Phytochemical Analysis, Antioxidant Potentialand Antimicrobial Activities of *Stemonagriffithiana*Kurz.

KhinSoe Aye[1], KhinMyo Aye[2],KhaingKhaing[3]

Abstract

The present study was carried out to evaluate the phytochemical, antioxidant and antimicrobial activities of *Stemona griffithiana* Kurz. belonging to the family Stemonaceae. The phytochemical analysis were also carried out to identify the presence of different phytochemicals in the extracts of leaves and roots of *Stemona griffithiana* Kurz..And then the extracts were evaluated for the antioxidant activities by DPPH assay.The leaves and roots were also analyzed to check for their antimicrobial activity by agar well diffusion method and was assessed on tested organisms; *Enterococcus faecalis, Echerichia coli, Staphylococcus aureus, Bacillus cereus* and *Candida albicans*. The results showed the presence of alkaloids, glycosides, phenolics, and tannins. The DPPPH assay was also conducted to exhibit highest antioxidany activity which was compared to ascorbic acid,standard drug. IC_{50} values for ascorbic acid and extracts of leaves and roots were found to be 84.78 ± 0.39,17.95 ± 1.64 and 64.13 ± 7.10 µg/ml respectively in DPPH method. As the results of antimicrobial analysis, the leaves extracts showed against on Gram positive bacteria and the roots extracts had no zone of inhibition on tested organisms.So, the present study indicated that the plants are of the therapeutic potential due to the presence of variousphytochemicals.

Keywords: :*Stemona griffithiana*,phytochemicals,antioxidant, DPPH assay, antimicrobial activity

Introduction

The Stemonaceae is a family of monocotyledonous perennial herbs. *Stemona griffithiana* Kurz. in the family Stemonaceae is a wild plant and commonly known as Thamya-ni.

Myanmar is rich in varieties of medicinal plants due to the presence of different climate zones. Medicinal plants have been used for the treatment of bacterial infections. (Kress *et.al* ,2003).

The family Stemonaceae is the only source of *Stemona* alkaloids. Several workers have reported various chemical constituents of *Stemona* species from the other parts of the world. *Stemona tuberosa* ,*S.japonica*, and *S.sessilifolia* have been used in China and Japan for various medicinal and biological properties.

However there was scanty literature on chemical constituents of the species *Stemona griffithiana* Kurz.in Myanmar yet . Therefore, present study has been undertaken. In this study,*Stemona griffithiana* Kurz. was selected to test the phytochemical, antioxidant and antimicrobial activities of the plant.

The main aims of this research was to provide the taxonomic support for this species being classified under the Stemonaceae family, to bridge the information on preliminary pharmacognostic investigation of the studied species, to fullfill the need of incomplete information of the family Stemonaceae naturally grown in Myanmar and to explore the potent and qualitative medicineto promote the health of Myanmar people.

Materials and Methods

Plant materials

[1] Lecturer, Dr.Department of Botany,Yadanabon University
[2] Associate Professor, Dr. Department of Botany,Shwebo University
[3] Lecturer, Dr.Department of Botany,Yadanabon University

The fresh plant was collected from Lat-Moung-Kway Hill, Patheingyi Twonship, Mandalay Region during the months of Jaunary to February and identified inthe Department of Botany, Yadanabon University. The leaves and roots were washed thoroughly and dried at room temperature about one month.The dried materials were grounded well into fine powder in a mixer grinder and sieved. The powders were stored in air sealed container at room temperature until further use.

Preparation of extracts

The air-dried leaves and roots powder (100g) were percolated with 500 ml of ethanol for one week and filtered with filter paper for three times respectively. The filtrates were evaporated by using rotary evaporators at 50 °C. Then the filtrates were dried in a beaker placed on a water bath at 60°C.The obtained extracts were subjected to phytochemical investigation and pharmacological investigation.

Preliminary Phytochemical Analysis

Phytochemical screening to detect the presence of bioactive compounds was performed by using analytical grade solvents and reagents. The respective yields and preliminary phytochemical investigation results were given in the Table 1.

Antioxidant activity

The antioxidant activity of plant extracts were determined by the1,1-diphenyl-2-picrylhydrazyl(DPPH) free radical scavenging assay.The samples were dissolved in DMSO (10mg/ml) and diluted with 50% EtOH for various concentrations.Briefly , the reaction mixture containing 50µl of diluted test sample of various concentrations and 50µl of DPPH (300 µmol) dissolved in ethanol , was taken in 96-well micro-titer plate and kept standing at 37°C for 30 min.The absorbance was measured at 517 nm by using 96 well microplate reader.Ascorbic acid was used as a standard. 50% EtOH was used as the control and added to the 96-well plate instead of the sample. Percentage inhibition activity was calculated from $[(A_0-A_1)/A_0]^{\times}100$ where A_0 is the absorbance of control, and A_1 is the absorbance of the extract or standard.The antioxidant activity of the extract was expressed as IC_{50}.The experimental work was performed triplicates and the graph was plotted with the average of three observations.

The Anti-microbial Activity

Tested microorganisms: One Gram-negative bacterium (*Escherichia coli*), three Gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*, *Bacillus cereus*) and one fungal strain (*Candida albicans*) were used as a tested microorganisms for this experiment.Some bacterial strains were kindly supported byPublic Health Laboratory (PHL) Mandalay.

Agar well diffusion method

The agar well diffusion method was used for antimicrobial activity evaluation. Tested microorganisms were inoculated in Muller Hinton Broth at 37°C for overnight. The next day, the overnight broth culture was diluted with Normal saline to obtain the OD_{600} at 0.08 to 0.1 with the approximate cell density of 1.5×10^8CFU/ml. Muller Hinton Agar plates were prepared and sterilized by autoclaving at 121°C for 15 min. The broth inoculums were prepared and sterilized by autoclaving at Muller Hinton Agar plates to obtain the uniform inoculums. After the plate was inoculated, 8 – mm diameter wells were made on the agar medium by using a sterile cork borer. Each 50 µl of plant extract (500 µg/ 50 µl) was introduced into each well labelled. Chloremphenicol 30 µg/ well was used as the positive control. Then, the plates were placed in an incubator at 37°C for 16 to 18 hours. After incubation, the plates were examined and zone diameters of complete inhibition were measured and recorded to the closest millimeter.

Results

Morphological Study

Stemona griffithiana Kurz. In Journ As. Soc. Beng. 1873.

Local Name: :Thamya-ni
Family: :Stemonaceae
Flowering Period : June to August

Perennial erect herbs, 1-1.5feet high. Stem and branches terete or slightly compressed, solid, green, glabrous or slightly pubescent.Leave simple alternate or radical above, petiolate, exstipulate,pale green, glabrous, slightly shealthing, pulvinous; blades broadly ovate, cordate at the base, entire along the margin, caudately accuminate at the apex. Inflorescence terminal or axillary raceme. Flowers bisexual, actinomorphic,bracteate, tetramerous, green or dull purple,; bracts ovate-lanceolate, green, glabrous. Perianth segements 4, biseriate, unequal, lanceolate, acute at the apex, free, green with purple nerves, glabrous. Stamen 4, free, exserted; filament short,purple; anthers dithecous, linear-lanceolate,dorsifixed, introse, yellow, dehiscing by longitudinal slit. Ovary superior, glabrous, monocarpellary with six ovules on the basal placenta; style absent.Fruits capsule with valved. Seeds broadly ellipsoid, 1-2 seeds with airl. white.

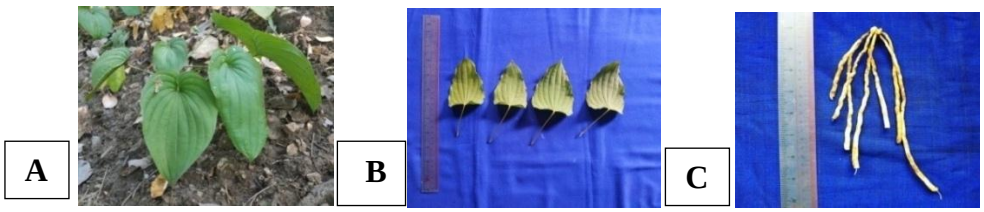


Figure 2.Morphological characters of *Stemonagriffithiana*Kurz.
A. Natural growing plant B. Leaves C. Tuberous root

Phytochemical Analysis

The results of phytochemical analysis of ethanol extracts of leaves and roots of *Stemona griffithiana* Kurz were shown in Table 1.As the results of phytochemical testalkaloids, gylcosides, polyphenols, aminoacids and tannins were present and flavonoids, phytosterols, saponins, , carbohydrates and cyanogenic glycosides were absent.Phenolics are present in leaves and absent in roots.And reducing sugars are present in roots but absent in leaves.

Table1. Preliminary Phytochemical test of leaves and roots of*Stemonagriffithiana*Kurz.

No	Test	Test Reagent	Observation		Result (+/-)	
			Leaves	Roots	EL	ER
1.	Alkaloids	Wagner's	Reddish brown ppt	Reddish brown ppt	+	+
2.	Flavonoids	Mg + HCL	No Red	No Pink	-	-
3.	Glycosides	10% Lead	White ppt	White ptt	+	+
4.	Phenolic	10 % $FeCL_3$	Dark green	Dark green	+	_
5.	Polyphenols	10% $FeCL_3$+ 1%$K_3Fe(CN)_6$	Dark green blue	Dark green blue	+	+
6.	Phytosterols	Acetic Anhydride + H_2SO_4 (conc:)	No Pink	No Pink	–	–

7	Saponins	Water (H_2O)		Foam	-	-
8.	Reducing sugars	Fehlling A+ B	No Red ptt	Red ptt	_	+
9.	Aminoacids	Ninhydrin		Purple	+	+
10.	Carbohydrates	10 % Napthanol	No Red ring	No Red ring	_	_
11.	Tannins	10 % Lead acetate	White ptt	White ptt	+	+
12.	Cyanogenic glycosides	Picric Paper	No colour change	No colour change	–	–

- Absent, + Present

Antioxidant Activity of Ethanolic Extracts of Leaves and Roots of *Stemon griffithiana* Kurz.

The results illustrate a decrease in the concentration of free radicals due to the scavenging ability of extracts.Leaves and roots of *Stemona griffithiana* and Ascorbic acid were exhibited as 97.92±1.43% inhibition with 250 µg/ml in leaves and 88.00 ± 2.80% ,94.63 ± 0.34 % inhibition with 500µg/ml in roots and ascorbic acid respectively.The IC_{50} value of ethanol extracts of leaves and roots and ascorbic acid were found to be 17.95±1.64,64.13 ±7.10,84.78 ± 0.39 µg/ml respectively.The resultsof Antioxidant activity were shown in Table 2 and 3.

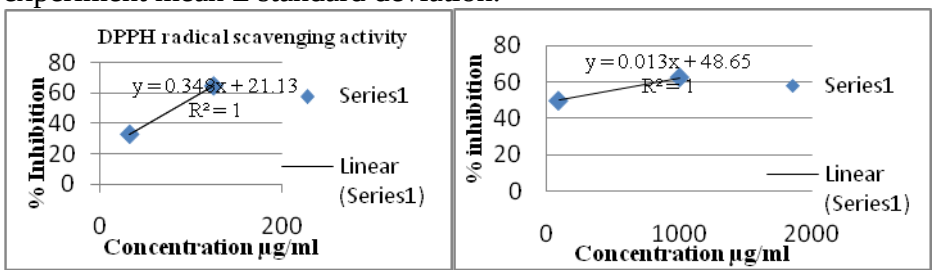
Table 2.DPPH scavenging activity of leaves of *Stemonagriffithiana*Kurz.

Sample(Concentration µg/ml)	125	62.5	31.25	15.63	7.81	3.91	IC_{50}(µg/ml) ±SD	Method
DPPH Scavenging (%) ± SD	97.92±1.43	89.04±0.65	71.28±1.43	46.15±3.00	25.55±1.43	16.48±4.02	17.95±1.64	DPPH Radical Scavenging Assay

Table 3.DPPH scavenging activity of roots of *Stemonagriffithiana*Kurz.

Sample (Concentration µg/ml)	1000	500	250	125	62.5	31.25	15.63	IC_{50}(µg/ml) ±SD	Method
DPPH Scavenging (%) ± SD	89.98 ± 2.13	88.00 ± 2.80	81.93 ± 1.80	68.17 ± 1.77	49.90 ± 3.12	36.15 ± 0.90	16.90 ± 1.18	64.13 ±7.10	DPPH Radical Scavenging Assay

Each value calculating in the table was obtained by calculating the average of 3 experiment mean ± standard deviation.



A.Test for leaves B.Test forroots

Figure 2.(A,B) DPPH scavenging activity of ethanol extract of leaves and roots of *Stemona griffithiana*Kurz.

Antimicrobial Activity

The results of antimicrobial activity of ethanolic extracts of leaves and tuberous roots of*Stemona griffithiana* Kurz were showed in table 4.The leaves extracts showed against 12 mm on *Bacillus cereus* and 16 mm *Enterococcus faecalis,*and in roots, no zone of inhibition on the testeddiscs and the solvent control disc. The zone of inhibition for chloramphenicol were 40 mm on*Echerichia coli* ,35 mm on *Enterococcus faecalis*,40mm on *Staphylococcus aureus*,32 mm on *Candida albicans* and 28mm on *Bacillus cereus* respectively

Table (4). Antimicrobial activity of ethanol extracts of leaves and roots of *Stemonagriffithiana*Kurz.

Sample	Inhibition Zone Diameter (mm)				
	Echerichia coli	*Enterococcus faecalis*	*Staphylococcus aureus*	*Bacillus cereus*	*Candida albicans*
Leaves	0	16	0	12	0
Roots	0	0	0	0	0
Chloramphenicol	40(++++)	35(++++)	40(++++)	28(++++)	32(++++)

Agar well - 8mm

slightly against activity, <12 (+) , moderately against activity, 13 - 15 (++)

more against activity,16- 20 (+++), most against activity 21 >24 (++++)

Echerichia coli *Enterococcus faecalis* *Staphylococcus aureus* *Bacillus cereus* *Candida albicans*

Figure 3.Antimicrobial activity of of *Stemonagriffithiana*Kurz.

Discussion

Medicinal plants have become very popular because they have very few side effects comparing to synthetic drugs. Phytochemical compounds were studied because they are highly abundant in nature and often used as parts of defence mechanisms in plants.Falvonoids and Phenolics are major classes of antioxidant compounds.The results of the present study showed that the extracts of leaves and roots have the phenolic compounds.These ethanolic extracts displayed free radical scavenging activity in the DPPH assay (IC_{50}17.95±1.64µg/ml and 64.13 ±7.10µg/ml) which is compared to that of ascorbic acid (IC_{50}84.78 ±0.39 µg/ml), a well-known standard antioxidant.

Several plant components like tannins are responsible for showing antioxidant property(Hasan.2009).As the results of phytochemical compounds,the ethanol extracts of leaves have slightly against antimicrobial activity but the roots were not against all test microorganisms.Therefore, this finding indicated that the ethanol extract of leaves of *Stemona griffithiana* Kurz. Was to be more antimicrobial activity than the roots extract.

Conclusion

The results demonstrated that theplant extracts have phenolics contents so it has a potent antioxidant activity as measured by DPPH assay. The leaves extracts demonstrated that significant antibiotic potential may be used for the treatment of several diseases caused by microorganism.Therefore, the ethanol extracts are good plant extracts for development of new drugs for preventing cell damage caused by free radical exposures and to cure various types of ailments, including microbial infections.

Acknowledgements

I am deeply grateful to acting Rector Dr Maung Maung Naing,Pro-rectors Dr Si Si Khin and Dr Tin Moe Thuzar, Yadanabon University for their permission to work on this research.I am greatly indebted to Dr Htar Lwin, Professor, Head of Department of Botany, Yadanabon University, for her advice in this work.

References

1. Harbone,JB.(1998).Phytochemical methods.3rd ed. Chapmen & Hall. London, UK.
2. Heywood, V.H. 1978.Flowering Plants of the World.Clarendon Press. Oxford.
3. Hooker, J.D. 1885. Floral of British India. Vol. VI. Oxford. Clarendon Press. 9.
4. Harvey, RA., Cornelissen, CN. And Fisher, BD. (2013).Vaccines and AntimicrobialAgents, The Microbial World Illustrated Reviews. Microbiology. 3rd ed p. 40-41
5. Hasan SM, Jamila M, Majumder MM, Akter R, Hossain MM, Mazumder ME,et al. Analgesic and antioxidant activity of the hydromethanolic extract of Mikania scandens (L.) Wild.Leaves.Am J Pharmacol Toxicol 4 (1):2009;1-7
6. Lee, S., Son, D., Ryu, J., Lee, Y.S., Jung. S.H., Lee. S.Y.,Shin, K.H. (2004). Antioxidant activities of Acanthopananax senticosus stems and their lignin components. Archives of Pharmacal Research, 27, 106-110