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COMPARATIVE STUDIES ON THE PHYTOCONSTITUENTS, ANTIBACTERIAL AND PESTICIDAL ACTIVITIES OF BLUE AND WHITE VARIETIES OF *CLITORIA TERNATEA* LINN

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ABSTRACT

Medicinal plants are the wonderful source for the development of new therapeutic compounds which contains potential therapeutic value. *Clitoria ternatea* L. is one such medicinal plant contained medicinal properties. In health care large number of antimicrobials shown side effects but antibacterial from plant origin shown therapeutic efficiency to cure infectious disease with no side effects. *Clitoria ternatea* L. are rich in large group of phytochemicals namely flavonoids, phenolic compounds and different secondary metabolites exert various therapeutic effects such as diuretic laxative effect, antioxidant, antibacterial ,anti-inflammatory, antidiabetics, antipyretic etc. In this present investigation we have screened phytochemical of *Clitoria ternatea* L of blue and white verities by employing methanolic extract, which shows the presence of secondary metabolites such as terpenoids, phlobatannins, tannins, flavonoids, and phenols. It's antibacterial activity against *Eschericia coli* (MTCC 433) and *Pseudomonas aeruginosa* (MTCC 424) were examined by well diffusion method and its pesticidal activity were carried out against *Sitophilus oryzae*. The results revealed that the white flowered leaves shown more antioxidant potential compared to blue flowered leaves of *Clitoria ternatea* L. Antibacterial activity was confirmed by the presence of zone of inhibition against the test microorganisms at different concentration of methanolic extract. Pesticidal activity was observed as 100% mortality against *Sitophilus oryzae* within a hour.

INTRODUCTION

Traditionally plants have been used as a source of therapeutic compounds which possess numerous biological properties that plays an important role in the health care. *Clitoria ternatea* Linn, is a a well-known medicinal plant commonly called as Butterfly pea is a perennial leguminous twiner belonging to the family Fabaceae and papilionaceae [1]. Since ancient times *Clitoria ternatea* L has referred as "Shankpushpi" used as reputed drug of Ayurveda medicinal system as a brain tonic ,antipyretic, anti-inflammatory, antidiabetic, antiproliferate, antioncogenic effect and to cure infertility [2]. It has two species blue and white colour flower varieties. Root, leaves, stem and flower of both varieties are used for medicinal purpose from ancient times. *Clitoria ternatea* L. are rich in major phytoconstituents of pentacyclic triterpenoids such as taraxerol and taraxerone [4]. In plants naturally occurring large group of phytochemicals namely

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flavonoids and other group of phenolic compounds are rich in *Clitoria ternatea*.Linn [5] and also it contains very effective antioxidant, antibacterial and pesticidal activity.

In the present research methanolic and aqueous extract of both varieties of *Clitoria ternatea*.L was prepared and the phytochemical constituents were analysed. Antioxidant activity for the methanolic extract of *Clitoria ternatea* were determined by DPPH free radical scavenging assay using ascorbic acid as a standard and its antibacterial activity was tested against *Eschericia coli* (MTCC 433) and *Pesudomonas aeruginosa* (MTCC 424) by well diffusion method. Pesticidal activity for the methanolic extract of *Clitoria ternatea*.L against *Sitophilus oryzae* has been carried out.

MATERIALS AND METHODS

Sample Collection

The mature leaves of White and Blue varieties of *Clitoria ternatea* L. (Fig 1) were collected from Erode District, Tamil Nadu, India. The plant material was washed using distilled water to remove the debris. The plant material was shade dried for 8 days and then powdered. The fine powder of both varieties were stored in air tight container.

PREPARATION OF CRUDE PLANT EXTRACT:

Methanolic Extract

One gram of both varieties of *Clitoria ternatea* L was taken separately. The plant extract was prepared in Soxhlet apparatus using methanol as a solvent for 3 hours. The methanolic extract was collected and concentrated by rotary evaporator at 54^{o}C.

Aqueous Extract

Aqueous extract of both varieties of *Clitoria ternatea* L was prepared by mixing 5grams of plant material with 50ml of distilled water and boiled for 2minutes and then filtered using Whattman No.1 filter paper and the phytochemical analysis was carried out immediately without further storage.

Phytochemical Analysis

The aqueous extract of both varieties were tested for the presence of Phytochemicals namely Terpenoids, Phlobatannins, Tannins, Flavonoids, Phenols [7].

Test for Tannins

To test the presence of tannins in the plant extract, few drops of 0.1% ferric chloride was added to the filtrate and the colour change to brownish green or blue black confirmed the tannins presence in the leaf extract.

Test for Phlobatannins

10ml of the aqueous extract of both varieties were boiled with 1% HCL in a test tube separately and the presence of Phlobatannins was confirmed by the presence of red precipitate in the tube.

Test for Flavonoids

To a few drops of 1% liquor ammonia in two different test tube to which aqueous extract of both varieties was added and the yellow colouration confirmed the presence of Flavonoids.

Test for Terpenoids

To the extract around 2ml of Chloroform and 3ml of sulphuric acid was added consecutively and the reddish brown interface denoted the presence of terpenoids.

Test for Phenols

To the 5ml of plant filtrate a few drops of Ferric chloride was added and the formation of bluish black confirmed the phenols presence.

DPPH Radical Scavenging Activity

Different aliquots of methanolic extract of both varieties were taken in test tubes and the total volume was made up with methanol. One ml of DPPH (4mg/ml) was added to the filtrate and the tubes were kept in dark for 20minutes at room temperature. Absorbance was read in the spectrophotometer at 517nm using ascorbic acid as a standard. The Percentage free radical scavenging was calculated using the formulae as follow [6, 9].

% Radical scavenging=(Ac-As/Ac) X 100

Where, Ac = Absorbance of control and As= Absorbance of test sample.

ANTIBACTERIAL ACTIVITY

The modified agar well diffusion method [10] was employed. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25 µL of the plants extracts and blanks.24 hours fresh culture of test microorganisms such as *E.coli(MTCC 433)* and *P. aeruginosa* (MTCC 424) were swapped and wells are punched using gel puncture.10µl,20µl,30µl of both varieties of plant extract were added into the well respectively and incubated at 37 ^{o}C for 24 hours. Antibacterial compounds in the *Clitoria extract.* L extract diffuse into the medium and inhibit the growth of test microorganism which shows the zone of clearance in the plates and measured the centimetre of inhibition zone formed around the well. The test was carried out by triplicate.

PESTICIDAL ACTIVITY

The adult pests of *Sitophilus oryzae* were collected from naturally infected rice grains through Public Distribution System, Erode District, Tamil Nadu. These insects were reared on clean and uninfected rice grains in a jar with sufficient aeration. One ml of the *Clitoria ternatea* L leaf extract of both varieties were taken in a dry sterile clean petri dish and allowed to dry respectively. Then a

plug of cotton was used to wipe the extract from the plate. The cotton plug was placed in the petri dish containing adult *Sitophilus oryzae* (30 numbers) along with one gram of rice and the plates were sealed. The death rate of rice weevil was observed after an hour of incubation and reported as percent mortality [11].

Percent mortality= (number of rice weevil dead/number of rice weevil introduced)X100

RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS

Phytochemicals such as Tannins, Phlobatanninis, Flavonoids, Terpenoids and Phenols were obtained in the blue and white verities of leaves of *Clitora ternatea* L. (Table 1).

DPPH Radical Scavenging Activity

In DPPH assay, 1.1-Diphenyl-2-picrylhydrazyl (deep violet) act as stable free radical react with antioxidant compound present in the plant extract of both varieties and converts it in to 1,1-Diphenyl-2-picrylhydrazine respectively. Five different aliquots of sample extract were tested to identify the significance of extract concentration on the scavenging activity.0.1mM Ascorbic acid was used as standard for antioxidant activity.

The standard graph between the concentration (µg/ml) verses absorbance for both variety at 517nm shows that absorbance increase with increase in concentration was shown below (Figure.1).Maximum percentage of radical scavenging activity of Blue leaf extract and White leaf extract was found to be 73% and 76% respectively. (Fig 2-4)

ANTIBACTERIAL ACTIVITY

In this method both variety of *Clitoria ternatea* L leaf extract were inhibited the growth of test microorganisms and the zone of inhibition was measured (Figure 5). High range of inhibition was produced by both variety of methanolic extract against *E.coli*(MTCC 433) and the lower range of inhibition was produced by both variety against *P. aeruginosa*(MTCC 424).The zone of inhibition was measured in centimetre (Table 2,3).

PESTICIDAL ACTIVITY

Hundred percent mortality *of Sitophilus oryzae* was recorded for the methanolic extract of both variety of *Clitoria ternatea* L. at the concentration of 0.5mg/ml at one hour interval. After an hour total number of *Sitophilus oryzae* died were shown below (Figure 6).

Fig 1. Habitat of White and Blue varities of *Clitoria ternatea* Linn

Fig 2. Antioxidant activity of Ascorbic acid by DPPH method

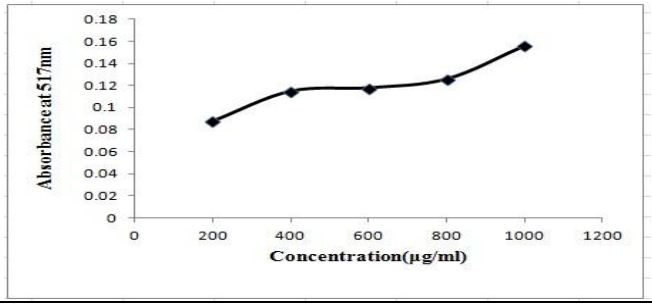
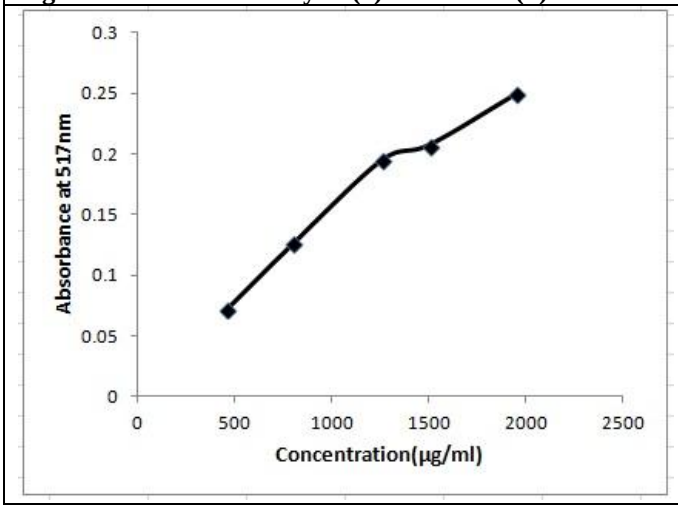


Fig 3. Antioxidant activity of (a) White and (b) Blue leaves extract of *Clitoria ternatea.L* by DPPH method



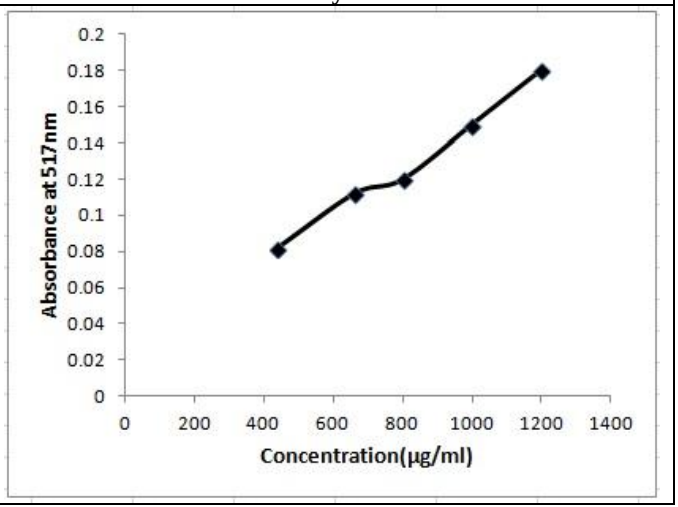


Fig 4. Maximum percentage of radical scavenging (a) Blue leaf extracts (b) White leaf extract

*BL-Blue leaf extract, WL-White leaf extract

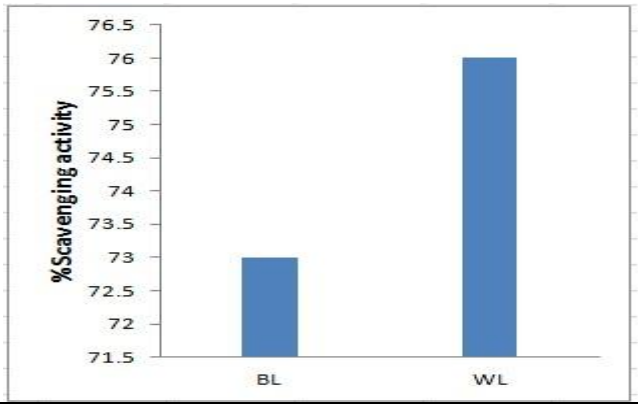


Fig 5. Antibacterial activity of (a) White and (b) Blue leaves extract of *Clitoria ternatea* L against *P.aeruginosa* (MTCC 424) by well diffusion method

(a) White leaves extract

(b) Blue leaves extract

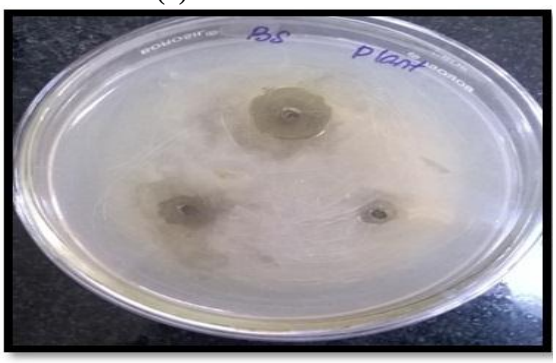


Fig 6. Pesticidal activity of (a) White and (b) Blue leaves extract of *Clitoria ternatea* L against *Sitophilus oryzae*

(a) White leaves extract

(b) Blue leaves extract

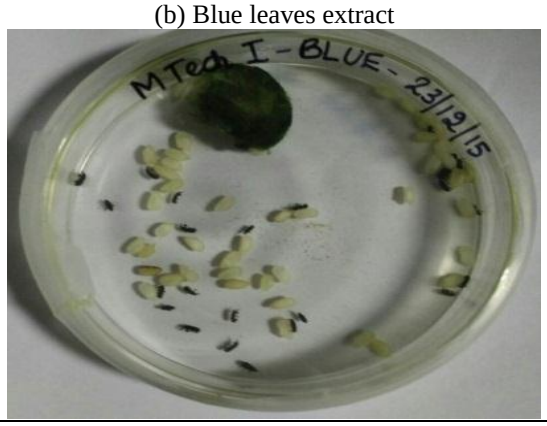


Table 1. Phytochemical analysis of White and Blue variety of *Clitoria ternatea.L*

S.No	Tests	Leaves of *Clitoria ternatea*(White variety)	Leaves of *Clitoria ternatea* (Blue variety)
1.	Tannins	+	+
2.	Phlobatannins	+	+
3.	Flavonoids	+	+
4.	Terpenoids	+	+
5.	Phenols	+	+

*(+) Presence of phytochemicals

Table 2. Antibacterial activity of White leaves of *Clitoria ternatea* L against *E.coli* (MTCC 433) and *Pseudomonas aeruginosa* (MTCC 424)

S. No	Extract Concentration (µl)	Zone of inhibition (cm)	
		E. coli	*P. aeruginosa*
1	10	0.3	0.2
2	20	0.8	0.7
3	30	1.5	0.9

Table 3. Antibacterial activity of blue leaves of *Clitoria ternatea.* L against *E. coli* (MTCC 433) and *Pseudomonas aeruginosa* (MTCC 424)

S. No	Concentration (µl)	Zone of inhibition (cm)	
		E. coli	*P. aeruginosa*
1	10	0.9	0.3
2	20	1.4	0.8
3	30	1.8	1.5

CONCLUSION

Clitoria ternatea L. of White and Blue colour flowered plant leaves are major source of phytochemical responsible for its therapeutic value[4]. Previous studies revealed that the methanolic extract of *C.ternatea* showed potential free radical scavenging activity [8]' Antioxidant activity of white variety show more potential radical scavenging activity compared to Blue variety of *C .ternatea* [12]. Khatoon et al extensively studied the pharmacognostical aspects on Blue and white flower varieties of *Clitoria ternatea* L [4]. They have reported the following phytochemical viz: as Steroids, Titerpenoids, Flavonoids, Alkaloids, Carbohydrates, Glycosides, Tannins and saponins. In our present study on the same plant and the blue and white verity collected form Tamil nadu also contains Tanninis, Phlobatannins, Flavonoids, Terpenoids and phenols. In addition to that,we have been reported that methanolic extract of *C. ternatea* showed that white variety have more Percentage of free radical scavenging activity than blue variety and the antibacterial activity of both variety of *C.ternatea* against *E.coli*(MTCC 433) and *P.aeruginosa* (MTCC 424) showed higher range inhibition against *E.coli*(MTCC 433) by both varieties of methanolic extract and Lower range of inhibition against *P.aeruginosa(MTCC 424).*A principle compound Quercetin which is present in the *Clitoria ternatea* L may be reason for its high antibacterial activity. It was previously reported that a small protein finotin present in seed showed high toxic to insect and pest [3]. Methanolic extract of both variety showed potential pesticidal activity against *Sitophilus oryzae* within an hour .Our present work revealed that methanolic extract of White and Blue colour flowered leaves of *Clitoria ternatea* have different biological properties such as antioxidant, antibacterial and pesticidal activity. The organic extract of *Clitoria ternatea* L could be further exploited in the future as a source of useful phytochemical compounds for pharmaceutical industry as well as the development of new antibacterial and anti pesticidal agent. The extract should be further studied to gain more applications to use as natural antioxidant.

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CONFLICT OF INTEREST

No interest

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