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Investigation of phytoconstituensts and screening of some bioactivities of *Stemona Curtisii* Hook. F.

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ABSTRACT: Medicinal plants are abundant in Myanmar. In the present research, the root of *Stemona curtisii* Hook. F. was chosen for investigation of some phytochemical constituents and bioactivity studies. Acute toxicity of 95 % ethanol extract from roots sample 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. 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* species by agar well diffusion method. Among them, 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. According to the results, it can be inferred that root of *S. cutrisii* may be used as remedy for the treatment of diseases related to the microorganisms tested.

KEYWORDS: *Stemona curtisii* Hook. F., phytochemical constituents, acute toxicity, antimicrobial activity, microorganisms.

1 INTRODUCTION

Stemona curtisii Hook.F. is one of the important monocotyledon plants belonging to the family Stemonaceae and widely distributed in the northen 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 considerable interest because it is the only source of the unique alkaloids known as stemona alkaloids including stemocurtisine, stemocurtisinol and oxyprotostemonine have been isolated from a root extract of *Stemona curtisii.* [1] Mungkornasawakul *et al*. repoted that the root extracts and the pure alkaloid of *S. curtisii* especially oxyprotostemonine were shown larvicidal activity against *Anopheles minimus.* [2, 3] While, Kaltenegger *etal*. reported that the crude extract of *S. curtisii* had insecticidal activities against *Spodoptera littoralis.*[4]The main chemical constituent of the *S. curtisii* is a specific group of Stemona alkaloids, including stemofoline, 2'-hydroxy stemofoline, oxyprotostemonine, dehydroprotostemonine, protostemonine, stemocochinine, stemocurtisine(pyridostemin), stemocurtisinol and oxystemokerrine.

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

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Fig. 1. Stemona curtisii roots and leaves

USES OF S. CURTISII

S. curtisii, 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 screen acute toxicity, and antimicrobial activities of root of *S. curtisii*. 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, and antimicrobial activities of root sample

2 MATERIAL 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 *Stemona curtisii* Hook. F. (Thar-myaa-oo) by authorized Botanist from Department of Botany, Pathein University. A total of 5 Kilo grams 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 sample material dried, it was ground into powder using grinding machine. The powdered material obtained was stored in clean air tight container.

PREPARATION OF CRUDE EXTRACTS BY DIRECT EXTRACTION METHOD FOR SCREENING OF SOME BIOLOGICAL ACTIVITIES

Each dried powdered sample (50 g) was extracted with 150 mL of PE (60-80 °C) for 6 hours 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, 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 sample, preliminary phytochemical tests on roots of *S. curtisii* were carried out according to the test tube tests. [5]

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

(i) Theory

To determine the symptomatology consequent to injection of the sample 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 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.

(j) Procedure

Acute toxicity of different doses of EtOH extracts 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, weighting (25-30g) 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 hrs continuously for mortality and behavior changes. Then check the animals each 24 hrs for fourteen days. The mortality during this period was noted (Nil or percent death). [6, 7] The results obtained from acute toxicity are described in Table 1.

(b) Screening of antimicrobial activity of different crude extracts of *S. curtisii*

The antimicrobial activities of different crude extracts such as PE, EtOAc, 95% EtOH, MeOH and H_2O extracts from roots of *S. curtisii* 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 method [8, 9] at Fermentation Department, Central Research and Development Centre, Ministry of Industry, Yangon, Myanmar.

Procedure

The antimicrobial activity of the crude extracts was performed by the agar well diffusion assay. The pathogenic test organisms were incubated in trypticase soy broth at appropriate temperature for 24 h. Nutrient agar medium containing meat extract (0.5 g), peptone (0.5 g), sodium chloride (0.25 g), agar (1.5 g) and 100 mL of distilled water were placed in a beaker and the contents were heated for 30 minutes. The nutrient agar medium was put into sterilized conical flask and plugged with cotton wool and then autoclave at 121 °C for 15 minutes. After cooled down to 40 °C, one drop of suspended strain was inoculated to the nutrient agar with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri-dishes and allowed to set the medium. Once solidified the dishes were stored for 2 h in a refrigerator. Five wells of 10 mm diameter each were cut out in the inoculated agar to place extract samples to be tested. The volume of each extract sampled placed in each well was 0.2 mL. Five samples, namely, PE, EtOAc, 95 % EtOH, MeOH and H_2O extracts were tested. The petri-dishes were then incubated at 37 °C for 24 h, and the diameters of clear inhibition zone around the well, if appeared were measured with calipers in millimeter. The antimicrobial activity was determined by measuring the clear zones around the wells.

3 RESULTS

Results of preliminary photochemical analysis of the root extract of *S. curtisii* showed the presence of alkaloids, flavonoids, terpenoids, steroids, starch, saponins, reducing sugars, phenolic compounds, glycosides, tannins, carbohydrates and α-amino acids. But cyanogenic glycosides were found to be absent in root of *S. curtisii*. The constituents such as phenolic compounds, terpenoids, flavonoids and steroids present in the sample may contribute to possess bioactivities such as antimicrobial, antioxidant, anticancer, antitumor, antipyretic, antiulcer and diuretic properties in root of *S. curtisii*.

(a) Acute Toxicity Test

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 minutes with symptoms of toxicity like restlessness, convulsion, coma and death. The results of other groups show no lethality of the mice was observed 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 1. Acute Toxicity Effect of Ethanolic Extract of S. curtisii Root on Albino Mice Model after two Weeks Administration

No	Groups	Extract Administration	Dosage	No. of death at day fourteen	% of death at day fourteen
1	Group 1	Ethanolic extract of *Stemona* root		5/6 (Dead= 5, Alive = 1)	83.33 %
2	Group 2	Ethanolic extract of *Stemona* root	300 mg/kg	Nil	0 %
3	Group 3	Ethanolic extract of *Stemona* root	50 mg/kg	Nil	0 %
4	Group 4	No administration	Nil	Nil	0 %

Fig. 2. Acute toxicity effect of ethanolic extract of Stemona root on albino mice model

(b) *In virto* Antimicrobial Activity of some Crude Extracts of Root of *S. curtisii* by Agar Well Diffusion Method

In virto 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 3 and the observed data are summarized in Table 2. Among the tested crude extracts of *S.curtisii* H, 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.

Table 2. In vitro Atimicrobial Activity of S.curtisii root by Agar Well Diffusion Method

Microorganism	Inhibition Well Diameter(mm)					
	S.curtisii					
	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

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

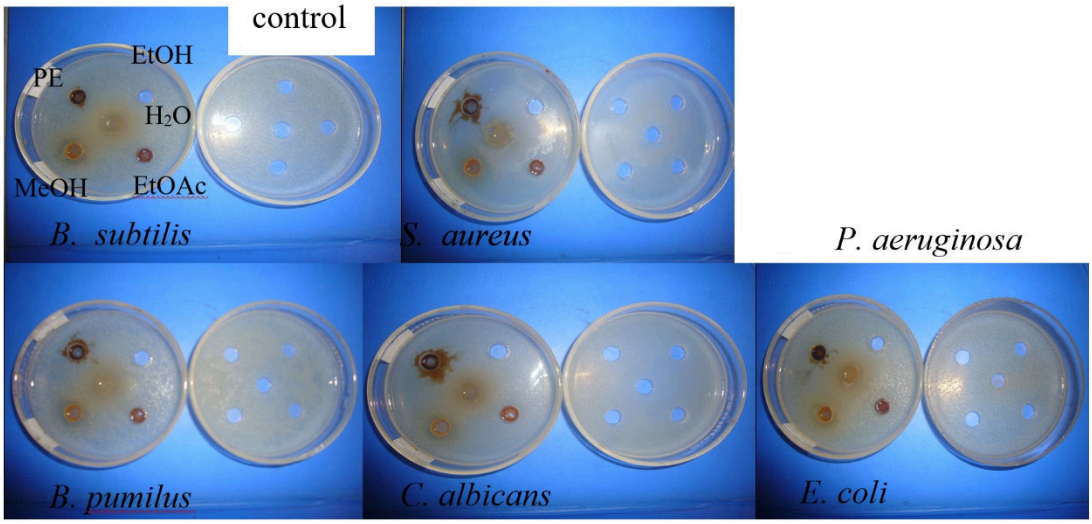

Fig. 3. Inhibition well diameter of root extracts of S.curtisii

4 CONCLUSION

From the overall assessments of the present work, the following inferences could be deduced. The roots of *S. curtisii* possess various chemical components such as alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugars, saponins, steroids, tannins and terpenoids.

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 show no lethality of the mice was observed up to fourteen days administration. From the acute toxicity test on roots of *S.curtisii*, extracts were found to be free from acute toxic under the dosage of 2000 mg / kg.

In a study on antimicrobial activity, among the five tested crude extracts of *S.curtisii*, MeOH extract exhibited the highest antimicrobial activity against all tested microorganisms. Therefore, bioactivity of *S. curtisii* is probably due to presence of phytochemical constituents such as terpenoids, saponins, alkaloids and flavonoids. The result obtained from this study strongly indicated that *S. curtisii* extract may play an important role in medicinal properties used *in vitro* and may be effective.

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