Comparative Investigation of Phytochemical Constituents and Antimicrobial Activity of *Clitoria ternatea* L. (Aung-me-nyo) Leaf, Flower, Root, Seed and Stem

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Abstract

Clitoria ternatea L., also known as butterfly pea flower, has antimicrobial properties that are beneficial to human health. In Myanmar, Aung-me-nyo plant has been of keen interest due to its wide spectrum of medicinal uses and biological activities. In the present research, parts of Clitoria ternatea L. (flower, leaf, root, seed and stem) were selected for chemical analysis. Firstly, the preliminary phytochemical screening of each part of Clitoria ternatea L. was been carried out. Moreover, the antimicrobial activity of watery extract of white flower, blue flower, leaf, root, seed and stem of Clitoria ternatea L. were respectively tested against six species of microorganisms such as Staphylococcus aureus, Escherichia coli, Pseudomonas fluorescens, Saccharomyces cerevisiae, Bacillus pumilus and Bacillus subtilis by employing agar well diffusion method. From the results, it could be suggested that it has medicinal uses not only flower but also all parts of the Clitoria ternatea.

Keyword: *Clitoria ternatea L., phytochemical constituents, antimicrobial activity*

1. Introduction

Clitoria ternatea L. is an attractive perennial climber with conspicuous blue or white flowers. [1]. All part of *Clitoria ternatea* L. have potential medicinal properties, the roots are with cathartic, diuretics and has laxative effects and it's juice used for chronic bronchitis [2], the roots has been evaluated for the medicinal values like anti diarrhea [3], antihistamic and cholinergic activity. The flowers are used for collyrium, anti-inflammatory and analgesic [4]. Fresh flower showed hypoglycemic and hypolipidemic effect [5]. Seeds are cathartic and useful in visceralgia. They are considered safe for colic, dropsy and enlargement of abdominal viscera [6]. The root, stem and flower are recommended for the treatment of snakebite and scorpion sting [7].

1.1. Scientific Classification of *Clitoria ternatea* L.

Kingdom : Plantae
Family : Fabaceae
Genus : *Clitoria*
Species : *C. ternatea*
Botanical name : *Clitoria ternatea* L.
Common Name : Butterfly pea
Myanmar Name : Aung-me-nyo
Distribution : South-east Asia, tropical Asia

Figure 1. Plants of *Clitoria ternatea* L.

Figure 2. White flower, blue flower, leaf, root, seed and stem of *Clitoria ternatea* L.

2. Materials and Method

2.1. Sample Collection

The plant parts (leaf, flower, root, seed and stem) of *Clitorea ternatea* L. were collected from Kyaukse University Campus, Taw-ma *Village*, Sintgaing Township, Mandalay Region. They were identified at Botany Department, Kyaukse University. These collected plant parts were shade dried, powdered in a mechanical grinder and stored in the air-tight container for chemical and biological investigation.

2.2. Preliminary Phytochemical Investigation

Screening of preliminary phytochemical constituents was carried out on the dried powdered sample to investigate the presence or absence of primary metabolites and secondary metabolites, such as alkaloids, glycosides, polyphenols, carbohydrates, starch, flavonoids, organic acids, tannins, cyanogenic glycosides, phenols, reducing sugars, saponins, and α-amino acids by using standard procedure [8]&[9].

2.3. Determination of Antimicrobial Activity of *Clitoria ternitea* L.

The preliminary study for antimicrobial activities by agar well diffusion assay was carried out by the method of Hokkaido University, 1988. The assay medium for the test organisms (1.0 g of glucose, 1.5 g of agar, 0.3 g of yeast extract, 0.2 g of peptone, 0.001 g of KH_2PO_4, 0.001 g of $MgSO_4.7H_2O$, 0.001 g of KNO_3, and 100 mL of distilled water were prepared at pH 7. The six kinds of test organisms were been used for the antimicrobial activities**.** 20 µL of watery extract of six parts of *Clitoria ternatea* L. were put on agar well diffusion and placed on assay plate containing test organisms [10]. These test organisms, *Staphylococcus aureus* (AHU 8465), *Saccharomyces cerevisiae* (NITE 52847), *Escherichia coli* (AHU 5437), *Pseudomonas fluorescens* (IFO 94307), *Bacillus pumilus* (IFO 905771) *and Bacillus subtilis* (IFO 90571) were obtained from Biotechnological Resource Development Center, (BDC), Pathein University.

3. Results and Discussion

3.1. Phytochemical Constituents

Phytochemicals are very important in medicine and constitute most of the valuable drugs. The present study reveals flower, leaf, root, seed and stem of *Clitorea ternatea* L. exhibited the presence or absence of alkaloids, glycosides, polyphenols, flavonoids, starch, carbohydrates, organic acids, tannin, phenols, reducing sugars, saponins, cyanogenic glycosides and α-amino acids. The results were presented in Table 1 to 5 and showed in Figure 3 to 7.

Table 1. Results of phytochemical constituents of flower

No.	Phytochemicals	Test Reagent	Remark
1	Alkaloids	Dragendorff's	+
		Wagner's	
		Mayer's	
2	Glycosides	10 % lead acetate	+
3	Polyphenols	5 % $FeCL_3$, 1 % $K_3Fe(CN)_6$	+
4	Flavonoids	Mg, HCl	+
5	Carbohydrates	α-naphthol, H_2SO_4	+
6	Organic acids	Bromothymol blue	+
7	Starch	1 % I_2	+
8	Tannin	1 % geltin	+
9	Phenols	5 % $FeCL_3$	+
10	Reducing sugars	Benedict's solution	+
11	Saponins	Water	+
12	Cyanogenic glycosides	H_2SO_4, Sodium picrate paper	-
13	α-amino acids	Ninhydrin	+

(+) = Presence, (-) = absence

Figure 3. Photograph of phytochemical investigation of flower

Table 2. Phytochemical constituents of leaf

No.	Phytochemicals	Test Reagent	Results
1	Alkaloids	Dragendorff's	+
		Wagner's	
		Mayer's	
2	Glycosides	10 % lead acetate	+
3	Polyphenols	5 % $FeCL_3$, 1 % $K_3Fe(CN)_6$	+
4	Flavonoids	Mg, HCl	-
5	Carbohydrates	α-naphthol, H_2SO_4	+
6	Organic acids	Bromothymol blue	+
7	Starch	1 % I_2	+
8	Tannin	1 % geltin	+
9	Phenols	5 % $FeCL_3$	-
10	Reducing sugars	Benedict's solution	+
11	Saponins	Water	+
12	Cyanogenic glycosides	H_2SO_4, Sodium picrate paper	-
13	α-amino acids	Ninhydrin	+

(+) = Presence, (-) = absence

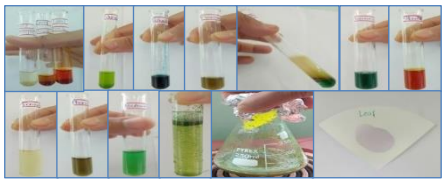

Figure 4. Photograph of phytochemical investigation of leaf

Table 3. Phytochemical constituents of root

No.	Phytochemicals	Test Reagent	Results
1	Alkaloids	Dragendorff's	+
		Wagner's	
		Mayer's	
2	Glycosides	10 % lead acetate	+
3	Polyphenols	5 % $FeCL_3$, 1 % $K_3Fe(CN)_6$	+
4	Flavonoids	Mg, HCl	+
5	Carbohydrates	α-naphthol, H_2SO_4	+
6	Organic acids	Bromothymol blue	+
7	Starch	1 % I_2	+
8	Tannin	1 % geltin	+
9	Phenols	5 % $FeCL_3$	-
10	Reducing sugars	Benedict's solution	-
11	Saponins	Water	+
12	Cyanogenic glycosides	H_2SO_4, Sodium picrate paper	+
13	α-amino acids	Ninhydrin	+

(+) = Presence, (-) = absence

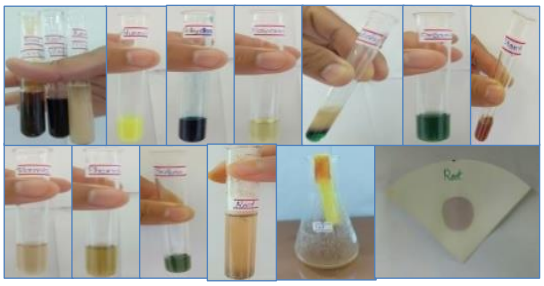

Figure 5. Photograph of phytochemical investigation of root

Table 4. Phytochemical constituents of seed

No.	Phytochemicals	Test Reagent	Results
1	Alkaloids	Dragendorff's	+
		Wagner's	
		Mayer's	
2	Glycosides	10 % lead acetate	+
3	Polyphenols	5 % $FeCL_3$, 1 % $K_3Fe(CN)_6$	+
4	Flavonoids	Mg, HCl	-
5	Carbohydrates	α-naphthol, H_2SO_4	+
6	Organic acids	Bromothymol blue	+
7	Starch	1 % I_2	+
8	Tannin	1 % geltin	+
9	Phenols	5 % $FeCL_3$	+
10	Reducing sugars	Benedict's solution	-
11	Saponins	Water	-
12	Cyanogenic glycosides	H_2SO_4, Sodium picrate paper	-
13	α-amino acids	Ninhydrin	+

(+) = Presence, (-) = absence

Table 5. Phytochemical constituents of stem

No.	Phytochemicals	Test Reagent	Results
1	Alkaloids	Dragendorff's	+
		Wagner's	
		Mayer's	
2	Glycosides	10 % lead acetate	+
3	Polyphenols	5 % $FeCL_3$, 1 % $K_3Fe(CN)_6$	+
4	Flavonoids	Mg, HCl	+
5	Carbohydrates	α-naphthol, H_2SO_4	+
6	Organic acids	Bromothymol blue	+
7	Starch	1 % I_2	+
8	Tannin	1 % geltin	+
9	Phenols	5 % $FeCL_3$	+
10	Reducing sugars	Benedict's solution	-
11	Saponins	Water	+
12	Cyanogenic glycosides	H_2SO_4, Sodium picrate paper	-
13	α-amino acids	Ninhydrin	+

(+) = Presence, (-) = absence

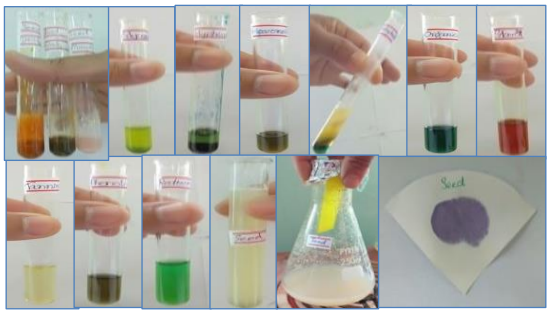

Figure 6. Photograph of phytochemical investigation of seed

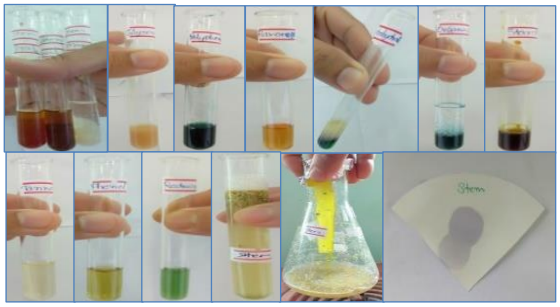

Figure 7. Photograph of phytochemical investigation of stem

3.2 Antimicrobial Activity of *Clitoria ternitea* L.

The results of antimicrobial activity of watery extract of leaf, white flower, blue flower, root, seed and stem were presented in Table 6 and showed in Figure 8.

Table 6. Results of antimicrobial activity of six parts of *Clitoria ternitea* L.

Types of Microorganisms	Inhibition Zone Diameter (mm)					
	I	II	III	IV	V	VI
Staphylococcus aureus	-	+ (12)	-	++ (16)	++ (17)	+ (11)
Escherichia coli	-	++ (15)	-	++ (16)	++ (17)	-
Pseudomonas fluorescens	-	++ (15)	-	++ (15)	+ (14)	+ (12)
Saccharomyces cerevisiae	-	++ (15)	-	++ (15)	+ (14)	+ (13)
Bacillus pumilus	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	-	-

Agar well diameter = 8mm
10 mm – 14mm (+) Low activity
15 mm – 19mm (++) Moderate activity
20 mm – above (+++) High activity
(-) No activity

I = leaf
II = white flower
III = blue flower
IV = root
V = seed
VI = stem

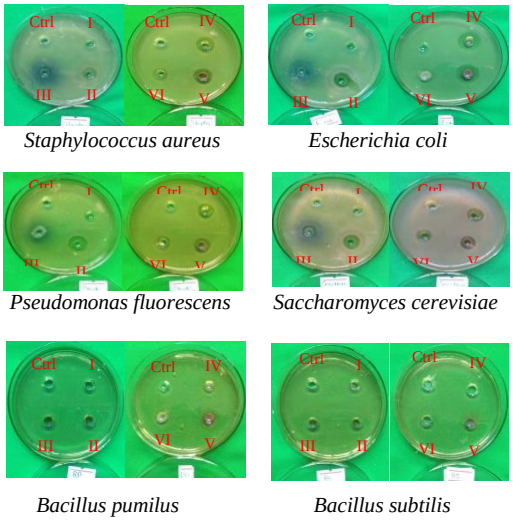

Figure 8. Antimicrobial activity of H_2O extracts of six parts of *Clitoria ternitea* L.

4. Conclusion

In preliminary phytochemical investigation, alkaloids, glycosides, carbohydrates, tannins, starch, organic acids, polyphenols and α-amino acids were present in all parts of *C. ternatea*. Some alkaloids have a bitter taste while many are toxic to other organisms. Tannins contributed the property of astringency leading to faster healing of wounds and inflamed mucous membranes. Bioactive polyphenols protect the human body from the oxidative stress which may cause many diseases. Flavonoids, that possess many useful properties, including anti-inflammatory, oestrogenic, enzyme inhibition, antimicrobial, anti-allergic, antioxidant, vascular and cytotoxic anti-tumour activity, were present in flower, root and stem but absent in leaf and seed. Phenolic compounds, that are of great importance as cellular support material because they form the integral part of cell wall structure, were present in flower, seed and stem but absent in leaf and root. Reducing sugars were present in flower and leaf but absent in root, seed and stem. Saponins were present in all parts of *C. ternatea* except seed. Traditionally sponins are extensively used as detergents, pesticides and molluscicides, in addition to they also have beneficial health effects. Cyanogenic glycosides were absent in all parts of *C. ternatea* except root. They are natural plant toxins that are present in several plants. Cyanide is formed following the hydrolysis of cyanogenic glycosides that occur during crushing of the edible plant material.

The antimicrobial activity of watery extract of six parts of *C. ternatea* was screened by Agar Well diffusion method. According to the results, it was found that white flower, root, seed and stem exhibited antimicrobial activity against four test species except *Bacillus pumilus and Bacillus subtilis*. In addition, white flower and root showed moderated activity but seed and stem showed low activity. From the data obtained in this study, it can be concluded that it has medicinal uses not only flower but also all parts of the *Clitoria ternatea*.

Clitoria ternitea will be a new antimicrobial agent by enhancing the existing of antibiotics with the extracts of *C. ternatea* as the new development of antimicrobial action. In the medical sector, it is hoped that further studies will be carried out in order to develop and use the beneficial compounds in *C. ternatea* to formulate new and powerful antimicrobial drugs of natural sources.

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