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Research Article

Phytochemical Analysis and Antimicrobial Screening of *Clitoria ternatea* L

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Abstract

North-eastern India is known for its rich biodiversity. Phytochemical are non-nutritive, chemical compounds occurs naturally on plants during metabolic processes and they have diverse proactive properties or disease preventive properties. Plants are known to produce these chemicals to protect them. While recent research demonstrates that they can also play an important role in protecting humans against diseases. Even some of these plants are in use as traditional medicine for centuries now. Most phytochemical like flavonoid, carotenoids and polyphones have antimicrobial activity and serve as a source of antimicrobial agents against pathogens. The current study was focused on the plant species like *Clitoria ternatea* L. to investigate type of phytochemicals present in it and its antimicrobial activity on disease causing microbs like *Salmonella typhi* and *S. aureus* In the present study, the phytochemical screening for leaves of *clitoria ternata* showed the presence of active component like saponins, tannins, flavonoids, terpenoids, glycosides, amino acid, antraquanine, phenols, alkaloids, proteins and steroids. The control value of ZOI is 9 mm and 3 mm with respect to *S. aureus* and *S. typhi*. The zone of inhibition of all plant extract shows more antimicrobial activity in Hexane as compared to other plant extract at streptomycin disk ranges between 10-12 mm.

Keywords: Phytochemical; Antimicrobial; *Clitoria ternatea*; *S. aureus*; *S. typhi*

Introduction

Phytochemicals are the chemicals that present naturally in plants. Now- a-days these phytochemicals become more popular due to their countless medicinal uses. Phytochemicals play a vital role against number of diseases such as asthma, arthritis, cancer etc. unlike pharmaceutical chemicals these phytochemicals do not have any side effects. Since the phytochemicals cure diseases without causing any harm to human beings these can also be considered as "man-friendly medicines". This paper mainly deals with collection, extraction, qualitative and quantitative analysis of phytochemicals.

North-eastern India is known for its rich biodiversity. Phytochemical are non-nutritive, chemical compounds occurs naturally on plants during metabolic processes and they have diverse proactive properties or disease preventive properties. Plants are known to produce these chemicals to protect them. While recent research demonstrates that they can also play an important role in protecting humans against diseases. Even some of these plants are in use as traditional medicine for centuries now. Most phytochemical like flavonoid, carotenoids and polyphones have antimicrobial activity and serve as a source of antimicrobial agents against pathogens. *C. ternatea* L., commonly known as Asian pigeon wings, bluebellvine, blue pea, butterfly pea, cordofan pea and Darwin pea, is a plant species belonging to the Papillionaceae family. It is a perennial herbaceous plant, with elliptic, obtuse leaves. It grows as a vine or creeper, doing well in moist, neutral soil. The most striking feature about this plant is the color of its

flowers, a vivid deep blue; solitary, with light yellow markings. Some varieties yield white flowers. The fruits are 5–7 cm long, flat pods with six to ten seeds in each pod. The current study was focused on the plant species like *Clitoria ternatea* L. to investigate type of phytochemicals present in it and its antimicrobial activity on disease causing microbs like *Salmonella typhi* and *S. aureus*.

Material and Method

Collection of plant materials

This plant *Clitoria ternatea* L. collected from our college campus of D.B.F. Dayanand college of Arts and science, Solapur.

Cleaning of plants

After plants collection they have to be cleaned properly. The cleaning process involve the following steps. Cleaning, washing, peeling or stripping leaves from stems. Cleaning has to be done by hands in order to get better results.

Drying

The main purpose of drying is to remove the water content from plants so that the plants can be stored. Plants have to be dried immediately as soon as the plants collection or this will lead to spoilage of plant materials. The drying done by using natural process.

Natural drying

Natural process includes sun- drying. Sometimes plants are placed on drying frames or on stands, to be air-dried in barns or sheds. But this may take few weeks for complete drying. The time depends on temperature and humidity.

After complete drying of plants they have to be powdered well for further analysis using mixture machine.

Preparation of plant extracts

Plant extract made in different solvents like distilled water, ethanol, and hexane and petroleum ether as follow.

Extraction of plant material

Various organic solvents were used for the extraction of bioactive compounds. The 25-30gm *C. ternata L.* powder of leaves was separately extracted with different solvents like D. W, Ethanol, Hexane, and P. Ether in a soxhelt apparatus. With the rotary evaporator evaporate the extracts. The concentrated extracts were used for antimicrobial activity. The concentrated extracts were subjected to qualitative test for the identification of various phytochemicals constituents as per standard procedures.

Analysis of phytochemical

- **Screening for Alkaloids:** Few ml of extract and add a drop of Mayer's reagent added by side of test tube. A white and creamy precipitate indicates presence of alkaloids.
- **Amino acid**: 1 ml of extract was treated with few drops of Ninhydrin reagent. Appearance of purple color shows the presence of amino acid.
- **Steroids :** 1 ml of extract + dissolve 10 ml of chloroform and 10ml of conc. sulphuric acid by slide of TT. Upper layer turns red and sulphuric acid layer showed yellow with green fluorescence.
- **Cardiac glycosides**:- To the solution of extract in glacial acetic acid, few drops $Fecl_3$ and conc. Sulphuric acid observed reddish brown color at the junction of two layer shows presence of CO_2.
- **Antraqanine**: 5 ml extract boiled with 10 ml sulphuric acid filtrate while hot shaken with 5ml chloroform. Chloroform layer was pipette into another test tube and 1 ml of dilute NH3 added observed color changes.
- **Flavonoids**: To 1 ml of the extract, few drops of dilute sodium hydroxide were added. An intense yellow color was produced in plant extract becomes colorless on addition of dilute acid. It indicates flavonoids.
- **Tannin**: 5 ml of extract + few drops of 1% lead acetate were added. A yellow precipitate was formed which shows presence of tannin.
- **Phenol**: 2 ml of extract + 2 ml of $Fecl_3$ and appearance of deep bluish green color solution presence of phenol.
- **Saponin**: 5 ml of extract boiled in 10 ml of D.W. in test tube, shake vigorously for 30 sec. and allowed to stand for half an hr formation of forth indicates the presence of saponins.
- **Terpenoids**: 5 ml extract + 2 ml chloroform + 3ml conc. Sulphuric acid observed reddish brown color show presence of terpenoids.
- **Reducing sugar**: 1 ml of extract + 5-8 drop of felhings reagent and boiled it. A brick red color precipitated is observed.

- **Proteins**: 1 ml of methanolic extract of *C. ternatea L.* was taken and added a few drops of nitric acid to the sides of the test tube very gently. Within few seconds the formation of yellow color indicates the presence of proteins in the sample (Xanthoprotein test).

Microbial screening

The following strains were used for the study and were maintained by the Microbiology department of microbiology of D. B. F. Dayanand college of Arts and Science, Solapur.

- *Staphylococcus aureus* (*S. aureus*)
- *Salmonella typhi* (*S. typhi*)

These cultures were maintained on sterile nutrient agar slants and stored at 4 ºC until further use.

Observations

Figure 1: Leaf powder.

Figure 2: Plant extracts after soxhelt extraction.

Figure 3: Phytochemical investigation.

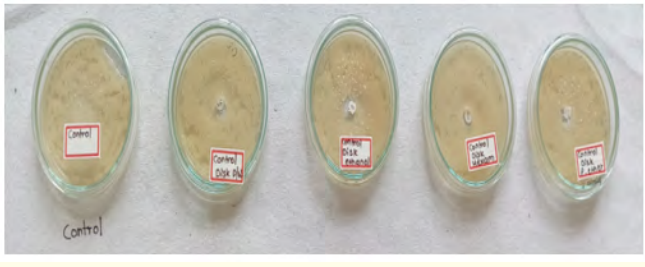

Figure 4: *S. aureus* of Ethanol Of concentrations of 100 µl, 200 µl, 500 µl and control disk.

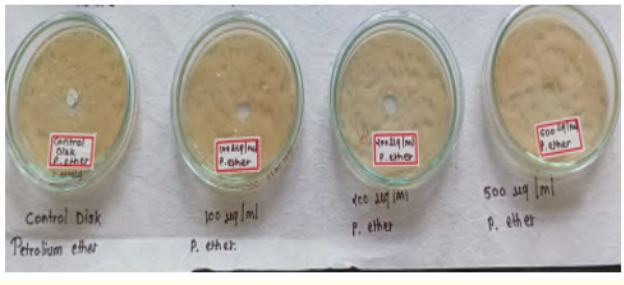

Figure 5: *S. aureus* of petroleum ether of concentrations of 100 µl, 200 µl, 500 µl and control di.

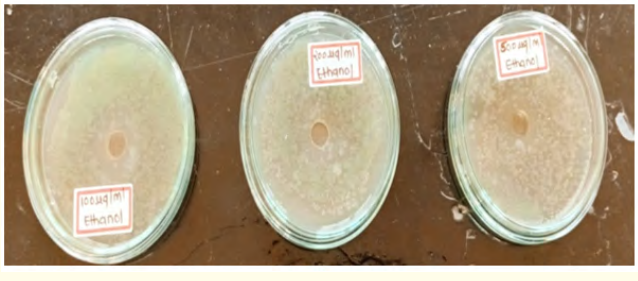

Figure 6: *S. typhi* of Ethanol Of concentrations of 100 µl, 200 µl, 500 µl.

Observation tables

Sr. No.	Phytochemicals analysis	D.W.	Ethane	Hexane	Ether
1	Alkaloids	-	++	-	+
2	Amino acid	++	+	-	-
3	Steroids	++	++	++	+
4	Cardiac glycosides	+++	+	+	-
5	Antraquanine	-	-	-	-
6	Flavonoids	-	++	-	++
7	Tannin	+	++	-	-
8	Phenol	-	+++	+++	++
9	Saponine	+++	+	+	-
10	Terpenoid	+++			-
11	Carbohydrates	+			
12	Proteins	-	++	+	-

Table 1: Phytochemical investigations of *Clitoria ternatea*

sequential extraction.

Sr. No.	Organisms	Inhibition zone of concentration (µl)	D. W. (mm)	Ethanol (mm)	Hexane (mm)	P. Ether (mm)
1	*S. aureus*	Streptomycin disk	7	13	12	-
2		100	1	14	9	2
3		200	4	16	3	3
4		500	3	20	4	3
5	*S. Typhi*	Streptomycin disk	-	-	7	2
6		100	3	11	2	3
7		200	-	4	10	-
8		500	5	8	4	5

Table 2: Antimicrobial assay of *C. ternatea.*

Result and Discussion

The extraction was performed by successive hot continuous soxhlet extraction in order to the polarity of the solvents i.e. Petroleum Ether for defatting, hexane, ethanol and Distilled Water etc. The results on the antimicrobial activity of medicinal formulations showed that all the formulations were effective against tested microorganisms with different zone of inhibition (Table 2). A total number of 8 plant extracts at three concentrations (100, 200, 500 µl) and one control disc for each solvents of *Clitoria ternatea L.* Were tested for their antimicrobial activity against the 2 bacterial *S. aureus and S. typhi.* In the present study, (Table 1) the phytochemical screening for leaves of *Clitoria ternata* showed the presence of active component like saponins, tannins, flavonoids, terpenoids, glycosides, amino acid, antraquanine, phenols, alkaloids, proteins and steroids. The control value of ZOI is 9 mm and 2 mm with respect to *S. aureus* and *S. typhi*. The zone

of inhibition of all plant extract shows more antimicrobial activity in Hexane as compared to other plant extract at streptomycin disk ranges between 10-12 mm. The ethanol extract in all plants shows maximum zone of inhibition ranges between 5-20 mm. The D. W. extract showed more antibacterial activity in *S. aureus* at control disk. The D. W. Extract also showed more antibacterial activity in *S. aureus* at 200 µl. The D. W. and petroleum ether does not show zone of inhibition in *S. typhi* at 200 and in *S. typhi* at 500 µl. In hexane extract showed more antimicrobial activity in *S. aureus* at 100 µl and *S. typhi* 200 µl ranges between 5-10 mm. In petroleum ether, does not show zone of inhibition against *S. aureus* at streptomycin disk. In P. ether extract, it show low zone of inhibition ranges between 1-5 mm against *S. aureus* and *S. typhi* [1-12].

Conclusion

In the present study, the phytochemical screening for leaves of *Clitoria ternata* showed the presence of active component like saponins, tannins, flavonoids, terpenoids, glycosides, amino acid, antraquanine, phenols, alkaloids, proteins and steroids. Antimicrobial investigation reveals that the current plant extract is found to be useful against the *S. typhi* and s. aurous basically the ethanolic and petroleum ether extracts are effective against *S. typhi* and *S. aureus*.

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