ethyl acetate Formic acid (5:4:1)	**Distance of solute**	***Rf* value**
1.	**(Quercetin)** Dis. travel by mobile phase = 6 cm No. of spot at normal light = 1 No. of spot at short UV = 1 No. of spot at long UV = 1	Normal Light- 3.8 Short- 3.8 Long- 3.8	Normal- 0.63 Short- 0.63 Long- 0.63
2.	**Hexane extract** Dis. travel by mobile phase = 6 cm No. of spot at normal light = 0 No. of spot at short UV = 1 No. of spot at long UV = 6	Normal Light- 0 Short- 3.4 Long- 3.1, 3.5, 3.8, 4, 5.1, 6	Normal- 0 Short- 0.56 Long- 0.51, 0.58, 0.63, 0.66, 0.85, 1
3.	**Chloroform extract** Dis. travel by mobile phase = 6 cm No. of spot at normal light = 2 No. of spot at short UV = 5 No. of spot at long UV = 6	Normal Light- 3.7, 4 Short- 3.4, 3.7, 4, 4.4, 4.9 Long- 3, 3.3, 3.7, 4, 4.4, 5.2	Normal- 0.61, 0.66 Short- 0.56, 0.61,0.66, 0.73, 0.81 Long- 0.5, 0.55, 0.61, 0.66, 0.73, .81
4.	**Ethyl acetate extract** Dis. travel by mobile phase = 6 cm No. of spot at normal light = 1 No. of spot at short UV = 5 No. of spot at long UV = 9	Normal Light- 3.6 Short-2.9, 3.4, 3.7, 4, 4.4	Normal- 0.6 Short- 0.48, 0.56, 0.61, 0.66, 0.73

		Long- 2.7, 2.9, 3,3.4, 3.7, 4, 4.4, 5.5, 6	Long- 0.45, 0.48, 0.5, 0.56, 0.61, 0.66, 0.73, 0.91,1
5.	**Ethanol extract** Dis. travel by mobile phase = 6 cm No. of spot at normal light = 1 No. of spot at short UV = 6 No. of spot at long UV = 10	Normal Light- 3.6 Short- 2.8, 3.2, 3.5, 3.7, 4, 4.5 Long- 2.3, 2.6, 2.9, 3, 3.4, 3.7, 4, 4.2, 5.5, 6	Normal- 0.6 Short-0.46, 0.53, 0.58, 0.61, 0.66, 0.75 Long- 0.38, 0.43, 0.48, 0.5, 0.56, 0.61, 0.66, 0.7, 0.91, 1
6.	**Aqueous extract** Dis. travel by mobile phase = 6 cm No. of spot at normal light = 0 No. of spot at short UV = 0 No. of spot at long UV = 0	Normal Light- 0 Short- 0 Long- 0	Normal- 0 Short- 0 Long- 0



Normal light **Short UV** **Long UV**

Figure 1: TLC of Flavonoids in *Clitoria ternatea*

1st spot= Standard Quercetin, 2nd spot= Hexane extract, 3rd spot= Chloroform extract, 4th spot= Ethyl acetate extract, 5th spot= Ethanol extract, 6th spot= Aqueous extract

Table 4: Results of total phenol and flavonoids content of roots extract of *Clitoria ternatea*

S. No.	Extracts	Total phenol content	Total flavonoids content

		mg/100mg	
1	Chloroform	0.34	0.57
2	Ethyl acetate	0.50	0.71
3	Ethanolic	1.04	1.65
4	Aqueous	-	1.41

Conclusion

The present investigation demonstrated that *Clitoria ternatea* roots are a rich source of bioactive phytochemicals, particularly flavonoids and phenolic compounds. Among the tested solvents, ethanol proved to be the most effective for extraction, yielding the highest phenolic and flavonoid content, followed by aqueous and ethyl acetate extracts. TLC profiling confirmed the presence of multiple flavonoid constituents, including quercetin-like compounds. These findings suggest that ethanolic root extract of *Clitoria ternatea* holds significant potential for further pharmacological evaluation and therapeutic applications, particularly in areas related to antioxidant and neuroprotective activities.

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