STUDY ON QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL CONSTITUENTS AND SOME BIOLOGICAL ACTIVITIES OF *CLITORIA TERNATEA* L.(AUNG – MAE –NYO) FLOWERS

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Abstract

The purpose of the research is to study the qualitative and quantitative phytochemical constituents and some biological activities such as antimicrobial activity, antitumor activity and acute toxicity activity of *Clitoria ternatea* L. (Aung-mae-nyo) flowers. The qualitative phytochemical screening of *C. ternatea* was examined by test tube method which revealed the presence of various bioactive components like alkaloids, flavonoids, carbohydrates, glycosides, phenol, saponin, terpenoids, tannin, quinones, amino acid and sterols. From the results of quantitative phytochemical screening such as total phenol contents determined Folin's Ciocalteu's method were observed to be 43.22 mg of GAE/ g in water extract and 75.72 mg of GAE /g in ethanol extract. The total flavonoids content in water and ethanol extracts determined by aluminium chloride method were observed to be 33.75 mg QE/g and 58.44 mg QE/g. The values for the water and ethanol extracts of total carbohydrate content determined by Anthrone method were found to be 374.68 mg GE/g and 265.23 mg GE/g. The total tannin contents in water and ethanol extracts were presented as 3.23 g TAE/100 g and 7.45 g TAE/100 g. The antimicrobial activity of the ethanol and water extracts of *C. ternatea* flowers was investigated against six tested microbial strains: *B. subtilis, S. aureus, P. aeruginos, B. pumilus, C. albicans* and *E. coli* by agar well diffusion method. It can be observed that ethanol extract is more active on tested microbial strains than water extract. Antitumor activity was carried out with water and ethanol extracts by Potato Crown Gall (PCG) test. From this experiment, both extracts were found to prevent the tumor formation with the dose of 0.1 and 0.15 mg/disc. The ethanol and water extracts of sample were also studied on acute toxicity by the Organization for Economic Co-operation and Development (OECD) Guideline. The acute toxicity test on albino mice indicated no toxic effect in both extracts of sample.

Keywords: *Clitoria ternatea* L., qualitative and quantitative phytochemical constituents, extractable matter, antimicrobial, antitumor, antioxidant, acute toxicity

Introduction

In many parts of the world, traditional knowledge and biodiversity still play an important role in health care, culture, religion, food security, environment and sustainable development. In herbal medicine, the one or more active ingredients are derived from the aerial and non-aerial parts, juices, resins and oils of the plant either in crude state or as pharmaceutical formulation (Gupta *et al.,* 2010). Currently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs. The use of herbal remedies is more prevalent in patients with chronic diseases such as cancer, diabetes, asthma and end-stage renal disease (WHO, 1991).

Clitoria ternatea L. is an appealing perennial climber with conspicuous blue or white flower. It is commonly known as "Aparajita", "butterfly pea", "shankhapuspi" and belongs to the Fabaceae family. It is traditionally used to deal with diverse illness (Manjula *et al*., 2013). The plant is native to South-East Asia and allotted in tropical Asia including India, the Phillipines and Madagascar. In Myanmar, it is found in Kachin, Mandalay, Sagaing and Yangon. It is commonly known as pe-nauk-ni, aung-me-hpyu and aung-me-nyo (Robert and Gary, 2018).

The whole plant extract has potential medicinal values such as anti-helmintic, anti-inflammatory, antipyretic, antibacterial, analgesic, antidepressant, anxiolytic, sedative, anticonvulsant, anticancer and antioxidant activity. Especially, *C. ternatea* contains an

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antioxidant called flavonoids, anthocyanin, phenolic compounds which activate antioxidant activity helps decrease oxidative stress caused by disease causing and aging free radicals. And also, it contains proanthocyanidin which increases blood flow to the capillaries of the eyes, useful in treatment of glaucoma, blurred vision, retinal damage or tired eyes (Chakraborty *et al*., 2017). In several Indian studies, butterfly pea exhibited significant antimicrobial effects against *Staphylococcus aureus*. Moreover, *C. ternatea's* cyclotides has efficient anti-cancer and anti-tumor activity which can cause cancer cell death by disrupting cell membrane integrity (Divya *et al.*, 2018).

Materials and Methods

Collection of Plant Sample *Clitoria ternatea* L.

The flowers sample of *C. ternatea* (Figure 1) was collected from Tharkayta Township, Yangon Region. These samples were identified at Department of Botany, University of Yangon. The collected samples were cleaned and air-dried at room temperature. The dried sample was ground into powder using grinding machine (Figure 2). The dried powdered samples were used for chemical and biological experiment.

Figure 1 Photographs of *Clitoria ternatea* L. flowers

Fresh sample Dried sample Dried powder

Figure 2 Preparation of dried powder sample of *Clitoria ternatea* L. flowers

Preliminary Qualitative Phytochemical Investigation of *C. ternatea* Flowers

In order to find out the types of phytoorganic constituents such as alkaloids, flavonoids, carbohydrates, phenols, saponins, tannins, quinones, terpenoids, oxalate compounds, glycosides,

amino acids, sterols and resins in the sample, preliminary phytochemical tests were carried out according to the appropriate reported methods. The results are shown in Table 1.

Determination of Total Phenolic Contents in Crude Extracts of *C. ternatea* Flowers

The total phenolic content (TPC) assay was performed in accordance with modifications. A 1 mL of each sample solution was mixed with 5 mL of Folin- Ciocaltea Reagent in a test tube covered with aluminium foil. After 5 min, 5 mL of 10% Na_2CO_3 was added to each test tube. The sample was then incubated for 90 min at room temperature. The absorbance was measured at 765 nm spectrophotometrically (KWF UV-7504). A standard curve of Gallic acid solutions (range from 6.25 – 100 µg mL^{-1}) was used for calibration. The experiment was done in triplicate. Concentrations of Gallic acid equivalent (GAE) in the plant extracts were calculated from the linear regression equation explored from standard curve construction for Gallic acid (Table 2 and Figure 4). TPC in the water and ethanol extracts of plant sample were expressed as (mg GAE/g). The resultant data are presented in Table 3.

Determination of Total Flavonoids Contents in Crude Extracts of *C. ternatea* Flowers

The total flavonoids content was determined by the aluminium chloride calorimetric assay. 0.3 mL of plant extracts were mixed with 0.15 mL of $AlCl_3.6H_2O$ (0.3 M), 0.15 mL of $NaNO_2$ (0.5 M), and 3.4 mL of 30 % methanol in a test tube, after 5 min, 1mL of NaOH was added. Then the absorbance was measured at 415 nm and quercetin was used as a standard solution of flavonoids. The absorbance data and calibration curve are shown in Table 4 and Figure 5. The total flavonoid content was examined with the quercetin equivalents consistent with mg QE/g of dried fraction. The data are described in Table 5.

Determination of Total Carbohydrate Contents in Crude Extracts of *C. ternatea* Flowers

The 100 mg of the sample was into a boiling tube. Then the sample was hydrolyzed by putting in a boiling water bath for 3 h with 5 mL of 2.5 M HCl then cooled to room temperature. And then, it was neutralized with solid Na_2CO_3 until the effervescence ceases. The volume was made up to 100 mL and centrifuge at 10,000 rpm for 20 min. Then the 0.5 and 1 mL aliquots were taken for further analysis. The standard was prepared by 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mL of the glucose. The absorbance data and calibration curve are shown in Table 6 and Figure 6. The volume was made up to 1.0 mL in all the tubes by adding distilled water. Then 4 mL of anthrone reagent was added (Dissolve 200 mg anthrone in 100 mL of ice-cold 95 % H_2SO_4, which prepare fresh before use). Then the mixture was heated for 8 min by using boiling water bath. Cooled rapidly and read the green color at 620 nm. The observed data are shown in Table 7.

Determination of Total Tannin Contents in Crude Extracts of *C. ternatea* Flowers

One hundred milligrams (100 mg) each of the powdered sample was weighed and put into 150 mL conical flask. 10 mL of distilled was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 mL conical flask and made up to the mark. The freshly prepared extract (1 mL) of sample was diluted to 10 mL with distilled water, and mixed with 0.5 mL of 0.1 M $FeCl_3$ in 0.1 M HCl and 0.5 mL of 0.008 M $K_3Fe(CN)_6$. The mixture was allowed to stand for 1 minute for color development, and absorbance was read at 720 nm. Tannin content was extrapolated from a standard curve (prepared with tannic acid at concentration of 0.0625, 0.125, 0.25, 0.5 and 1.0 mg/mL) and absorbance versus concentration of standard tannic acid (Table 8 and Figure 7). The tannin content was determined by the method of Van-Burden and Robinson, 1981. The tannin content of sample was calculated and data are described in Table 9.

Screening of Antimicrobial Activity of *C. ternatea* Flowers by Agar Well Diffusion Method

Antimicrobial activity of water and ethanol extracts of (Aung-Mae-Nyo) flowers were studied by agar well diffusion method at Pharmaceutical Research Department, Insein, Yangon Region. The agar well plate diffusion method was used to test the antibacterial action of the extracts on 24 h broth culture of the organisms used. At first, the extracts (1 g each for 6 species of bacteria) were introduced into sterilized Petri-dishes and dissolved in 1mL of respective solvents: H_2O and EtOH. And then, 1 mL each of the bacterial suspension of 24 h of nutrient agar was streaked evenly onto the surface of trypticase soy agar plates with sterile cotton swab. Immediately after hardening of the agar well were made with a 10 mm sterile cork borer from each seeded agar. After removing the agar, the wells were filled with the drug extracts (0.1 mL) to be tested. The plates were incubated at 37 °C for 18 - 24 h. The diameters of the inhibition zone were measured and recorded in mm. The results are shown in Table 10 and Figure 8.

Screening of Antitumor Activity of *C. ternatea* Flowers by Potato Crown Gall Test

Isolated *Agrobacterium tumefacien* has been maintained as solid slants under refrigeration. For inoculation into the potato discs, 48 hours broth cultures containing 5×10^7 - 5×10^9 cell/mL was used. Fresh, disease free potatoes were purchased from a local market. Tubers of moderate size were surface sterilized by immersion in 0.1 % sodium hypo chloride for 20 min. Ends were removed and the potatoes were soaked an additional 10 min. A core of the tissue was extracted from each end and discarded. The remainder of the cylinder was cut into 0.5 cm thick discs with a surface sterilized scalpel. The discs were then transferred to agar plates (1.5 g of agar dissolved in 100 mL sterile distilled water (DW), autoclaved for 20 min at 121°C, 20 mL poured into each petri-dish). Each plate contained four potato discs and 4 plates, were used for each sample dilution.

Each sample of 0.05, 0.1 and 0.15 mg was separately dissolved in DMSO (2 mL) and filtered through Millipore filters (0.22 µm) into sterile tube. This solution (0.5 mL) was added to sterile DW (1.5 mL), and broth culture of *A. tumefaciens* in PBS (2 mL) was added. Control was made in this way; DMSO (0.5 mL) and sterile DW (1.5 mL) were to the tube containing 2 mL of broth culture of *A. tumefaciens*. Using a sterile disposable pipette, 1 drop (0.05 mL) from these tubes was used to inoculate each potato disc, spreading it over the disc surface. After inoculation, Petri dishes were sealed by per film and incubated at 27 - 30 °C for 3 weeks. Tumors were observed on potato discs after 21 days under stereo-microscope followed by staining with Lugol's iodine (10 % KI and 5 % I_2) after 30 min and compared with control. The antitumor activity was examined by observation of tumor produced or not. The results are shown in Table 11 and Figure 9.

Acute Toxicity of *C. ternatea* Flowers Crude Extracts on Albino Mice Model

The acute toxicity of different doses of crude extracts was evaluated by the methods of OECD Guidelines for the Testing of Chemicals 423 (Diener *et al*., 1995). According to the test description, total number of 15 adult female albino mice, weighing (25-30 g) were selected and divided into five groups and each group contained three animals. They were maintained in accordance with the recommendation of the guide for the Care and Used of Laboratory Animals published by the US National Institutes of Health (NIH Publication N. 85-23, revised 1996) for studies involving experimental animals. They had free access to feed and clean drinking water during the three days' acclimatization period and throughout the experimental period. They were fasted for 18 h before giving the extracts. Groups (I) and (II) mice were orally administrated with ethanol extract of sample 300 mg/kg and 2000 mg/kg (b.wt) dose. Groups (III) and (IV) mice were giving orally with water extract of sample 300 mg/kg and 2000 mg/kg (b.wt) dose. Group

(V) mice performed as a control group. All groups of mice were kept in the three mice in the separated room at the room temperature and they were treated with cleaned water and normal animal food as shown in Figure 3. After administration of extract on each group of animals were observed first 6 hours continuously for motility and behavior changes. The motility during 14 days was noted (number of death or percent death) and the results obtained from acute toxicity effect are described in Table 12.

(a) (b)

Figure 3 Acute toxicity test on albino mice (DDY strain)
(a) Mice were put in standardized boxes, natural light and temperature, allowed free access to both water and animal feed
(b) Oral toxicity test on albino mice

Results and Discussion

Sample Collection and Preparation

The sample *C. ternatea* flowers was collected from Tharkayta Township, Yangon Region during December 2018 to February 2019 and identified by an authorized botanist, Department of Botany, University of Yangon. The fresh samples were cleaned by washing with water and air dried. The dried samples were kept in the sealed air tight container to prevent moisture changes and other contamination. These samples were used for chemical and biological investigation throughout the research work.

Qualitative Phytochemical Constituents of *C. ternatea* Flowers by Test Tube Method

Preliminary phytochemical screening of *C. ternatea* powder revealed the presence of several compounds. The main phytochemical constituents include alkaloids, flavonoids, carbohydrates, glycosides, phenol, saponin, terpenoids, oxalate compounds, tannin, quinones, amino acid and sterols. These compounds are described as potent biologically active compound found in used medicinal plant parts which are precursors for clinically useful drugs. However, resin was not detected at the assay conditions. The obtained data are shown in Table 1.

Table 1 Qualitative Phytochemical Results of *Clitoria ternatea* L.

No.	Test	Extract	Test reagent	Observation	Results
1	Alkaloids	1% HCl	Mayer's reagent	Pale yellow ppt	+
			Dragendorff's reagent	Orange ppt	+
			Wagner's reagent	Brown ppt	+
2	Flavonoids	EtOH	Dilute HCl and 20% NaOH	Intense yellow color	+
3	Carbohydrate	H_2O	Molisch's reagent and conc: H_2SO_4	Red ring	+
4	Phenol	EtOH	5% $FeCl_3$	Deep blue color	+
5	Saponins	H_2O	Distilled water	Frothing	+
6	Tannins	H_2O	Gelatin ,1% $FeCl_3$ and 1% HCl	Cherry red color	+
7	Quinones	EtOH	Conc: HCl	Yellow color	+
8	Terpenoids	H_2O	Chloroform and conc: H_2SO_4	Reddish brown ppt	+
9	Oxalate compounds	H_2O	Glacial acetic acid	Violet color	+
10	Glycosides	H_2O	Glacial acetic acid, 5% $FeCl_3$ and conc: H_2SO_4	Brown ring	+
11	α-amino acids	H_2O	Ninhydrin reagent	Purple color	+
12	Sterols	H_2O	Chloroform, acetic anhydride, conc: H_2SO_4	Intense pink color	+
13	Resins	H_2O	Distilled water	No turbidity	–

+ = Present, - = Absent

Total Phenol Contents of *C. ternatea* Flowers by Folin-Ciocalteu Method

In this study, the total phenolic content of *C. ternatea* was estimated by Folin-Ciocalteu method. Phenols react with an oxidizing agent phosphomolybdate in F-C reagent under alkaline conditions and result in the formation of blue colored complex, the molybdenum blue which is measured at 765 nm colorimetrically. Total phenolic content (TPC) was expressed as micro gram of Gallic acid equivalent (GAE) per milligram of crude extract (mg GAE/g). The total phenol content of ethanol extract (75.72 ± 0.20 mg GAE/ g) was found to be higher than water extract (43.22 ± 1.20 mg GAE/ g). The observed absorbance data of different concentrations of standard concentrations of standard gallic acid solution are summarized in Table 2 and standard calibration curve is shown in Figure 4. The resulting data are shown in Table 3.

Table 2 Absorbance of Different Concentrations of Standard Gallic Acid Solution

No.	Concentrations (µg/mL)	Absorbance (Mean value) at 765 nm
1	6.25	0.168
2	12.50	0.233
3	25.00	0.326
4	50.00	0.510
5	100.00	0.868

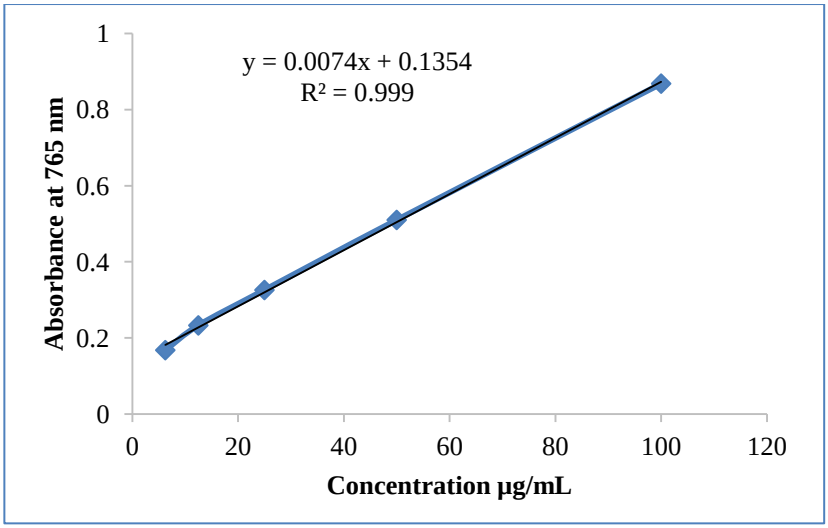


Figure 4 Calibration curve for the standard gallic acid solution

Table 3 Total Phenol Content (TPC) of Ethanol and Water Extracts of *C. ternatea*

No.	Extracts	TPC (mg GAE/g ± SD)
1	Ethanol	75.72 ± 0.20
2	Water	43.22 ± 1.20

Total Flavonoids Contents of *C. ternatea* Flowers by Aluminium Chloride Colorimetric Method

The total flavonoids contents in the examined different crude extract using the aluminium chloride colorimetric assay were expressed in terms of quercetin equivalent. The values obtained for the concentration of total flavonoid were expressed as mg of quercetin equivalent/g of dry mass. The total flavonoid content of water extract (33.75 ± 0.54 mg QE/g) was observed to be lower than ethanol extract (58.44 ± 1.12 mg QE/ g). The observed absorbance data of different concentrations of standard quercetin solution are summarized in Table 4 and standard calibration curve is shown in Figure 5. The resulting data are expressed in Table 5. In this case ethanol extract of sample have high content of flavonoid.

Table 4 Absorbance of Different Concentrations of the Standard Quercetin Solution

No.	Concentrations (µg/mL)	Absorbance (Mean value) at 415 nm
1	0.62	0.008
2	1.25	0.016
3	2.50	0.025
4	5.00	0.041
5	10.00	0.071
6	20.00	0.130

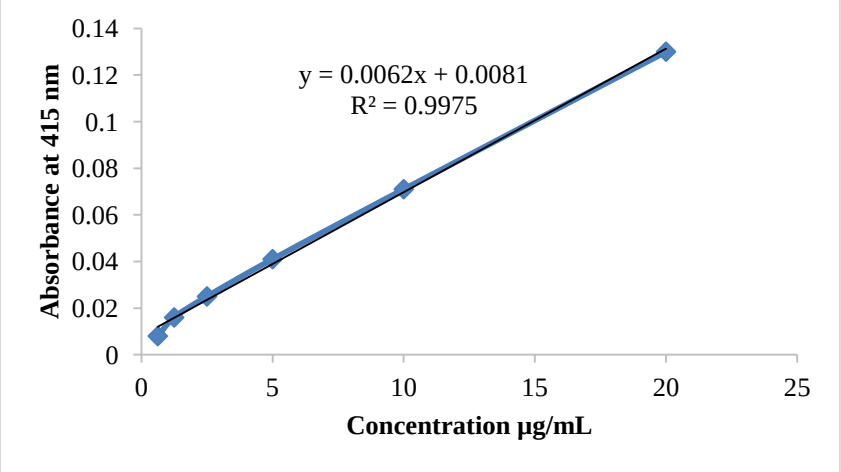


Figure 5 Calibration curve for the standard quercetin solution

Table 5 Total Flavonoids Content (TFC) of Ethanol and Water Extracts of *C. ternatea*

No.	Extracts	TFC (mg QE/g ± SD)
1	Ethanol	58.44 ± 1.12
2	Water	33.75 ± 0.54

Total Carbohydrate Contents of *C. ternatea* Flowers by Anthrone Method

The carbohydrate content in different plant extract using the Anthrone method was expressed in terms of glucose equivalent. The observed absorbance data of different concentrations of standard glucose solution are summarized in Table 6 and standard calibration curve is shown in Figure 6. The total carbohydrate content of water extract (374.68 ± 0.86 mg GE/g) was observed to be higher than ethanol extract (265.23 ± 1.14 mg QE/ g). The resulting data are shown in Table 7.

Table 6 Absorbance of Different Concentrations of the Standard Glucose Solution

No.	Concentrations (µg/mL)	Absorbance (Mean value) at 620 nm
1	3.13	0.143
2	6.25	0.227
3	12.50	0.341
4	25.00	0.531
5	50.00	0.898

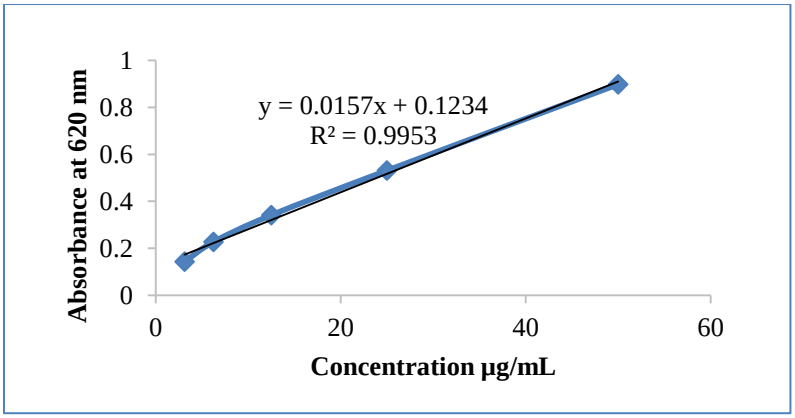


Figure 6 Calibration curve for standard glucose solution

Table 7 Total Carbohydrate Content (TCC) of Ethanol and Water Extracts of *C. ternatea*

No.	Extracts	TCC (mg GE/g ± SD)
1	Ethanol	265.23 ± 1.14
2	Water	374.68 ± 0.86

Total Tannin Contents of *C. ternatea* Flowers

Tannin is widely distributed in plant and occurs in solution in cell sap, often in distinct vacuoles. Tannins are readily soluble in water or alcohol given as stringent solution that is useful in medicine (Ibrahim *et al.*, 2004). In this research, tannins content for flowers of *Clitoria ternatea* L. were determined. For the analysis of tannin content, it is necessary to prepare a calibration curve from a series of standard tannic acid solutions at 720 nm. It was found that the plot of absorbance *vs* concentration of tannic acid is distributed in Figure 7 and the data are expressed in Table 8. After that the tannin contents were calculated by using standard comparison method. It can be obtained that tannin content is evidently high in the ethanol extract of sample (7.45 ± 0.32 g TAE/100 g) compared to the water extract of sample (3.23 ± 0.54 g TAE/100 g). The obtaining data are shown in Table 9.

Table 8 Absorbance of Different Concentrations of the Standard Tannic Acid Solution

No.	Concentrations (mg/mL)	Absorbance (Mean value) at 720 nm
1	0.062	0.087
2	0.012	0.143
3	0.250	0.289
4	0.500	0.535
5	1.000	0.986

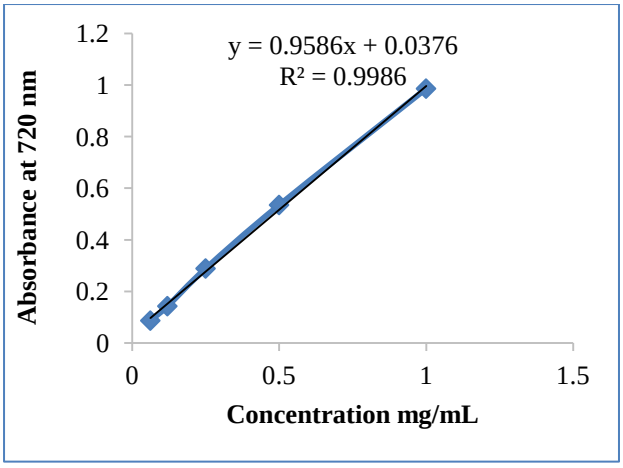


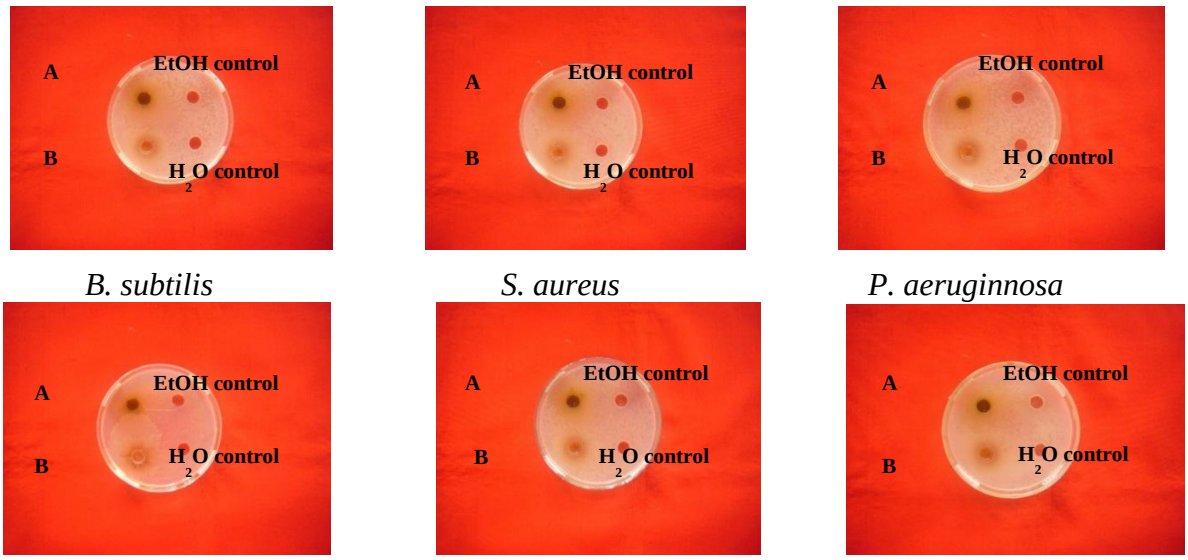
Figure 7 Calibration curve for standard tannic acid solution

Table 9 Total Tannin Content (TCC) of Ethanol and Water Extracts of *C. ternatea*

No.	Extracts	TTC (g TAE/100g ± SD)
1	Ethanol	7.45 ± 0.32
2	Water	3.23 ± 0.54

Antimicrobial Activity of Ethanol and Water Extracts of *C. ternatea* Flowers by Agar Well Diffusion Method

The ethanol and water extract of samples were tested on six species of microorganisms including *B. subtilis*, *S. aureus*, *P. aeruginosa*, *B. pumilus*, *C. albicans and E. coli*. The results are shown in Figure 8 and Table 10. According to the results, ethanol extract detected more effective in water extract. It can be found that stronger antibacterial effect for gram positive bacteria such as *B. subtilis*, *S. aureus* and *B. pumilus* than others and the inhibition zone diameters of tested samples against six microorganisms were found in the range of 19 to 26 mm in ethanol extract and 15 to 19 mm in water extract.



A = ethanol extract sample, B = water extract sample

Figure 8 Antimicrobial activity screening of ethanol and water extracts of *C. ternatea*

Table 10 Inhibition Zone Diameter (mm) of Crude Extracts of *C. ternatea* Flowers Against Tested Microorganisms

Sample	Extracts	Inhibition zone diameter (mm) against different microorganisms					
		B. subitilis	***S. aureus***	***P. aeruginosa***	***B. pumilus***	***C. albicans***	***E. coli***
C. ternatea	EtOH	26 (+++)	24 (+++)	19 (++)	23 (+++)	19 (++)	20 (+++)
	H_2O	19 (++)	17 (++)	15 (++)	17 (++)	15 (++)	16 (++)
Control	EtOH	–	–	–	–	–	–
	H_2O	–	–	–	–	–	–

Agar Well – 10 mm
10 mm ~ 14 mm (+) - low activity
15 mm ~ 19 mm (++) - medium activity
20 mm ~ above (+++) - high activity

Antitumor Activity of *C. ternatea* Flowers by Potato Crown Gall Test

The antitumor activity of ethanol and water extracts of *C. ternatea* Flowers were investigated by using PCG test with bacterium *A. tumefaciens*. For inoculation of potato disc, 48 h broth cultures containing 5×10^9 cells/mL were used. The tested samples were dissolved in DMSO, diluted and mixed with bacterial culture for inoculated on the cleaned and sterilized potato disc, and incubated for 7 days, at room temperature. After that the tumors were appeared on potato discs and checked by staining the knob with Lugol's solution. In the control, the formation of white knob on the blue background indicated the presence of tumor cells because there is no protein in tumor cells. The activities of test samples did not form any tumor on the potato discs and its surface remained blue. Tumors were counted with the aid of dissecting scope after staining with Lugol's solution.

From this experiment, it was found that both ethanol and water extracts of *C. ternatea* were good for preventing the tumor formation with the dose of 0.10 and 0.15 mg/disc in *vitro* potato disc assays. In addition, both ethanol and water extracts were not significantly inhibited the formation of tumor with the dose of 0.05 mg/disc. The quantitative criteria and results are expressed as (-) for high inhibition, (+) for less inhibition and (+++) for non-inhibition of tumor growth after visual comparison with the control (Table 11 and Figure 9).

Table 11 Antitumor Activity of Crude Extracts of *C. ternatea* Flowers by PCG Test

Sample	Extracts	Concentration (mg)/disc		
		0.05	**0.10**	**0.15**
Clitoria ternatea	Ethanol	+	-	-
	Water	+	-	-
Control	D/W	++		

Tumor Inhibition: (++) = non activity, (+) = less activity, (-) = high activity

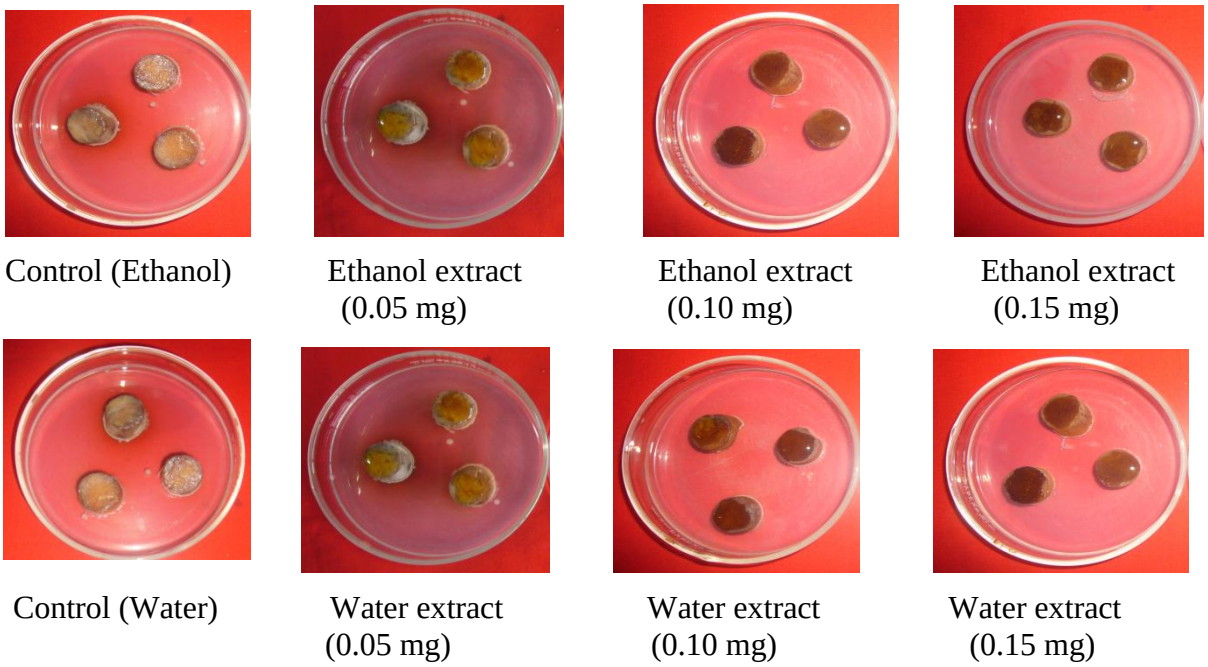


Figure 9 Antitumor screening of ethanol and water extracts of *C. ternatea* flowers incubated for 7 days

Acute Toxicity Study of Crude Extracts of (Aung-Mae-Nyo) Flowers

The acute toxicity screening of ethanol and water extract of *C. ternatea* flowers were prepared with the dose of 300 mg/kg and 2000 mg/kg body weight in each of albino mice. The conditions of mice groups were recorded after fourteen days' administration. The results shown no lethality of the mice was observed up to fourteen days. Each group of animals was also observed still alive and did not show any visible symptom of toxicity like restlessness, respiratory disorders, convulsion, aggressive activities, coma and death. Even with the dose up to 2000 mg/kg body weight administration, there is no lethality after 14 days. Therefore, ethanol and water extracts of *C. ternatea* flowers was free from acute toxic effect under condition. The results are distributed in Table 12.

Table 12 Acute Toxicity Effect of Crude Extracts of *C. ternatea* Flowers on Albino Mice Model after 14 Days Administration

No	Groups	Drug Administration	Dosage mg/kg (b. wt)	No. of death per tested mice	% of death
1.	I	Ethanol extract	300	0/3	0
2.	II	Ethanol extract	2000	0/3	0
3.	III	Water extract	300	0/3	0
4.	IV	Water extract	2000	0/3	0
5.	V (control)	Distilled water	-	0/3	0

Note: Each group contains 3 no: of mice
There is no acute toxicity in ethanol and water extracts of *C. ternatea* Flowers.

Conclusion

From the overall assessments of the research work, the following inferences could be deduced. In the qualitative phytochemical screening of *Clitoria ternatea* L. (Aung-Mae-Nyo) flowers, alkaloids, flavonoids, carbohydrates, glycosides, phenol, saponin, terpenoids, oxalate compounds, tannin, quinones, amino acid and sterols are present. But, a resin is not detected. From the results of quantitative phytochemical screening in ethanol and water extract, the total phenol content, total flavonoids and total tannin content (75.72 mg GAE/g, 58.44 mg QE/g and 7.45 g TAE/100g) in ethanol extract were evidently higher than (43.22 mg GAE/g, 33.75 mg QE/g and 3.23 g TAE/100g) in water extract. But, the total carbohydrate content of water extract (374.68 mg GE/g) was observed to be higher than ethanol extract sample (265.23 mg GE/g). In the studying on the antimicrobial activity of *C. ternatea* flowers, the ethanol extract showed more active on tested microbial strains than water extract. Among them, ethanol extract sample was more effective on gram positive bacteria such as *B. subtilis, S. aureus and B. pumilus* than the other. By the screening of antitumor activity, both extracts of *C. ternatea* flowers were found to prevent the tumor formation with the dose of 0.10 and 0.15 mg/disc. From acute toxicity test, it was observed that the ethanol and water extracts of sample were free from toxic effect even with the dose up to 2000 mg/kg body weight administration to the tested albino mice. Based on this scientific investigation, some biological activities of *C. ternatea* flowers can be well known and it is useful for people's health as natural medicine.

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