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Phytochemical screening of different solvent leaf extract of *Clitoria ternatea* and antimicrobial activity of ethanolic leaf extract of *Clitoria ternatea*

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Abstract

The goal of this work was to assess antibacterial activity and phytochemical components of various solvent extracts of Clitoria ternatea leaves. Ethanol, aqueous, acetone, and chloroform solvents were used to collect, shade dry, grind, and extract leaf samples. To find bioactive substances such as carbohydrates, tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenols, terpenoids, and steroids, preliminary phytochemical screening was carried out. The findings showed that all solvent extracts contained carbohydrates and alkaloids, but the ethanol extract had a high concentration of tannins, flavonoids, quinones, phenols, terpenoids, and steroids. Using the agar well diffusion method, antibacterial activity of the ethanolic leaf extract was assessed against the bacterial strains Proteus mirabilis, Bacillus subtilis, Escherichia coli, and Streptococcus pneumoniae. The extract was tested at three different concentrations (50, 75, and 100 µg/mL), and the highest zone of inhibition against all tested species was found at 100 µg/mL. This study demonstrates that Clitoria ternatea leaves include phytochemical components and antibacterial activity, indicating their possible use in antimicrobial formulations and natural medicinal agents.

Keywords: *Clitoria ternatea*, Chloroform extract, Acetate extract, Ethanolic extract, Aqueous extract, Phytochemicals and Antimicrobial activity.

1. Introduction

Clitoria ternatea, commonly known as Butterfly pea, belongs to the family Fabaceae and sub-family Papilionaceae, is a perennial leguminous twiner, which originated from tropical Asia and later was distributed widely in South and Central America, the East and West Indies, China and India [1]. Where it has become naturalised, *C. ternatea* contains antifungal proteins and is homologous to plant defensins. Using a passive avoidance test and a spatial learning T-maze, researchers have shown that the aqueous root extract of C. ternatea enhances memory in rats [2]. *Clitoria ternatea* is cultivated throughout India but is naturalised in the more tropical regions [3]. It is known by a number of common names, including butterfly pea. All parts of the plants (roots, seeds and leaves) are used medicinally and

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are purported to have a range of actions, including effects to improve cognitive function and reduce dementia, treatment of respiratory conditions such as asthma and bronchitis, treatment of inflammation and laxative and diuretic actions [4]. Therefore, in this study, we focus on the studies on the antimicrobial activities of extracts from *Clitoria ternatea* L., belong to the Fabaceae family, against general food spoilage and human pathogens, so that new food preservatives can be explored and developed on the basis of the natural resources [5]. The roots are useful in asthma, burning sensation, ascites, inflammation, leucoderma, leprosy, hemicrania, amentia, pulmonary tuberculosis, ophthalmology and reported as bitter, refrigerant, ophthalmic, laxative, diuretic, cathartic, aphrodisiac, tonic [6].

In addition to the identification of various anthocyanins and flavonol glycosides, other components such as 6-malonylastragalin, phenylalanine, coumaroyl sucrose, tryptophan and coumaroyl glucose were determined [7]. These effects are attributed to the bioactive compounds in butterfly pea flowers, namely polyphenols and anthocyanins, which inhibit the enzyme alpha-amylase in the pancreas and alpha-glucosidase activity [8]. These findings support the potential of butterfly pea extract as an antidiabetic agent [9]. butterfly pea flower extract has been found to inhibit the cyclooxygenase enzyme, thereby impeding arachidonic acid metabolism, prostaglandin production, and inflammatory processes [10].

2. Experimental selections

2.1 Collection of plant material

The leaves of *Clitoria ternatea* were freshly collected from a well-matured plant from Aadhiur, a village in Tirupattur District, Tamil Nadu. The leaves are washed with water and dried carefully in the absence of sunlight to remove the water molecules present in the leaves. The dried leaves are made into a fine powder using a blender. Then the fine powders are stored properly in an airtight container for future use.

Fig. 1. *Clitoria ternatea* (Butterfly Pea) plant and leaves

2.1.1 Taxonomical Classification

Kingdom: Plantae

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)

Division : Magnoliophyta (Flowering plants)

Class : Magnoliopsida (Dicotyledons)

Subclass : Rosidae

Order : Fabales

Family : Fabaceae (Legume family)

Genus : *Clitoria*

Species : *Clitoria ternatea L.*

2.2 Extraction of the sample

About 5 grams of fine powder of the leaf was mixed with 50 mL of ethanol. Then the suspension is left to 24 hours

for incubation; meanwhile, occasional shaking is allowed. After 24 hours of incubation, the contents are filtered through what man No. one filter paper. Then the filtrate was collected separately and left for evaporation using vacuum distillation and dried. The residues Ethanol, Aqueous, Acetone and Chloroform extract of *Clitoria ternatea* were collected and stored in an airtight container for further analysis [11].

2.3 Qualitative Phytochemical Screening

The qualitative tests were carried out in the leaves of *Clitoria ternatea* by adopting the standard procedure [12] [13]. The Ethanol, aqueous, acetone and chloroform extracts were screened for the presence of phytochemicals.

1. Test for alkaloids

Mayer's test: a small portion of solvent-free extract was stirred with a few drops of diluted HCl and filtered. The filtrate was then tested for the following colour test. (a) 1.36 g of mercuric chloride was dissolved in 60 mL of distilled water. (b) 5 g of potassium iodide was dissolved in 20 ml of distilled water, and (a) and (b) were mixed, and the volume was adjusted to 100ml with distilled water. Appearance of cream-coloured precipitate with Mayer's reagents showed the presence of alkaloids.

2. Test for flavonoids

Shinoda's test: 5 ml of 20% sodium hydroxide was added to an equal volume of the extract. A yellow solution indicates the presence of flavonoids.

3. Test for steroids

Liebermann Buchard test: A small amount of sample is treated with 2ml of acetic anhydride, followed by the addition of 3ml of H_2SO_4 Solution. Colour changes from violet to green or blue indicate the presence of steroids.

4. Test for terpenoids

Salkowski Test: To 1ml of extract, add 0.5ml of chloroform followed by a few drops of concentrated sulphuric acid. The formation of reddish-brown precipitate indicates the presence of terpenoids.

5. Test for Saponins

Froth test: 5ml of extract is diluted with 20ml of distilled water and agitated for 10 minutes. Foam is formed, which indicates the presence of saponins.

6. Test for Carbohydrates

Fehling test: Two millilitres of each plant extract were hydrolysed with dilute HCl, neutralised with alkali, and then heated with Fehling's solution A and B. The formation of a red precipitate was an indication of the presence of reducing sugar.

7. Test for tannins and phenolic compounds:

Lead Acetate test: 10% lead acetate solution, 0.5g of the extract was added and shaken to dissolve. A white precipitate observed indicates the presence of tannins and phenolic compounds.

8. Test for glycosides

Keller-Killani test: To 2ml of extract, glacial acid, one drop 5% ferric chloride and concentrated sulphuric acid were added. Appearance of reddish-brown colour at the junction of the two liquid layers indicates the presence of glycosides.

9. Test for Quinones

Sulfuric acid test: One drop of concentrated sulfuric acid was added to 5 mL of each extract dissolved in isopropyl alcohol. Formation of red colour indicates the presence of quinones.

10. Test for Phenols

The sample solution is treated with a few drops of 10% ferric chloride. Formation of blue or green colour indicates the presence of phenols.

11. Test for saponins

Two millilitres of distilled water were added to the sample solution and shaken well. Formation of foams indicates the presence of saponins.

Antibacterial activity

Antibacterial activity was analysed with the standard agar well diffusion method. Different concentrations of the methanolic extract (50, 100, 150 µg/mL) were prepared by using 5% DMSO (Dimethylsulphoxide). 10µL of 24h test cultures, such as Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Streptococcus pyogenes, and Bacillus subtilis, were spread onto respective Muller-Hinton agar medium by the spread plate method. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was analysed by measuring the diameter of the zone of inhibition formed around the well [14,15].

3. Result and Discussion

3.1 Phytochemical analysis

Using various solvent extracts of *Clitoria ternatea* leaf was studied for the analysis of phytochemical compounds. It shows the presence of carbohydrates & alkaloids in all various solvents. Table 1 indicates some of the phytochemical compounds present in the various solvents. [16].

Table 1: Phytochemical analysis of various solvent extracts of *Clitoria ternatea* leaf

S. No.	Phyto Chemical Compounds	*Clitoria ternatea* ethanol extract of leaf	*Clitoria Ternatea* aqueous extract of leaf	*Clitoria Ternatea* acetone extract of leaf	*Clitoria ternatea* chloroform extract of leaf
1	Carbohydrate	+	+	+	+
2	Tannins	+	+	+	–
3	Saponins	–	–	–	+
4	Alkaloids	+	+	+	+
5	Flavonoids	+	–	+	–
6	Glycosides	–	–	–	–
7	Quinones	+	–	–	–
8	Phenols	+	+	–	–
9	Terpenoids	+	+	–	–
10	Steroids	+	+	+	–

3.2 Photochemical analysis of ethanol extract of *Clitoria ternatea* Leaf

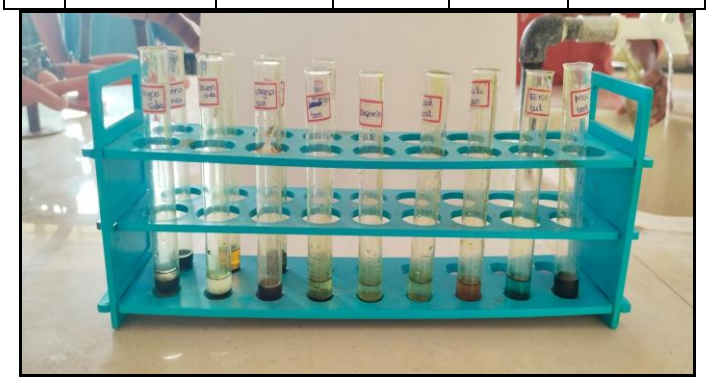

Fig. 2. Phytochemical analysis of ethanol extract of *clitoria ternatea* leaf

The analysis of preliminary phytochemical screening using Ethanol extract of *Clitoria ternatea* concludes that presence of Carbohydrates, Tannins, alkaloids, Flavonoids, Quinones, phenols, terpenoids & Steroids. The analysis of preliminary phytochemical screening using ethanol extract of *Clitoria ternatea* concludes that absence of saponins & glycosides. The analysis of preliminary phytochemical

screening using aqueous extract of *Clitoria ternatea* Leaf concludes the presence of Carbohydrates, tannins, alkaloids, phenols, terpenoids, & saponins.

3.3 Phytochemical analysis of aqueous extract of *Clitoria ternatea* L.

The analysis of preliminary phytochemical screening using aqueous extract of *Clitoria ternatea* leaf concludes the absence of flavonoids, Quinones, saponins & glycosides.

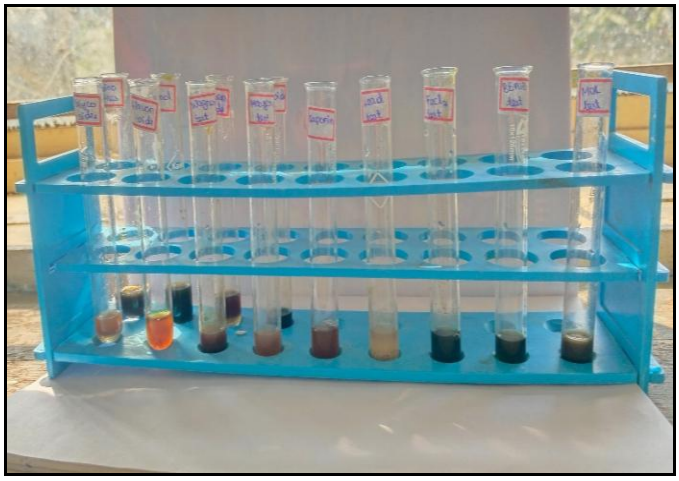

Fig. 3. Phytochemical analysis of aqueous extract of *clitoria ternatea* leaf

3.4 Phytochemical analysis of the acetone extract of *Clitoria ternatea* leaf

Fig. 4. Phytochemical analysis of acetone extract of *clitoria ternatea* leaf

The analysis of preliminary phytochemical screening using the acetone extract of *Clitoria ternatea* leaf concludes that presence of Carbohydrates, Tannins, Alkaloids, flavonoids, Phenol & Steroids. The analysis of preliminary phytochemical screening using the acetone extract of *Clitoria ternatea* concludes the absence of saponins, glycosides, quinones, phenols & terpenoids.

3.5 Phytochemical analysis of chloroform extract of *Clitoria ternatea* of leaf

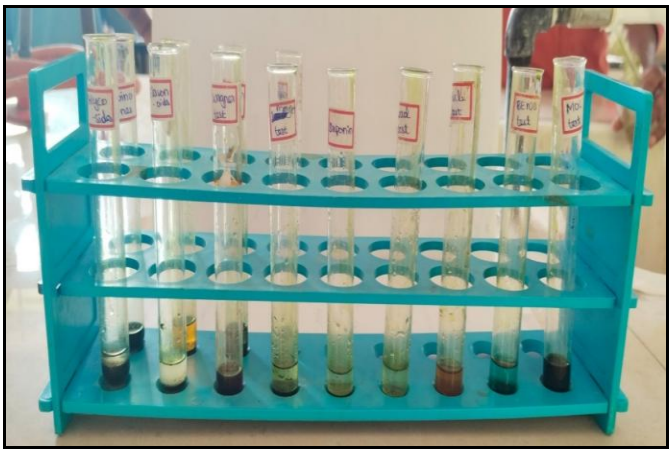

Fig. 5. Phytochemical analysis of the chloroform extract of *clitoria ternatea* leaf

The analysis of preliminary phytochemical screening using Chloroform extract of *Clitoria ternatea* leaf concludes the presence of Saponins & Alkaloids. The analysis of preliminary phytochemical screening using chloroform extract of *Clitoria ternatea* concludes the absence of tannins, flavonoids, glycosides, quinones, phenols, terpenoids, & Steroids.

3.6 Antibacterial activity

The antibacterial activity of the ethanolic extract of *Clitoria ternatea* leaf is mentioned below in Table 2

Table 2 Antibacterial activity of ethanolic extracts of *Clitoria ternatea* leaf

Name of the Microbes	Ethanolic extract of *Clitoria ternatea* leaf		
Concentration (µg /ml)	50	75	100
Bacteria	ZONE OF INHIBITION (mm)		
Streptococcus pneumoniae	**14**	**18**	**20**
Escherichia coli	**15**	**17**	**19**
Bacillus subtilis	**15**	**18**	**20**
Proteus mirabilis	**13**	**17**	**20**

By analysing the size of the inhibition zone surrounding a well containing the test material, the agar well diffusion technique is a widely used, straightforward, and reliable way to gauge how well a substance inhibits bacterial growth [17].

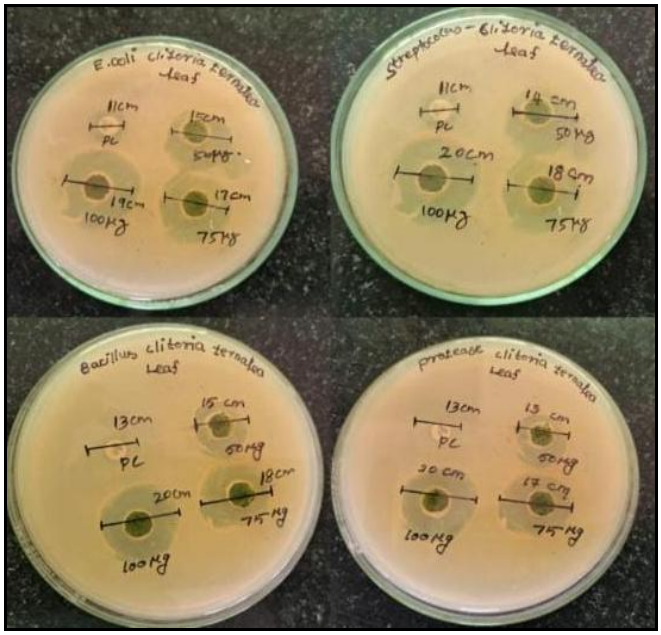

Fig. 6. Antibacterial activity of ethanolic leaf extracts of *Clitoria ternatea*

According to the above table, ethanolic extracts from *Clitoria ternatea* leaves at different concentrations (50, 75, and 100 µg/mL) have been demonstrated to have antibacterial properties against a variety of bacteria, including Escherichia coli, Streptococcus pneumoniae, Bacillus subtilis, and proteus mirabilis (Figure 6). The ethanolic extract of *Clitoria ternatea* leaf show the greatest concentration of inhibitory zone (mm) at 100 µg/mL.

4. Conclusion

Using various solvent extracts of *Clitoria ternatea* leaf is abundant in phytochemicals, including carbohydrates & alkaloids present in the all various solvents like ethanol, aqueous, acetone and chloroform. The analysis of preliminary phytochemical screening using Ethanol extract of *Clitoria ternatea* concludes that presence of Carbohydrates, Tannins, alkaloids, Flavonoids, Quinones, phenols, terpenoids & Steroids. The analysis of preliminary phytochemical screening using aqueous extract of *Clitoria ternatea* Leaf concludes that presence of Carbohydrates, tannins, alkaloids, phenols, terpenoids, & saponins**.** The analysis of preliminary phytochemical screening using Acetone extract of *Clitoria ternatea* leaf concludes that presence of Carbohydrates, Tannins, Alkaloids, flavonoids, Phenol & Steroids. The analysis of preliminary phytochemical screening using Chloroform extract of *Clitoria ternatea* leaf concludes that presence of Saponins & Alkaloids. The ethanolic extracts of *Clitoria ternaea* leaf is form inhibition zones against various bacterial strains like *Streptococcus pneumoniae, Escherichia coli, Bacillus subtilis* and *proteus*

mirabilis. The maximum zone of inhibition is formed in the concentration at 100µg/ml.

Conflict of Interest: Nil

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