

DETERMINATION OF ANTIMICROBIAL ACTIVITIES OF CRUDE ALKALOID EXTRACTED FROM THE STEMS OF *Tinospora cordifolia* Miers.

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Abstract: *Tinospora cordifolia* Miers. is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae. The qualitative phytochemical screening of the stems of *Tinospora cordifolia* Miers. was determined by using standard methods. The stems of *Tinospora cordifolia* Miers. revealed the presence of alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, tannins, glycosides and carbohydrates, and showed the absence of steroids and reducing sugars. The quantitative crude alkaloid was extracted from the stems of *Tinospora cordifolia* Miers. by using Harbone J.B. Method. The percentage yield of the crude alkaloid was found to be 2.008%. And the antimicrobial activities of crude alkaloid extracts in various solvent systems were tested by using agar well diffusion method on six selected organisms, such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli*.

Keywords: *Tinospora cordifolia* Miers., stems, crude alkaloid, Harbone J.B. method and antimicrobial activities

1. INTRODUCTION

Mankind has used many plants for medicinal purposes for thousands of years. Natural products have been traditionally accepted as remedies for many diseases. The beneficial medicinal effects of plant products typically result from the combinations of secondary metabolites present. Plant extracts have been known since antiquity to possess notable biological activities, including antibacterial, antioxidant, and anticancer properties (Tilburg JC, Kapchuk TJ.,2008).

An alkaloid is a naturally occurring nitrogen-containing compound, which shows a basic. It is assumed that alkaloids are produced by the plant as a type of defense against insects and herbivores. They are usually bitter tasting, and often have psychoactive properties. About 80% of plant alkaloids possess anti-inflammatory properties but isoquinoline (berbamine, berberine, cepharanthe and tetrandrine) is the most active (Yang L, Stöckigt J.,2010).

Among the medicinal plants, *Tinospora cordifolia* Miers. belongs to the family Menispermaceae which consists of about 70 genera and 450 species that are found in tropical lowland regions. This family is a rich source of alkaloid and terpenes. Myanmar and India are its native countries. It is widely available for sale in local markets, herbal stores etc. Flowers and fruits grow during summer and during winter. The leaves afford a good fodder for cattle. *Tinospora cordifolia* Miers., has showed no side effect and toxicity. It is reported to possess antispasmodic, anti-inflammatory and antiallergic properties (Bhalerao *et al.*, 2016).

Aim

The main aim of this research is to determine the antimicrobial activities of crude alkaloid extracted from the stems of *Tinospora cordifolia* Miers.

1.1 Botanical Description of *Tinospora cordifolia* Miers

Scientific name :	<i>Tinospora cordifolia</i> Miers	Locality :	Tropical regions of Myanmar
Family :	Menispermaceae	Habi :	Climber
English name :	Heart-leaved Moon seed	Parts used :	Stems
Myanmar name :	Sindone-ma-nwe		



2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The stems of *Tinospora cordifolia* Miers. were collected from Myitkyina University campus, Kachin state in August 2018. The stems of this selected medicinal plant were thoroughly washed with tap water, cut into small pieces and air dried for one month, and then stored in airtight glass bottles.



2.2 Preliminary Phytochemical Analysis of the stems of *Tinospora cordifolia* Miers.

Phytochemical screening of the stem of *Tinospora cordifolia* Miers. was performed for the qualitative detection of alkaloids, flavonoids, terpenoids, phenolic compounds, steroids, saponins, tannins, glycosides, carbohydrates and reducing sugar by using standard procedures.

2.3 Extraction of Alkaloids from the stems of *Tinospora cordifolia* Miers.

A 5 g of the ground stems of *Tinospora cordifolia* Miers. was added to a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 hr. it was filtered and this extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with 1% ammonium hydroxide solution and then filtered. The residue is the alkaloid, which was dried and weighed. As stated above, this extraction procedure was carried out for three times using (5) g of dried sample per extraction. About 0.04 g of the dried residue was dissolved in 3 mL distilled water. Dragendorff's reagent was added to it. The crude alkaloid was confirmed by performing which shows the appearance of orange colored precipitate using Dragendorff's reagent (Harborne, J.B.,1973). The percentage yield of crude alkaloid was calculated using the following formula:

$$\% \text{ yield} = \frac{\text{weight of the alkaloid residue} \times 100}{\text{weight of ground stems of } Tinospora \text{ cordifolia Miers.}}$$



Figure 1. 5 g of the sample in 200 mL of 10 % acetic acid in ethanol for 4 hr



Figure 2. Crude alkaloid residue on the filter paper

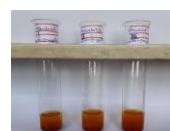


Figure 3. Crude Alkaloid test with Dragendorff's reagent

2.4 Determination of Antimicrobial Activities of Crude Alkaloid

The antimicrobial activities of the stems of *Tinospora cordifolia* Miers. were evaluated at Pharmaceutical Research Department, Yangon by using agar well diffusion method on six selected organisms that include three gram positive bacteria, *Bacillus subtilis*, *Bacillus pumilus* and *Staphylococcus aureus*, two gram negative bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*, and fungi, *Candida albicans*.

3. RESULTS AND DISCUSSION

In this research work, phytochemical screening and crude alkaloid extraction of the stems of *Tinospora cordifolia* Miers. were carried out. And the antimicrobial activities of crude alkaloid were also determined.

3.1 Phytochemical Screening of the Stems of *Tinospora cordifolia* Miers.

The results of the preliminary screening of the stems of *Tinospora cordifolia* Miers. are shown in Figure 4. and Table 1.

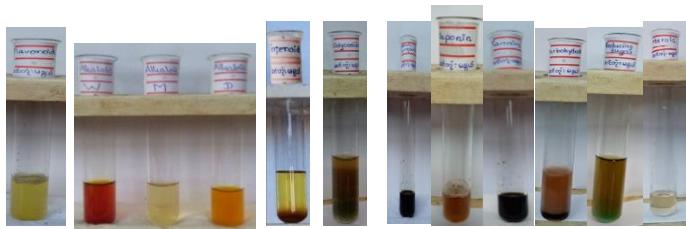


Figure 4. Phytochemical Analysis of the Stems of *Tinospora cordifolia* Miers.

Table1. Phytochemical Screening of the Stems of *Tinospora cordifolia* Miers.

No.	Tests	Extracts	Test reagents	Observation	Results
1.	Alkaloids	1 % HCl	Dragendroff's reagent Wagner's reagent	Orange colored ppt reddish brown colored ppt	+
2.	Flavonoids	EtOH	Mayer's reagent Mg turning, conc: HCl	cream colored ppt Greenish yellow color	+
3.	Terpenoids	EtOH	CHCl ₃ , conc: H ₂ SO ₄	Reddish brown colored ppt	+
4.	Phenolic compounds	EtOH	10 % FeCl ₃	Black color solution	+
5.	Steroids	CHCl ₃	Acetic anhydride, conc: H ₂ SO ₄	No Green color solution	-
6.	Saponins	H ₂ O	Distilled water	Frothing	+
7.	Tannins		10 % FeCl ₃	Dark brown color solution	+
		H ₂ O			
8.	Glycosides	H ₂ O	10 % lead acetate	White ppt	+
9.	Carbohydrates	H ₂ O	10 % α -naphthol, conc: H ₂ SO ₄	Violet color ring of the interface of the two layers	+
10.	Reducing sugar	H ₂ O	Benedict's solution	brick-red colored ppt	-

(+) = Presence of constituents (-) = Absence of constituents

According to these results, the stems of *Tinospora cordifolia* Miers. showed the presence of alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, tannins, glycosides and carbohydrates and the absence of steroids and reducing sugars. These phytochemicals of the stems of *Tinospora cordifolia* Miers. are unique health compounds that are essential to human health.

3.2 Determination of the Percentage Yield of Crude Alkaloid extracted from the Stems of *Tinospora cordifolia* Miers.

The results of crude alkaloid of *Tinospora cordifolia* Miers. are shown in Table2.

Table 2. The Percentage Yield of Crude Alkaloid extracted from the stems of *Tinospora cordifolia* Miers.

No	Weight of ground <i>Tinospora cordifolia</i> Miers. stems (gram)	Weight of the alkaloid residue (gram)	% yield
1	5	0.1003	2.006
2	5	0.1005	2.010
3	5	0.1004	2.008
Average	5	0.1004	2.008

The average yield percent of the crude alkaloid content in the stems of *Tinospora cordifolia* Miers. was quantitatively found to be 2.008%. This data signifies that the stems of *Tinospora cordifolia* Miers. in general has the highest percentage yield. The most popular reagent, Dragendorff's reagent reacts with tertiary and quaternary nitrogen atoms present in alkaloid molecules. Therefore, this crude alkaloid may contain berberine, colchicine, canadine, atropine, nicotine and tubocurarine, but may not

involve alkaloids containing primary and secondary amino groups (norephedrine and ephedrine). According to the current results, the stems of *Tinospora cordifolia* Miers. hold an important bioactive compounds.

3.3 Antimicrobial Activities of Crude Alkaloid

The results of antimicrobial activities of crude alkaloid extracted from the stems of *Tinospora cordifolia* Miers. are shown in Figure 5 and Table 3.

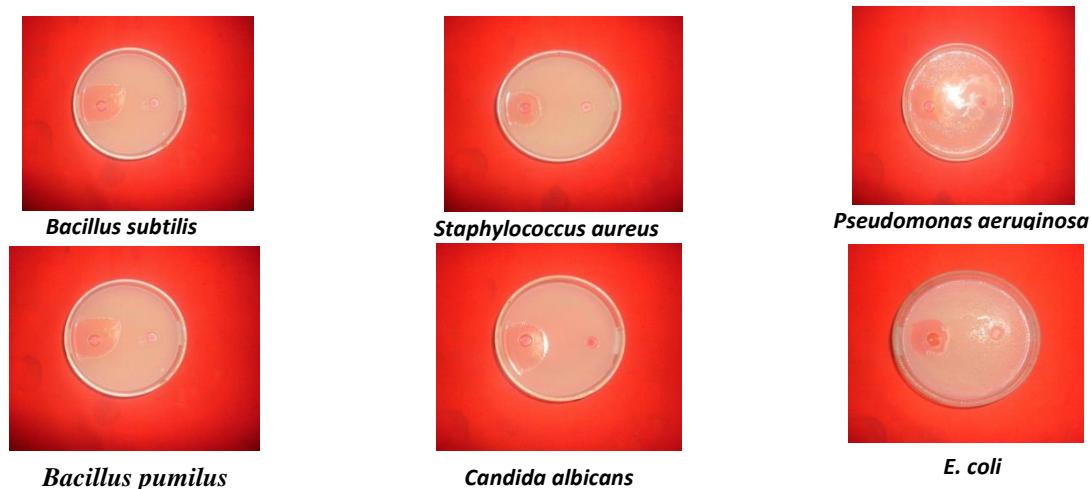


Figure 5. Antimicrobial activities of crude alkaloid extracted from the stems of *Tinospora cordifolia* Miers.

Table 3. Results of Antimicrobial Activities of crude Alkaloid Extracted from the Stems of

Tinospora cordifolia Miers.

Sample	Inhibition zone diameters of crude alkaloids against six microorganisms (mm)					
	I	II	III	IV	V	VI
crude alkaloid	30 mm (+++)	25 mm (+++)	30 mm (+++)	30 mm (+++)	30 mm (+++)	25 mm (+++)

Agar well – 10 mm

10 mm ~ 14 mm (+)

15 mm ~ 19 mm (++)

20 mm above (+++)

Organisms

(I) *Bacillus subtilis*

(II) *Staphylococcus aureus*

(III) *Pseudomonas aeruginosa*

(IV) *Bacillus pumilus*

(V) *Candida albicans*

(VI) *Escherichia coli*

According to these results, the crude alkaloid extracted from the stems of *Tinospora cordifolia* Miers. showed high activities on all tested organisms. The antimicrobial activities of crude alkaloid demonstrate that folk medicine could be as effective as modern medicine to combat pathogenic microorganisms.

4. CONCLUSION

Phytochemical screening of the stems of *Tinospora cordifolia* Miers. contained alkaloids, flavonoids, terpenoids, phenolic compounds, saponins, tannins, glycosides and carbohydrates but showed the absence of steroids and reducing sugars. These qualitative phytochemicals of the stems of *Tinospora cordifolia* Miers. are responsible for showing considerable antimicrobial, antioxidant and anticancer activities. The significant amount of crude alkaloid was found to be 2.008%. And the antimicrobial activities of crude alkaloid extracted from the stems of *Tinospora cordifolia* Miers. showed high activities on all tested organisms. The stems of *Tinospora cordifolia* Miers. could serve as a potential source of useful drugs. Further works are needed to isolate, characterize and elucidate the structures of the bioactive compounds in the stems of *Tinospora cordifolia* Miers. for pharmacological active constituents.

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