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SCREENING OF PHYTOCHEMICALS, *In Vitro* ASSESSMENT OF ANTIOXIDANT, ANTIBACTERIAL, TLC PROFILING ACTIVITY OF *Clitoria ternatea* LEAVES

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ABSTRACT

The current study is aimed to determine the efficacy of methanolic extract of *Clitoria ternatea* leaves for pharmacological properties. The qualitative phytochemical analysis was evaluated. The antioxidant and anti-bacterial properties were analyzed using different assays. The TLC profiling was also assessed to study the various phytochemicals. The phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols, steroids, tannins, saponins, terpenoids and glycosides. The DPPH antioxidant exhibited that the antioxidant capacity of the methanolic extract of *C. ternatea* leaves increased in a dose dependent manner. The TLC analysis exhibited the presence of different phytochemicals and the retention factor was also calculated. Hence, from this study the methanolic extract of *C. ternatea* can furthermore be explored for pharmacological properties.

Keywords: Antioxidant, Denaturation, Pharmacological, Phytochemical and Therapeutic

INTRODUCTION

Plant materials are indeed a precious asset in the fight against major diseases around the world. Traditional medicinal approaches, particularly the use of medicinal plants, continue to be important in meeting the basic health needs of poor countries **[1]**. These plants have therapeutic value as they contain chemical active compounds that have a specific physiological function on the human body. Alkaloids, tannin, flavonoid, and phenolic chemicals are the most important bioactive elements of plants. Individuals and communities benefit significantly from the use of herbal plants **[2]**. Many therapeutic herbs are also utilised as spices and food. Free radical scavenging ability has long been known for phenolic.

Tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids are some of the chemical compounds found in medicinal plants that have a specific physiological function on the human body **[3]**. Therapeutic methods are used to extract bioactive compounds for herbal plants, which are extracted utilising sophisticated bio assays. The use of bio assays has resulted in the discovery of a number of new medically important phytochemicals **[4]**. Many potential medications are taken from plants, which are used in a variety of therapeutic and innovative pharmacological sectors to create drugs employing the best active ingredients from herbal drugs. Natural products or their derivatives represented for about half of the top-selling medications in 1991 **[5]**. *C. ternatea* is a resourceful perennial climber with blue or white flowers that bloom profusely. It is also known as "butterfly pea" and "shankhapuspi" and belongs to the *Fabaceae* family. It has long been used to cure a variety of diseases **[6]**. The plant is native to Southeast Asia, although it can also be found in tropical Asia, such as India, the Philippines, and Madagascar. *C. ternatea's* roots, seeds and leaves are frequently employed in Ayurvedic medicine **[7]**.

This plant's extracts have been utilized in the Ayurvedic 'Medhya Rasayana' as a rejuvenating recipe for the treatment of neurological problems and are said to improve intelligence **[8]**. The entire plant and seed extracts are used in the treatment of stomatitis, piles, female infertility, hematemesis, sleeplessness, epilepsy, psychosis, leucorrhea. *C. ternatea* has been exhibited to possess numerous pharmacological activities such as possessing anxiolytic, antidepressant, anticonvulsant, antistress, sedative antipyretic, anti-inflammatory, analgesic **[9]**. Anthelmintic

and anti-microbial activities. The plant consist of various secondary metabolites like as kaempferol and its glucoside-clitorin, taraxerol and a lactone aparajitin.

METHODOLOGY

Plant collection

Fresh leaves of *C. ternatea* were collected from Arumbakkam, Chennai, Tamil Nadu. The leaves were rinsed thoroughly with water. The plant material was dried in the shade. Using an electric blender, the dried leaves were processed into powder. For extraction, the powdered substance was kept in an airtight container **[10]**.

Extraction of plant material

A 50g powdered sample was weighed and placed in a conical flask. A methanol solvent was used to extract the material. It was left alone for 48 hrs. The extract was filtered using No.1 Whatman filter paper. A rotary evaporator and a vacuum desiccator were used to concentrate the filtered extract. For further investigation, the extract was kept in an airtight bottle **[11]**.

Qualitative phytochemical analysis

The presence of secondary metabolites in the methanolic extract of *C. ternatea* was done using standard procedures **[12]**. The phytochemicals such as alkaloids, flavonoids, carbohydrates, saponins, glycosides, phenols, proteins and amino acids, tannins, steroids and terpenoids were tested in the methanolic extract of *C. ternatea*.

Test for alkaloids

3 ml methanolic extract was taken in a test tube and mixed with 3 ml of 1% HCl and heated on steam bath. Mayer's reagent was then added to the mixture. Turbidity of the solution indicates presence of alkaloids **[13]**.

Test for flavonoids

To 1 ml of methanolic extract, 1 ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as an evidence for the presence of flavonoids. 1 ml of the filtrate was taken in a test tube and left to boil in water both for few minutes. 1 ml of Fehling's solution A and B was added to the boiled filtrate. A red precipitate shows the presence of carbohydrates.

Test of carbohydrates

Fehling's test

1 ml of the filtrate was taken in a test tube and left to boil in water both for few minutes. 1 ml of Fehling's solution A and B was added to the boiled filtrate. A red precipitate shows the presence of carbohydrates.

Test for saponins

The crude methanolic extract was mixed with 5 ml of distilled water taken in a

test tube and it was shaken vigorously. Formation of foam is an evidence to the presence of saponins.

Test for glycosides

To 1 ml of extract a few drops of glacial acetic acid and ferric chloride and 3-4 drops of concentrated sulphuric acid were added. The appearance of blue-green color indicates the presence of glycosides **[14]**.

Test for phenols

Ferric chloride test

To 50 mg of the extract, 5 ml of distilled water was added and a few drops of neutral 5% ferric chloride was added to the mixture. A dark greenish colour indicates presence of phenols.

Test for proteins and amino acids

100 mg of the extract was taken and 10 ml of distilled water was added and the mixture was filtered through a Whatman No.1 filter paper.

Ninhydrin test

2 drops of the Ninhydrin reagent was taken in a test tube and 2 ml of the plant extract was added. Presence of proteins and amino acids indicates purple colour.

Test for tannins

4 ml of the methanolic extract was taken and treated with $FeCl_3$. Color change into green shows presence of tannins.

Test for steroids

1ml of methanolic extract was taken in a test tube and mixed with concentrated H_2SO_4 Formation of red color at the bottom of the test tube indicate steroids.

Test for terpenoids

2 ml of the methanolic extract was mixed in 2 ml of chloroform and allowed to dry. Concentrated H_2SO_4 was added and boiled for 2 mins. Formation of grey color indicates presence of terpenoids **[15]**.

Antioxidant activity of methanolic extract of *Clitoria ternatea*

DPPH free radical scavenging activity

The free radical scavenging activity was determined by DPPH assay described by with slight modifications. DPPH solution (0.1mM) was prepared in methanol and 2.4 ml of this solution was added to 1.6 ml of methanolic extract at different concentrations (20, 40, 60, 80, and 100 µl). The mixture was thoroughly mixed before being left undisturbed at room temperature for around 30 mins. The mixture's absorbance was measured at 517 nm. As a reference, ascorbic acid was used. The following formula was used to calculate the percentage of inhibition **[16]**.

$$\textbf{\% of inhibition} = A_0\text{-}A_1 / A_0 \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the methanolic extract.

Antibacterial activity

The antibacterial agar well diffusion assay was used to evaluate the antibacterial activity of the individual crude extracts and their combination, according to the procedures described in various literature. With a cotton swab, the standardised bacterial broth culture was streaked evenly on sterile Muller-Hinton agar (MHA) plates. Beef infusion (2.0 g), acid hydrolysate of casein (17.5 g), starch (1.5 g) and agar (17.0 g) comprise one litre of MHA, which has a pH of 7.3 ± 0.2. After thirty minutes, equidistant wells with a 6 mm diameter sterilised cork border were formed on each plate. The labeled wells were filled with the test extracts. For comparison, gentamicin (25 g/mL) and sterile distilled water (100 L/well) were used as a positive and negative control, respectively. After that, they were left to stand on the laboratory bench for 2 hrs to allow the extracts to properly diffuse into the media. Finally, the plates were incubated for 24 hrs at 37ºC. The sizes of zones of inhibition, including the diameter of the well, were measured with a ruler after incubation and reported in millimetre (mm). The experiment was carried out three times for each bacteria, with the mean of zones of inhibition calculated for each test extract and the standard antibiotic **[17]**. Following the agar well diffusion experiment for the individual extracts, crude extracts of three medicinal plants with better *in vitro* antibacterial effectiveness were mixed in proportions of 1:1 and 1:1:1, respectively, to generate combinations of two and three. The mixed extracts were then tested for antibacterial activity using the same agar well diffusion method.

Thin layer chromatography

Using capillary tubes, a methanolic extract of *C. ternatea* leaves was applied to the TLC plates and left to dry. The TLC plate was created in the chamber using the mobile phase of hexane, chloroform and methanol (6:3:1). The phytoconstituents were identified using ultraviolet (UV) light at (254nm). The standard utilised was quercetin. The sample's mobility was measured in retention factor (Rf) and estimated using the procedure below **[18]**.

R*f*= Distance traveled by the plant extract/distance traveled by the solvent system

RESULTS AND DISCUSSION

Preliminary screening for the presence of phytochemicals in methanol extract of *C. ternatea* revealed the phyto-compounds **(Figure 1)** such as phenols, terpenoids, alkaloids and steroids and tannins **(Table 1)**. The quantification of flavonoids

and phenols in the methanol extracts were also studied. The results of the present study revealed that methanolic extract possess high phenolic and flavonoid content. The total yield of flavonoid and phenol content varies due to different extraction techniques and solvents utilised. During the metabolic process in a biological system, a large number of free radicals are created **[19]**.

The radicals will be able to induce a variety of ailments. Antioxidant mechanisms are therefore required to protect the biological system against free radicals such as reactive oxygen species. Reactive oxygen species play a role in diseases such as diabetes and cancer, both directly and indirectly.

The antioxidant potential data demonstrated dose-dependent manner. The DPPH assay showed maximum inhibition of free radicals in the methanolic extract whereas the aqueous extract showed very less inhibition.

In DPPH assay the methanolic extract exhibited the inhibition percent 63.32 at the concentration of 100 µg/ml while aqueous extract showed only 29.13% inhibitions at the same concentration **(Figure 3)**. In the present study the free radicals were predominantly scavenged by methanolic extracts. Previous studies also suggested the use of methanol extracts enabled free radical scavenging ability of plants.

Antibacterial activity

The methanolic extract of *C. ternatea* leaves was selected to study the antibacterial activity. The results of the antibacterial activity are presented in **(Figure 4 and Figure 5)**. Three bacterial strains namely *Klebsiella pneumoniae*, and *E. coli* were studied for antibacterial activity utilizing the methanolic extract of *C. ternatea* leaves. The results revealed that the methanolic extract of *C. ternatea* were potentially effective in suppressing the growth of bacteria **[20, 21]**. Among the two bacterial strains the highest zone of inhibition was observed in *E. coli* **(Figure 3)** then *Klebsiella pneumoniae.*

TLC profiling

The results are summarized in **(Table 2)**. Thin layer chromatography is a technique for the identification of phytochemicals **(Figure 6)**. In this study the TLC analysis of methanolic extract of *C. ternatea* leaves revealed the presence of secondary metabolites such as alkaloids, flavonoids, phenols, and tannins **[22-24]**. The R*f* values provide information about the polarity of the compounds, which can be used to isolate chemicals from plant extracts using various chromatographic and spectroscopic techniques.

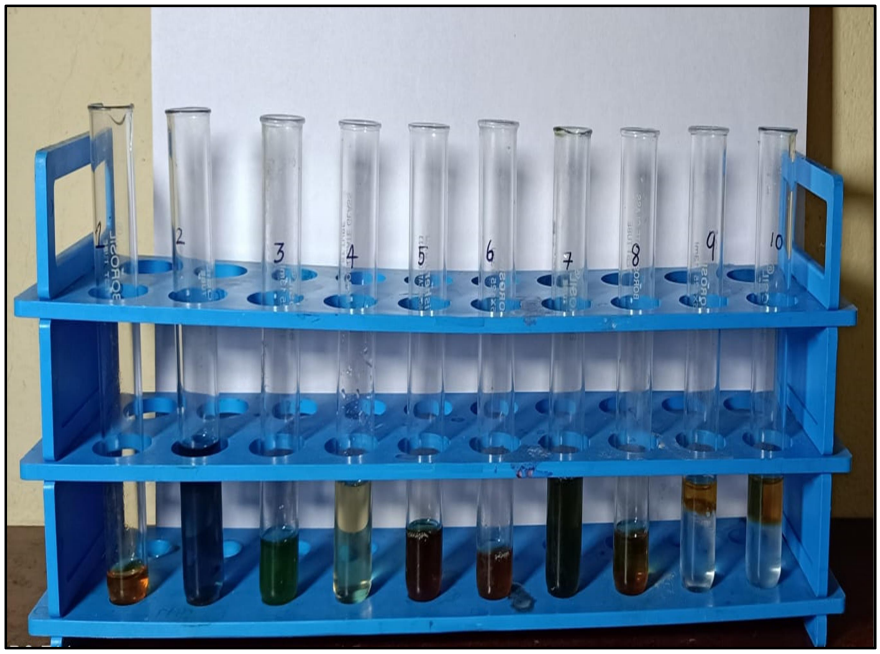


Figure 1: Phytochemical analysis of methanolic extract of *Clitoria ternatea*

Table 1: Qualitative phytochemical analysis of *C. ternatea* leaf extracts

Phytoconstituents	Name of tests	Methanol
Alkaloids	Dragendroff's test	+
Flavonoid	Aluminium chloride test	++
Carbohydrates	Fehling's test	-
Saponins	Foam test	+
Glycosides	Borntrager's test	-
Phenolic compounds	Ferric chloride	++
Amino Acids	Ninhydrin test	-
Tannin	Tannins test	++
Steroids	Steroids test	++
Terpenoid	Salkowski test	+++

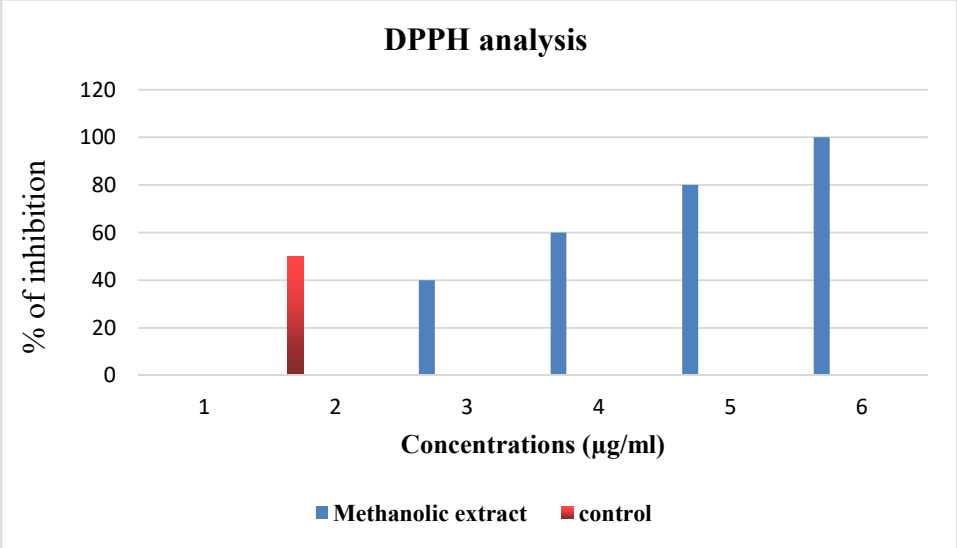


Figure 2: DPPH assay of *Clitoria ternatea* leaf extract

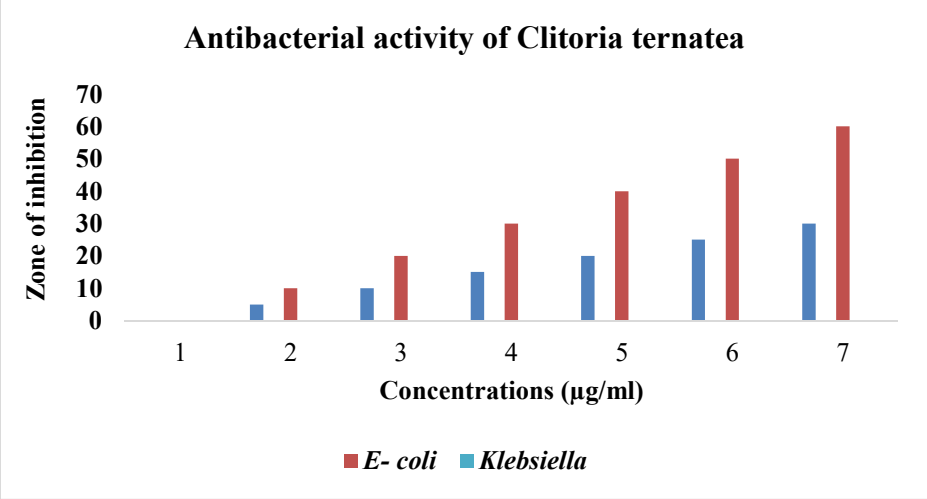


Figure 3: Antibacterial activity of *Clitoria ternatea* leaf extracts

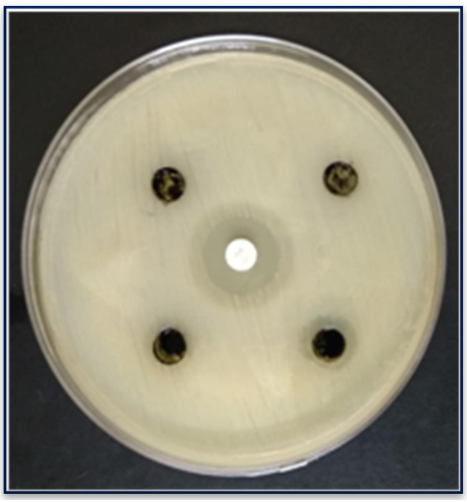


Figure 4: *Klebsiella pneumoniae*

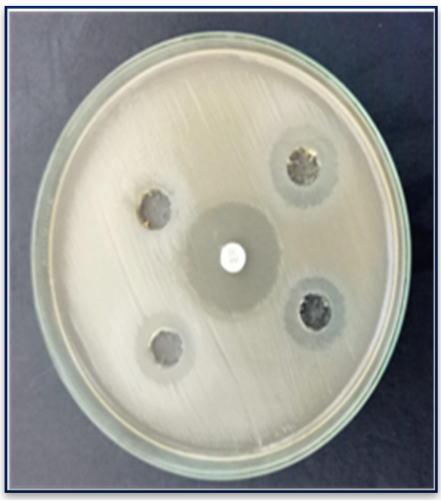
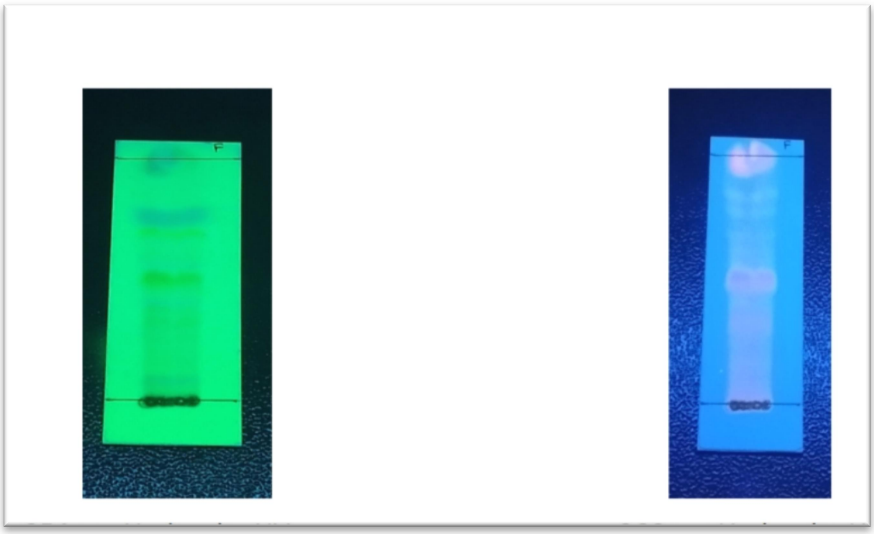


Figure 5: *E .coli*



254 nm under the UV **366 nm under the UV**

Figure 6: Thin layer chromatography methanol extracts of *Clitoria ternatea* leaf

Table 2: Thin layer chromatography of organic extracts obtain from *Clitoria ternatea* leaf

Solvent System	Ethyl acetate : Chloroform: Methanol (6:3:1)
Confirmatory Test	Mayer's test
Extract	Extract
R*f* Value	0.016,0.727,0.137,0.143,0.1,0.292,0.323,0.519,0.533,0.638,0.642, 0.729, 0.715

CONCLUSION

The results of this study show that the methanolic extract of *C. ternatia* leaves contain secondary metabolites that are biologically active. The antioxidant activity of the extracts also clearly reveal that it has potential antioxidants. The antibacterial activity also exhibited significant zone of inhibition. Therefore, this study has revealed the potential benefits of the *C. ternatia* leaves. Further research on *C. ternatia* can be a effective in the field of pharmacology.

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