



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(6): 1104-1108
Received: 12-09-2017
Accepted: 13-10-2017

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Phytochemical and antibacterial activities of crude leaf and root extracts of *Clitoria ternatea* varieties (Fabaceae)

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Abstract

Medicinal Plants are rich source of therapeutic compounds that have tremendous applications in pharmaceutical industry. *Clitoria* has three varieties *Clitoria ternatea* L. Var. *ternatea* Hook. F. (white), *Clitoria ternatea* L. Var. *ternatea* Hook. F. (Blue) and *Clitoria ternatea* L. Var. *Pleniflora* Fantz. (Blue var), *Clitoria* is an attractive perennial climber with conspicuous blue or white flowers. Preliminary phytochemical analysis for Carbohydrates, starch, chlorophyll, alkaloids, total phenolics, total flavonoids, tannins, steroides were made by following standard procedures. Our study also showed that the leaf and root extracts of three *Clitoria ternatea* varieties have some of the factors that may influence the sensitivity of the tested bacteria (*E.coli*).

Keywords: *Clitoria ternatea* varieties, Extract, Phytochemical analysis, Antibacterial activity

1. Introduction

Clitoria ternatea L. belongs to fabaceae family known as Butterfly pea; a perennial twining herb, found in tropical equatorial areas. The plants are seen much adaptive to various ranges of temperatures and humidity. They are tolerant to frost and dry conditions (Anonymous 1998). *Clitoria ternatea* is a vigorous, strongly persistent, herbaceous perennial legume; stem fine twining, sparsely pubescent, sub erect at base, and 0.5-3 m long. Leaves pinnate with 5 or 7 leaflets; petioles 1.5-3 cm long; stipules persistent, narrowly triangular, 1-6 mm long, subulate, prominently 3 nerved; rachis 1-7 cm long; stipules filiform, 2mm long; leaflets elliptic. *Clitoria* has three varieties *Clitoria ternatea* L. Var. *ternatea* Hook. F. (white), *Clitoria ternatea* L. Var. *ternatea* Hook. F. (Blue) and *Clitoria ternatea* L. Var. *Pleniflora* Fantz. (Blue var), *Clitoria* is an attractive perennial climber with conspicuous blue or white flowers. It is traditionally used to treat various ailments (Sivarajan and Balachandran 1994) [9].

C.ternatea varieties contain phytochemical constituents with important pharmacological activities. A wide range of secondary metabolites including triterpenoids, flavanol, glycosides, anthocyanins and steroids has been isolated from *C. ternatea*, its extracts possess a wide range of pharmacological activities including antimicrobial, antipyretic, anti-inflammatory, analgesic, diuretic, local anesthetic, antidiabetic, insecticidal, blood platelet aggregation-inhibiting and for use as a vascular smooth muscle relaxing properties. This plant has a long use in traditional ayurvedic medicine for several diseases and the scientific studies has reconfirmed those with modern relevance.

The fabaceae family member *Clitoria ternatea* L. varieties have been used in ayurveda and traditional medicine for curing a number of diseases. *Clitoria ternatea* has been shown to have number of pharmacological activities such as possessing anxiolytic, antidepressant, antistress, Sedative, anti-pyretic, anti-inflammatory, analgesic (Gomez and kalamani 2003), and antimicrobial activities (Kamilla *et al.*, 2009) [4].

Various parts of *Clitoria ternatea* have been reported to have tranquilizing property, anti-inflammatory, anti-pyretic property. The flavanol glycoside present in root is reported to have anti-bacterial activity, *Clitoria* have been reported to have anti-inflammatory, hepato protective anti hyperlipidemic and immune inhibitory activities (Mukherjee *et al.*, 2008) [6].

This study aims to determine the phytochemical compounds and anti bacterial properties in each variety. The varieties of *Clitoria* are:

- *Clitoria ternatea* L. Var. *ternatea* Hook. F. (white)
- *Clitoria ternatea* L. Var. *ternatea* Hook. F. (Blue)
- *Clitoria ternatea* L. Var. *Pleniflora* Fantz. (Blue var)

Materials and Methods

Collection and extraction of plant materials

The fully matured fresh leaves and roots of *Clitoria ternatea* L were collected from various parts of Thiruvananthapuram district, Kerala. The plant materials were washed thoroughly, shade dried and finely powdered. The dried powdered materials were extracted with two different solvents such as water and acetone.

Phytochemical analysis

The prepared plant extracts were analysed for the presence of alkaloids, carbohydrates, terpenoids, saponins, proteins, aminoacids, fixed oils, phenolic compounds, tannins, flavonoids, steroids etc.. (Harborne *et al.*, 1998) [3].

Preparation of plant extract for antimicrobial screening

For antimicrobial screening the concentrated, dried and powdered ethanol leaf and root extract was dissolved in 10 % dimethyl sulfoxide (DMSO) and were stored at 4^o C for further use.

Anti-bacterial property

(A) Reagents

1. Muller Hinton Agar Medium (1 L)

The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petriplates (25-30ml/plate) while still molten.

2. Nutrient broth (1L)

One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and

sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes.

3. Streptomycin (standard antibacterial agent, concentration: 10mg / ml)

(B) Procedure

Petriplates containing 20ml Muller Hinton Agar Medium were seeded with bacterial culture of *E. coli* (growth of culture adjusted according to McFards Standard). Wells of approximately 10mm was bored using a well cutter and samples (100µl) were added. The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well (NCCLS, 1993) [5]. Streptomycin was used as a positive control.

Results and Discussions

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutical and pharmaceutical industry. The present study contributes valuable information of bioactive compounds present in varieties of *clitoria ternatea*. Qualitative and quantitative analysis of crude leaf and root extract was carried out for Carbohydrates, Alkaloids, Tannins, Flavanoids, Phenols, Carbohydrates, Protein, Amino acids, and terpenoids

The phytochemical analysis of plant extracts were done by using Acetone, and water showed in the table 1,2 and 3. It was observed that most of the components were present in water. Carbohydrate were only found in the root, leaves, and flowers. The flavonoids, alkaloids, phenols are present in three varieties of *Clitoria* in water extract. In the Acetone solvent *Clitoria ternatea* (Blue) shows terpenoids, tannin, saponin in all parts. *Clitoria ternatea* (white) shows steroids is present only in their leaf and terpenoids present in the leaf in two solvents. In water solvent *Clitoria ternatea* L. Var. *pleniflora* (blue var) shows most of the compounds in the leaf.

Table 1: Qualitative analysis of *Clitoria ternatea* L. Var. *ternatea* (Blue) extract in Acetone and Water.

<i>Clitoria ternatea</i>	Acetone	Water	Acetone	Water	Acetone	Water
	Leaves	Leaves	Root	Roots	Flower	Flower
Carbohydrate	+	+	+	+	+	+
Protein	-	-	-	-	+	+
Aminoacids	-	-	-	-	-	-
Terpenoids	+	-	+	+	+	-
Alkaloids	+	+	+	+	-	-
Tannin	+	+	-	-	+	-
Saponin	+	+	+	-	-	+
Flavonoid	-	+	+	-	-	-
Steroids	-	-	-	+	+	+
Phenol	+	+	+	+	-	-

“+ve indicates presence and “-ve” indicates absence.

Table 2: Qualitative analysis of *Clitoria ternatea* L. Var. *ternatea* (White) extract in Acetone and Water

<i>Clitoria ternatea</i>	Acetone	Water	Acetone	Water	Acetone	Water
	Leaves	Leaves	Root	Root	Flower	Flower
Carbohydrate	+	+	-	+	-	+
Protein	-	-	-	-	-	+
Aminoacids	-	-	-	-	-	-
Terpenoids	+	+	-	-	-	-
Alkaloids	+	+	+	-	+	+
Tannin	+	+	-	-	-	+
Saponin	-	+	+	-	+	-
Flavonoid	+	+	+	+	-	+
Steroids	-	-	-	+	-	-
Phenol	+	-	+	+	-	-

“+ve indicates presence and “-ve” indicates absence.

Table 3: Qualitative analysis of *Clitoria ternatea* L. Var. *pleniflora* (Blue var) extract in Acetone and Water

<i>Clitoria ternatea</i>	Acetone	Water	Acetone	Water	Acetone	Water
	Leaves	Leaves	Root	Root	Flower	Flower
Carbohydrate	+	+	-	+	-	+
Protein	-	-	-	-	-	+
Aminoacids	-	-	-	-	-	-
Terpenoids	+	+	-	-	+	+
Alkaloids	+	+	+	-	+	-
Tannin	+	+	+	-	+	+
Saponin	+	+	-	-	+	-
Flavonoid	-	+	+	+	+	+
Steroids	-	+	-	+	+	+
Phenol	-	-	+	+	-	-

“+ve indicates presence and “-ve” indicates absence.

Chlorophyll is a pigment present in all plants which give green colour to them. *Clitoria ternatea* L. varieties leaves have high chlorophyll content, It possess anti mutagenic and anti-carcinogenic properties to a certain extent as it is a very good chelating agent due to its physical and chemical structures. Thus we don't have to rely on external chemicals or supplements for these benefits in keeping our body healthy

and for increasing our community. Also they stimulate red blood cells in uptake of oxygen and efficiently deliver magnesium ions to our body. The total chlorophyll present in *Clitoria ternatea* (Blue) is 4.504 mg, *Clitoria ternatea* (white) 3.581 mg and in *Clitoria ternatea* L.Var.*pleniflora* (blue var) is 6.58 mg.

Table 4

Sl no	Taxa studied	Chlorophyll a (mg)	Chlorophyll b (mg)	Total chlorophyll (mg)
1.	<i>Clitoria ternatea</i> L. (Blue)	0.971	3.532	4.504
2.	<i>Clitoria ternatea</i> L. (White)	0.204	3.377	3.581
3.	<i>Clitoria ternatea</i> L. Var. <i>pleniflora</i> (Blue var)	1.138	5.442	6.58

The role of carbohydrates is to provide energy needed for physical activity, brain function and operation of muscles in the organs. Carbohydrate content is more present in the *Clitoria ternatea* L. (White) leaf and root. *Clitoria* leaves contain more carbohydrate content and are beneficial in eye disorders and insect bite poison. Starch content is more

present in the *Clitoria ternatea* L. (White variety) leaf and *Clitoria ternatea* L.(Blue variety) Root. Starch is broken down into glucose and it plays a prominent role in providing energy, most of the caloric intake should come from this macronutrient.

Table 5

Sl no	Taxa	Sample	Concentration of carbohydrate/mg
1	<i>Clitoria ternatea</i> L. (Blue)	Leaf	2.51
		Root	5.12
2	<i>Clitoria ternatea</i> L. (White)	Leaf	5.77
		Root	6.15
3	<i>Clitoria ternatea</i> L. Var. <i>pleniflora</i> (blue var)	Leaf	1.18
		Root	3.15

Starch is a type of complex carbohydrate that undergoes several different steps during digestion. Starch content is more present in the *Clitoria ternatea* L.(White variety) leaf

and *Clitoria ternatea* L. (Blue variety) Root as shown in Table:6.

Table 6

Sl no	Taxa	Sample	Concentration of starch/mg
1	<i>Clitoria ternatea</i> L. (Blue)	Leaf	2.13
		Root	3.26
2	<i>Clitoria ternatea</i> L. (White)	Leaf	2.89
		Root	2.36
3	<i>Clitoria ternatea</i> L. Var. <i>pleniflora</i> (blue var)	Leaf	2.14
		Root	1.96

Flavonoid content was more in the *Clitoria ternatea* (White) leaf and *Clitoria ternatea* (Blue) root. Apart from *Clitoria ternatea* (White) leaf other parts are equally rich in flavonoids. Dietary flavonoids are noted to play effective role

in cancer prevention. Flavonoids together with other secondary metabolites identified in *C.ternatea* active against diverse pathogens, used traditionally as analgesis, antimicrobial and soothing herbs (Ponnusamy, 2013).

Table 7

Sl no	Taxa	Sample	Concentration of flavonoid/mg
1	<i>Clitoria ternatea</i> L. (Blue)	Leaf	5.56
		Root	5.9
2	<i>Clitoria ternatea</i> L. (White)	Leaf	6.03
		Root	4.89
3	<i>Clitoria ternatea</i> L. Var. pleniflora (blue var)	Leaf	4.91
		Root	5.23

Alkaloids were most abundant in *Clitoria ternatea* L. (White variety). Alkaloids are mainly used in medicines. They act as life drugs in some serious disorders like heart failure, cancer, and blood pressure. They have been used for mental

excitement and euphoria. *Clitoria ternatea* (white) leaf and root in compare with other parts of the plant gave greater percentage of alkaloids.

Table 8

Sl no	Taxa	Sample	Concentration of alkaloids/mg
1	<i>Clitoria ternatea</i> L. (Blue)	Leaf	2.03
		Root	3.13
2	<i>Clitoria ternatea</i> L. (White)	Leaf	2.1
		Root	3.19
3	<i>Clitoria ternatea</i> L. Var. pleniflora (blue)	Leaf	1.19
		Root	2.26

Phenol content is high in the *Clitoria ternatea* L. (White) leaf and *Clitoria ternatea* L. (Blue) root. Concentrated phenol liquids are commonly used for permanent treatment of

ingrown toe, fingernails and sore throat. *C.ternatea* varieties strongly highlights their use in various antimicrobial activities (Rabeta and Nabil, 2013) [8].

Table 9

Sl no	Taxa	Sample	Concentration of phenol/mg
1	<i>Clitoria ternatea</i> L. (Blue)	Leaf	2.33
		Root	4.29
2	<i>Clitoria ternatea</i> L. (White)	Leaf	3.23
		Root	3.19
3	<i>Clitoria ternatea</i> L. Var. pleniflora (blue var)	Leaf	2.54
		Root	4.14

Tannin content is high in the *Clitoria ternatea* L. (White) leaf and root. Comparatively *Clitoria* shows more tannin content

than other plants.. Tannin can serve as antidotes for poison.

Table 10

Sl no	Taxa	Sample	Concentration of tannin/mg
1	<i>Clitoria ternatea</i> L. (Blue)	Leaf	1.32
		Root	1.63
2	<i>Clitoria ternatea</i> L. (White)	Leaf	2.23
		Root	3.19
3	<i>Clitoria ternatea</i> L. Var. pleniflora (blue var)	Leaf	1.01
		Root	1.14

Anti-Bacterial Activity

The anti-bacterial activity test was performed by agar diffusion method by Muller Hinton agar medium. Anti-bacterial growth was determined by measuring the diameter of the inhibition zone (SD +/-mean). The anti-bacterial properties of *Clitoria ternatea* varieties was investigated by agar diffusion methods. The organic solvent (Acetone) extracts from the leaves and roots of *Clitoria* varieties were tested against *E.coli*. The results showed promising antibacterial activity against the tested microbial pathogens. Anti bacterial activity of *C.ternatea* varieties leaf and roots

shows inhibition at 100% and their zone of inhibition was recorded with in leaf 18, 16, 13 % and in the case of roots 12, 14, 16%. From the result it is clear that *Clitoria ternatea* (white root) 1L and *Clitoria ternatea* L. Var. *pleniflora* (blue var) shows maximum zone. The result of the study suggest that *Clitoria ternatea*-(white root) 1L and *Clitoria ternatea* L. Var. *pleniflora* (blue var) extracts of could be suitable for the treatment of various infection caused by *E.coli*. Anti-bacterial activity of *Clitoria ternatea* L. varieties Root against *E.coli* represented in Table: 11

Table 11

Sample	Concentration (μ l)	Zone of inhibition (mm)
<i>E. coli</i>	20 μ l	35
<i>Clitoria ternatea</i> L.(white root)1R	100 μ l	12
<i>Clitoria ternatea</i> L.(Blue root) 2R		14
<i>Clitoria ternatea</i> L. Var. pleniflora (blue var) 3R		16

Anti-bacterial activity of *Clitoria ternatea* L. varieties Leaf against *E.coli* represented in Table :12.

Table 12

Sample	Concentration (μ l)	Zone of inhibition (mm)
<i>E. coli</i>	20 μ l	35
<i>Clitoria ternatea</i> - (white leaf)1L	100 μ l	18
<i>Clitoria ternatea</i> (Blue leaf) 2L		16
<i>Clitoria ternatea</i> L. Var. pleniflora (blue var 3L)		13

Concentration of Sample stock: 1mg/ml DMSO

Summary and Conclusion

Various vegetative parts of Medicinal plants were the potent source of human health due to the presence of active phytochemical compounds that are responsible for its various pharmacological activities. On the basis of the results obtained, the present work conclude that the leaves and root of *Clitoria ternatea* varieties are rich in phytochemical constituents even though the phytochemical screening of the leaf and root extracts of samples had shown variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in aqueous extracts of leaves. The presence of various secondary metabolites such as alkaloids, terpenoids, saponins, proteins, aminoacids, fixed oils, phenolic compounds, tannins, flavonoids, steroids etc. were believed to exhibit the antibiotic properties of *Clitoria ternatea* L. leaves and roots confirmed their antimicrobial efficacy against *E. coli*. The present work highlights the possible use of *Clitoria ternatea* L. leaf and root extracts as a source of antioxidants and as antibacterial agents that can be used to prevent bacterial diseases. The study reveals that the results of extraction yield, total phenol and flavonoid compounds and bioactivity tests varied depending upon the type of solvent being used. Hence it can be concluded that the extract of this plant would direct to the establishment of some compounds that could be used to invent new and more potent anti microbial drugs of natural origin.

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