SCREENING OF THE ACUTE TOXICITY, ANTIMICROBIAL AND ANTITUMOR ACTIVITIES OF ROOTS OF *Stemona Curtisii* HOOK.F.

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ABSTRACT

In the present research, roots of *Stemona curtisii* Hook. F., family-Stemonaceae was chosen to be studied. Acute toxicity study of 95% ethanol extract from roots of *Stemona curtisii* Hook. F. was investigated by methods of OECD guidelines for the testing of Chemical 425. Screening of root extract was done with the dosage of 2000 mg/kg, 300 mg/kg and 50 mg/kg body weight in albino mice. Dosage of 2000 mg/kg was discovered lethality within 60 min with symptoms of toxicity like restlessness, convulsion, coma, and death. The results of other groups show no lethality of mice up to fourteen days administration. Antimicrobial activity of pet- ether, methanol, ethyl acetate, 95% ethanol and watery extracts from roots of *S .curtisii* was investigated against six species of microorganisms such as *Bacillus pumilus, Bacillus subtilis, Candida albicans, Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus* by agar well diffusion method. Ethyl acetate and methanol extracts of *S. curtisii* exhibited inhibition zone diameter in the ranges of (12~15 mm) and (11~20 mm) against all tested microorganisms, respectively. 95% ethanol extract of *S.curtisii* showed inhibition zone diameters (11 mm) only against *Pseudomonas aeruginosa.* On the other hand, pet-ether and watery extract of sample showed activity against five tested microorganisms in the range of (11~ 14 mm) and (11~20 mm) except *Bacillus subtilis*. Antitumor activity of methanol, ethyl acetate, 95% ethanol, and watery extracts of roots of *S.curtisii* was screened on *Agrobacterium tumefacien* by Potato Disc Assay method. All of extracts from root sample exhibited antitumor activity against *Agrobacterium tumefacien* after 5 days and 7 days periods of observation.

Keywords : *Stemona curtisii* Hook.F., acute toxicity, antimicrobial activity, antitumor activity

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INTRODUCTION

Stemona curtisii Hook.F. is one of the important monocotyledon plants belonging to the family Stemonaceae and widely distributed in the northern region of Myanmar. The roots of several species in the genus *Stemona* are widely used as medicinal purposes due to the occurrence of various alkaloids. The most important alkaloid is stemofoline which possess bio-insecticide properties. *Stemona* family is one of considerable interest because it is the only source of the unique alkaloids known as stemona alkaloids including stemocurtisine, stemocurtisinol and oxyprotostemonine (Figure 1) which have been isolated from a root extract of *S. curtisii* (Sastraruji, 2006).

The root extracts and the pure alkaloid of *S. curtisii* especially oxyprotostemonine were shown larvicidal activity against *Anopheles minimus* (Mungkornasawakul, 2003). Kaltenegger (2003) reported that the crude extract of *S. curtisii* had insecticidal activities against *Spodoptera littoralis*. The main chemical constituent of the *S. curtisii* Hook.F. is a specific group of Stemona alkaloids, including stemofoline, 2'-hydroxy stemofoline, oxyprotostemonine, dehydroprotostemonine, protostemonine, stemocochinine, stemocurtisine (pyridostemin), stemocurtisinol and oxystemokerrine (Mungkornasawakul, 2004).

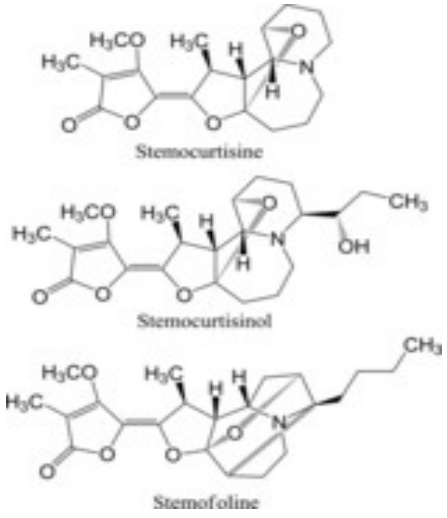

Figure 1 Structures of stemocurtisine, stemocurtisinol and stemofoline

Botanical Aspects of *Stemona curtisii* Hook.F.

Scientific Name	-	***Stemona curtisii*** Hook.F.
Family	-	Stemonaceae
Myanmar Name	-	Thar-myaa-oo
Common Name	-	Non Tai Yak in Thailand
Plant part used	-	Roots

Figure 2. *Stemona curtisii* plant, leaves and roots

Uses of *S.curtisii*

S.curtisii (Family Stemonaceae), a prominent species distributed in the south and southwest of Thailand, has widely been used as a natural pesticide and as treatment for head lice and skin diseases.

Aim and Objectives

The aim of this study was to evaluate acute toxicity, antimicrobial and antitumor activities of root of *S. curtisii*. (Thar-myaa-oo). To fulfill this aim, the research was carried out according to the following objectives.

(1) To extract the sample with various solvents

(2) To determine the phytochemical tests

(3) To investigate the acute toxicity, antimicrobial and antitumor activities of root sample

Materials and Methods

Collection and Preparation of *S. cutisii* Extracts

The roots of *S. curtisii* belonging to the family Stemonaceae were collected from Kalay Township, Sagaing Region in Myanmar, during January to February 2016. The collected roots samples were identified as *S. curtisii* (Thar-myaa-oo) according to the authorized botanist from Department of Botany, Myitkyina University. A total of 5 Kg of *S. cutisii* fresh root samples were scrutinized for any foreign matter and cleaned with distilled water. They were then chopped into small pieces and air dried under shade at the laboratory. When the plant material dried, it was ground into powder using grinding machine. The powdered plant material obtained was stored in clean air tight container.

Preparation of crude extracts by direct extraction methods for screening of some biological activities

Each dried powdered sample (50 g) was extracted with 150 mL of PE (60-80 °C) for 6 h by using soxhlet extractor. The filtrate was concentrated by removal of the solvent under reduced pressure to give the respective pet-ether crude extract. Preparation of ethyl acetate extract, 95% ethanol, methanol, dichloromethane and watery extracts were prepared by similar manner mentioned in above procedure. Each extract was dried at normal pressure on a water bath and stored under refrigerator for screening some bioactivities.

Qualitative screening of the phytochemicals

In order to classify the types of organic constituents present in root samples, preliminary phytochemical tests on samples were carried out according to the series of test tube tests.

(a) Screening of antimicrobial activity of different crude extracts of *S. curtisii* Hook.f.

The antimicrobial activities of different crude extracts such as PE, EtOAc, 95% EtOH, MeOH and H_2O extracts from roots of *Stemona curtisii* Hook.f.were determined against six species of microorganisms such as *Bacillus pumilus* (N.C.I.B - 8982), *Bacillus subtilis* (N.C.T.C - 8236), *Candida*

albicans, Escherichia coli (N.C.I.B - 8134), *Pseudomonas aeruginosa* (6749) and *Staphylococcus aureus* (N.C.P.C - 6371) by employing agar well diffusion at the Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar.

(b) Acute toxicity test of the samples on albino mice model

To determine the symptomatology consequent to injection of the plant and to determine the nature and degree of toxicity produced by these extracts and to find out the medium lethal doses (LD_{50}) of the extracts, acute toxicity test was done. Usually the acute lethality of a compound is determined on the basic of deaths occurring in 24 h but the survivors should be observed for at least seven days in order to detect delayed effects. In this study, acute toxicity effect of ethanol extracts of *Stemona* root (three doses) were determined on albino mice, at Laboratory Animal Services Division, Department of Medical Research (DMR), Yangon.

Acute toxicity of different doses of EtOH extract of sample was evaluated by the methods of OECD Guidelines for the Testing of Chemicals 425. According to the test description, total number of adult female albino mice, weighing (25-30 g) were selected and divided into four groups. Each group contained six animals. They were fasted for 18 h before giving the extracts. Group (1) mice were orally administrated with EtOH extract 2000 mg/kg dose. Group (2) mice were given orally with EtOH extract 300 mg/kg dose. Group (3) mice were also administered with EtOH extract 50 mg/kg dose and Group (4) mice performed as a control group and they were treated with clean water and normal laboratory animal food of Laboratory Animal Services Division, at Department of Medical Research. All groups of mice were kept in the four mouse cages in the separated room at the room temperature of 26 ± 1º C. After administration of extracts on each group of animals were observed first 6 h continuously for mortality and behavior changes. Then check the animals each 24 h for fourteen days. The mortality during this period was noted (Nil or percent death). The results obtained from acute toxicity are described in table 2.

(c) Screening of antitumor activity of crude extracts by potato crown gall test or potato disc assay method

The antitumor activity screening of different crude extracts such as ethanol, methanol, ethyl acetate and watery extracts of roots of *Stemona curtisii* Hook.f. was carried out against *Agrobacterium tumefacien* by Potato Crown Gall test or Potato Disc Assay method at the Pharmaceutical Research Department, Ministry of Industry, Yangon, Myanmar.

Fresh, disease free potato tubers were obtained from local market and used within 48 hours of transfer to the laboratory. Tubers of moderate sizes were surface-sterilized by immersion in 50 % sodium hypochlorite (Clorox) for 20 min. The ends were removed and soaked for 10 min more in Clorox. A core of the tissue was extracted from each tuber by using surface-sterilized (ethanol and flame) 105 cm wide cork borer. And, 2 cm pieces were removed from each end and discarded and the remainder of the cylinder is cut into 0.5 cm thick discs with a surface-sterilized cutter. The discs were then transferred to 1.5 % agar plates (1.5 g of Difco agar was dissolved in 100 mL of distilled water, autoclaved and 20 mL poured into each petri dish). Each plate contained three discs. The procedure was done in the clean bench in the sterile room.

100 mg, 200 mg and 300 mg of each extract was separately dissolved in 1 mL of dimethyl sulphoxide (DMSO); this solution was filtered through millipore filters (0.22 μm) into a sterile tube. 0.5 mL of this solution was added to 1.5 mL of sterile distilled water and 2 mL of broth culture of *A. tumefaciens* strain (48 h culture containing $3\text{-}5\times10^{9}$cells/mL) were added aseptically. Controls were made in this way; 0.5 mL of DMSO and 1.5 mL of sterile distilled water were added to the tube containing 2 mL of broth culture of *A. tumefaciens* (from the same 48 h culture).

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. The process of cutting the potatoes and incubation must be conducted within 30 min. The plates were sealed with tape to minimize moisture loss and incubated at room temperature for 12 days. After incubation, Lugol's solution (I_2-KI) was added and the tumors were counted with a microscope and

compared with control. The antitumor activity was examined by observation of tumor produced or not.

Results and Discussion

Results of preliminary photochemical analysis of the root extract of *S. curtisii* showed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds,reducing sugar, saponins, starchs, steroids, tannins, and terpenoids as shown in Table 1.

Table 1. Phytochemicals in Root Extract of *S. curtisii*

Phytochemical Components	**Root extracts of *Stemona curtisii***
Alkaloids	+
α-amino acid	+
Carbohydrates	+
Flavonoids	+
Glycosides	+
Phenolic Compounds	+
Reducing sugar	+
Saponins	+
Starch	+
Steroids	+
Tannins	+
Terpenoids	+
Cyanogenic glycosides	-

+ = Present; – = Absent

Acute Toxicity Study

Acute toxicity screening of EtOH extracts of *Stemona* root was done with the dosage of 2000 mg/kg, 300 mg/kg and 50 mg/kg body weight in albino mice. The condition of mice was recorded after administration in fourteen days. 2000 mg/kg group was discovered lethality within 60 min with symptoms of toxicity like restlessness, convulsion, coma and death. The results of other groups show no lethality of the mice up to fourteen days administration. Other groups of animals were also observed still alive and did not show any visible symptoms of toxicity like restlessness, respiratory disorders, convulsion, aggressive activities, coma and death (Table 2 and Figure 3).

Table 2. Acute Toxicity Effect of Ethanolic Extract of *Stemona* Root on Albino Mice Model after Two Weeks Administration

No	Groups	Dosage of EtOH extract (mg/Kg)	No. of death at day fourteen	% of death at day fourteen
1	Group 1	2000	5/6 (Dead= 5, Alive = 1)	83.33 %
2	Group 2	300	Nil	0 %
3	Group 3	50	Nil	0 %
4	Group 4	Nil	Nil	0 %

Figure 3. Acute toxicity study of ethanolic extract of *Stemona* root on albino mice model

In Vitro Antimicrobial Activity of some Crude Extracts of Root of *S.curtisii* by Agar Well Diffusion Method

In vitro antimicrobial activity of various crude extracts such as PE, MeOH, EtOAc, 95% EtOH and H_2O extracts was investigated by employing agar well diffusion method against six species of microorganisms. The inhibition zone diameter (ID) showed the degree of the antimicrobial activity. The larger the inhibition zone diameters, the higher the antimicrobial activity. The photographs illustrating the inhibition zones provided by crude extracts against six species of microorganisms are presented in Figure 4 and the observed data are summarized in Table 3. Among the tested crude extracts of *S.curtisii*, MeOH and EtOAc extracts showed highest antimicrobial activity against all tested microorganisms. EtOH extract has the antimicrobial activity against only one species of microorganisms, *Pseudomonas aureginosa* (ID: 11mm). However, H_2O extract of *S.curtisii* did not show activity against one species of microorganisms, *Bacillus subtilis*. From this observation, MeOH extract has the most potent antimicrobial activity.

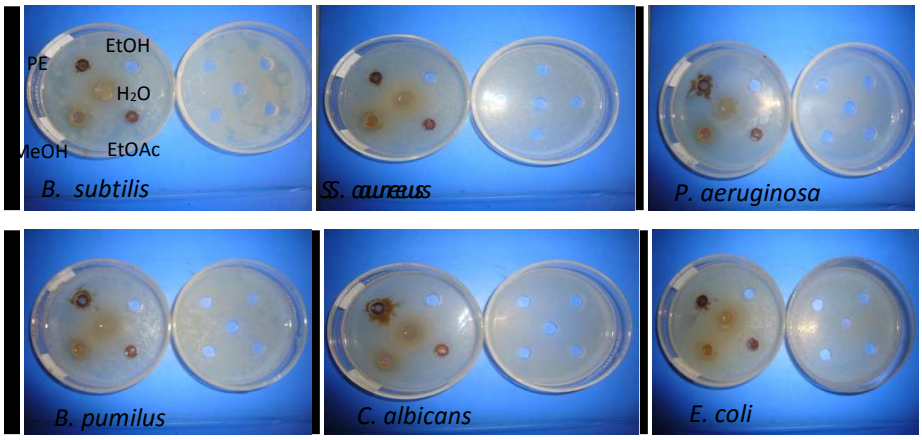

Figure 4. Inhibition well diameter of root extracts of *S.curtisii*

Table 3. *In vitro* Antimicrobial Activity of *S.curtisii* Root by Agar Well Diffusion Method

Table 3. *In vitro* Antimicrobial Activity of *S.curtisii* Root by Agar Well Diffusion Method

Microorganism	**Inhibition Well Diameter(mm)**					
	Crude Extracts of S.curtisii Root					
	I	**II**	**III**	**IV**	**V**	**Control**
Bacillus subtilis	–	11	13	_	_	_
Staphylococcus aureus	11	15	12	–	11	_
Pseudomonas aeruginosa	11	20	15	11	20	_
Bacillus pumilus	13	18	13	_	11	_
Candida albicans	14	16	13	_	11	_
Escherichia coli	12	15	14	_	12	_

I = PE extract
II = MeOH extract
III = EtOAc extract
IV = EtOH extract
V = H_2O extract
VI = Control

Agar Well Diameter-10mm
Inhibition Diameter -10~14mm (+)
Inhibition Diameter -15~19mm (++)
Inhibition Diameter -20 mm above (+++)

Screening of Antitumor Activity of some Crude Extracts from the Roots of S. *curtisii*

The antitumor activity screening of different crude extracts such as methanol, ethyl acetate, ethanol and watery extracts of root sample was carried out against *Agrobacterium tumefacien* by Potato Crown Gall test or Potato Disc Assay method. The photographs illustrating the different concentrations of plant crude extracts against *Agrobacterium tumefacien* are presented in Figures 5 and 6 and the observed antitumor activity of different crude extracts of root sample are summarized in Table 4. Among the tested crude extracts, all extracts of root exhibited antitumor activity against *Agrobacterium tumefacien.*

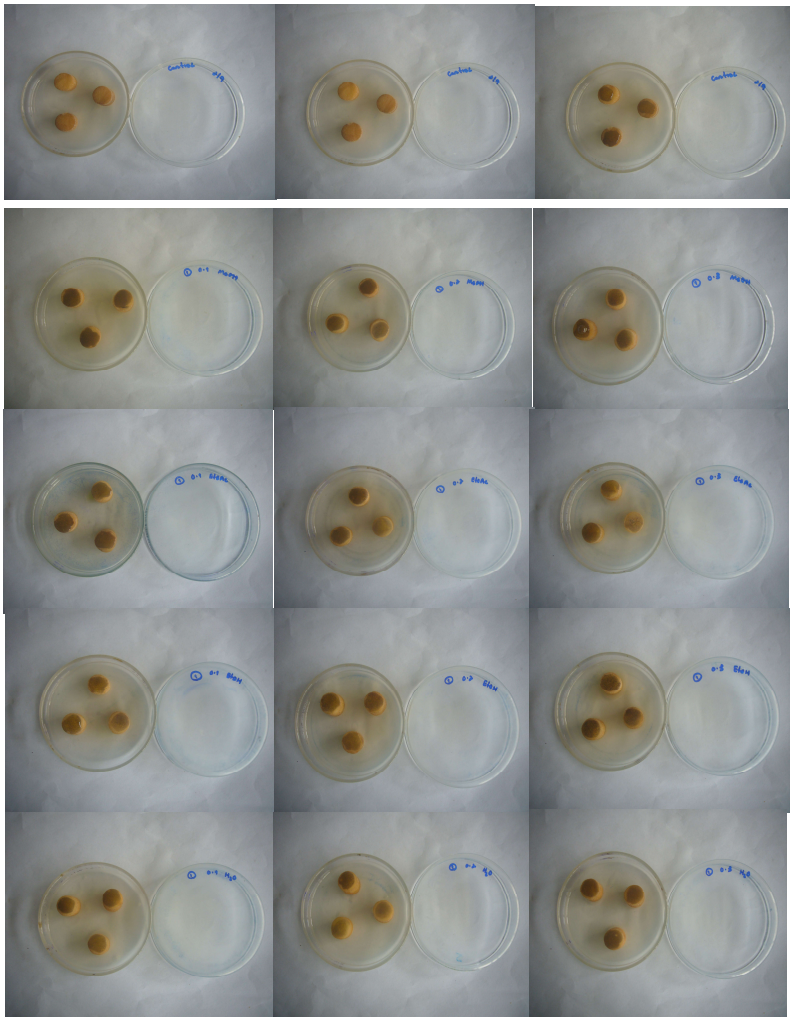

Figure 5. Photographs of observation on antitumor activity of different concentrations of MeOH, EtOAc, EtOH and H_2O extracts of *S. curtisii* on day 5

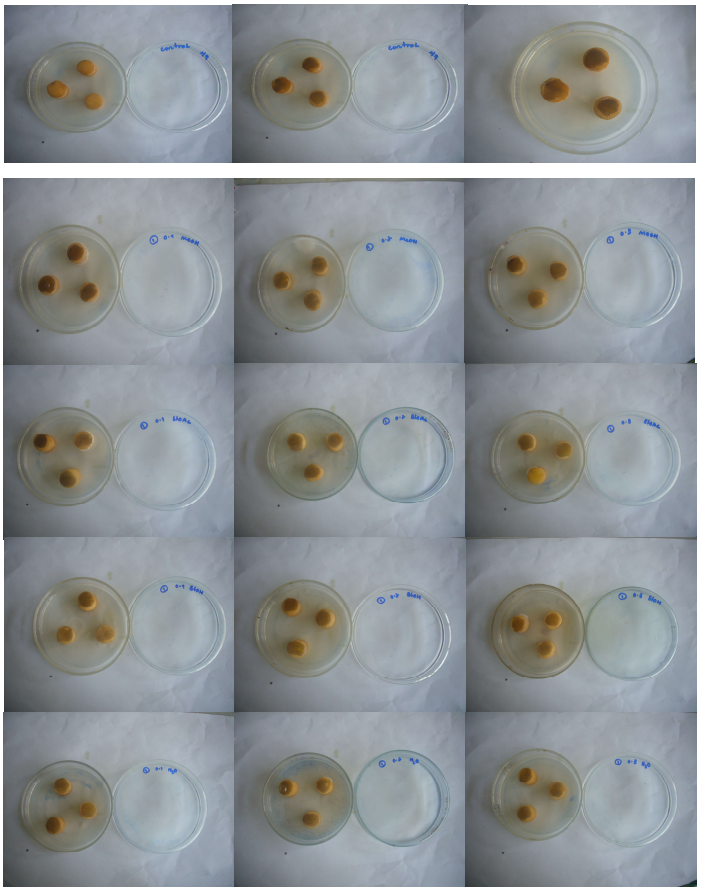

Figure 6. Photographs of observation on antitumor activity of different concentrations of MeOH, EtOAc, EtOH and H_2O extracts of *S. curtisii* on day 7

Table 4. Antitumor Activity of Different Crude Extracts from the Roots of *S. curtisii*

Samples	Concentration of extracts (mg /mL) DMSO	Antitumor Activity	
		Day-5	Day-7
MeOH extract	100	+	+
	200	+	+
	300	+	+
EtOAc extract	100	+	+
	200	+	+
	300	+	+
EtOH extract	100	+	+
	200	+	+
	300	+	+
H_2O extract	100	+	+
	200	+	+
	300	+	+
Control	100,200,300	–	–

(+) = exhibit antitumor activity

(–) = no antitumor activity

Conclusion

From the overall assessments of the present work, the following inferences could be deduced. According to acute toxicity test, 2000 mg/kg group was discovered lethality within 60 minutes with symptoms of toxicity like restlessness, convulsion, coma and death. The results of other groups showed no lethality of the mice up to fourteen days administration. In a study on antimicrobial activity, among the five tested crude extracts of *S.curtisii* Hook. F., MeOH extract exhibited the highest antimicrobial activity against all tested microorganisms. The antitumor activity of MeOH, EtOAc, EtOH and H_2O extracts of *S. curtisii* was screened on *Agrobacterium tumefacien* by Potato Disc Assay method. In *S.curtisii,* all extracts of root exhibited antitumor activity against *Agrobacterium tumefacien* after 5 days and 7 days periods of observation. Therefore, bioactivity of *S. curtisii* Hook. F. is probably due to presence of phytochemical constituents such as terpenoids, saponins, alkaloids and flavonoids. The result obtained from this study strongly indicated that tested crude extract of *S. curtisii* may play an important role in medicinal properties used *in vitro* and may be effective.

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