Extraction and Isolation of Organic Constituent and Evaluation of Antimicrobial Activity of *Urena lobata* Linn.

Khin Su Aye[1], Khin Mar Oo[2], Moh Moh Aye[3] and Mon Mon Thu[4]

Abstract

Urena lobata Linn., is an important plant belonging to the family Malvaceae and it is commonly known as aramina fibre, caesarweed or congo jute. It is locally known as "Kat-si-ne" in Myanmar. *U. lobata* is a sub-shrub, an annual in subtropic and perennial in the tropics. The present work is the investigation of some phytoconstituents from the root of *Urena lobata* Linn. (Kat-si-ne). According to the phytochemical investigation, Kat-si-ne root revealed the presence of alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, saponins, flavonoids, steroids, and starch were present. However, cyanogenic glycosides, tannins and reducing sugar were absent in the selected sample. Pure compound was isolated from Kat-si-ne root by column chromatographic separation. A steroidal glycoside pure compound was isolated (0.002 %, 0.00067 g) as brown powder. The antimicrobial activity of petroleum ether, ethyl acetate, ethanol, methanol and watery extracts was determined by agar well diffusion method against six species of microorganisms such as *Bacillus subtilis*, *Candida albicans, Escherichia coli, Malassezia furfur, Salmonella typhi* and *Staphylococcus aureus.* It was found that only ethanol extract showed antimicrobial activity.

Keywords: *Urena lobata,* phytochemical constituent, steroidal glycoside and antimicrobial activity

Introduction

Traditional medicine is the sum total of knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve, or treat physical and mental illnesses. Traditional medicine that has been adopted by other populations (outside its indigenous culture) is often termed complementary or alternative medicine (CAM) (Gurib, 2006). The World Health Organization (WHO) reported that 80 % of the emerging world's population relies on traditional medicine for therapy.

During the past decades, the developed world has also witnessed an ascending trend in the utilization of CAM, particularly herbal remedies (Chintamunnee & Mahomoodally, 2012). *U. lobata* may be one of the good sources of therapeutic phytochemicals. Medicinal plants and their derived products are one of the potential sources of modern medicines. The use of medicinal plants or their parts is long standing and increasing day by day (Islam *et. al*., 2016).

The aim of present research are collect the root of *Urena lobata* from Sittwe Township, to investigate the phytochemical constituent by standard method, Isolation of the organic compound and to evaluate antimicrobial activity from root of *Urena lobata.*

Materials and methods

Sampling of Root Materials

The plant of *Urena lobata* Linn. (Kat-si-ne) root was chosen to be studied and collected from Sittway University Campus. Sittway Township, Rakhine State, Myanmar. After collection, the scientific name of the plant was identified by authorized botanist at Botany Department, Sittway University.

[1] Demonstrator, Daw, Department of Chemistry, University of Mandalay
[2] Demonstrator, Daw, Department of Chemistry, University of Mandalay
[3] Associate Professor, Dr, Department of Chemistry, Sittwe University
[4] Professor, Dr, Department of Chemistry, University of Mandalay

The roots were washed with water and air dried at room temperature. The dried plants were cut into small pieces, and then they were grounded into powder in an electric motor grinder. The dried powdered samples were stored in the air-tight containers to prevent moisture changes and contamination (Trease and Evans, 1978; Robinson, 1983; Vogel, 1996).

Figure 1. Nature of *Urena lobata* (Kat-si-ne)

Preliminary Phytochemical Screening of the Root of Kat-si-ne

Test for alkaloids

The dried powdered sample (1.0 g) was boiled with 1 % HCl for about 10 min. It was allowed to cool and filtered. The filtrate was divided into four portions and tested with Mayer's reagent, Dragendorff's reagent, sodium picrate solution and Wagner's reagents, respectively. Observation was made to see if precipitate or turbidity occurred.

Test for α-amino acids

The dried powdered sample (1.0 g) was boiled with distilled water for about 10 min and then filtered. An aliquot portion of filtrate was transferred to a filter paper with the help of the micropipette and allowed to dry. Then this filter paper was sprayed with ninhydrin reagent and allowed to dry at 100 ℃ in an oven a few minutes. Observation was made to see if a violet coloured spot appeared on the filter paper.

Test for carbohydrates

The dried powdered sample (1.0 g) was boiled with 20 mL of distilled water for about 20 min and filtered. The filtrate was placed into a test tube and a few drops of 10 % α-naphthol was added and shaken. This test tube was kept inclined at an angle of 45 ℃ and about 1 mL of concentrated sulphuric acid was slowly introduced along the inner side of the test tube. Observation was made to see if a red ring formed between the two layers.

Test for cyanogenic glycosides

Dried powdered sample (3 g) was boiled with purified water (10 mL) for min. It was then filtered and 5 drops of concentrated sulphuric acid were added and sodium picrate paper was trapped in the neck of the test tube by means of a loosely closed cock. The resultant mixture was heated by using a spirit burner. Observation was made to see if the paper turned absent of brick-red.

Test for flavonoids

(1.0 g) of dried material was extracted with ethanol and evaporated to dryness and treated with 15 mL of petroleum-ether (60-80 ℃). The defatted residue was dissolved in methanol and mixed with 0.5 mL of concentrated hydrochloric acid in a

test tube, followed by a few pieces of magnesium turnings. Observation was made to see if pink colour within 3 min.

Test for glycosides

The dried powdered sample (1.0 g) was boiled with distilled water for about 10 minutes, and then allowed to cool and filtered. The filtrate was treated with 10 % lead acetate solution. Observation was made to see if precipitation took place on addition of the reagent.

Test for phenolic compounds

The dried powdered sample (1.0 g) was boiled with distilled water as usual and filtered. The filtrate was treated with ferric chloride and ferricyanide test solution. Observation was made to see if dark blue colour appeared.

Test for reducing sugar

About 3 g of dried powdered sample were boiled with 25 mL of dilute sulphuric acid for about 10 min and filtered. The resultant solution was added sodium hydroxide and boiled with Benedict's reagent for two minutes to see the formation, on cooling down the solution, indicating the presence of reducing sugars.

Test for saponins

The material of dried powdered samples was put into a test tube and some distilled water was added. Then the mixture was vigorously shaken for a few minutes. Observation was made to see if frothing took place.

Test for starch

Dried powdered sample (3 g) was boiled with purified water (10 mL) for 30 min. It was then filtered and two drops of iodine solution were added to the filtrate. Observation was made to see if dark blue precipitate was formed.

Test for steroids

3 g of dried powdered sample was refluxed with benzene and the solvent was removed by distillation under reduced pressure. 3 drops of acetic anhydride was added and the mixture was shaken. Then, a few drops of concentrated sulphuric acid were carefully added and shaken. Observation was made to see if the solution turned to green colour.

Test for tannins

3 of 4 drops of 2 % NaCl solution were added to the ethanolic extracted of plant material, followed by filtration. About 3 mL of the filtrate was transferred to test tube and added 2-3 drops of 1 % gelatin solution. Observation was made to see if precipitates were formed.

Test for terpenoids

Petroleum-ether extract from the flavonoid test tube above was dissolved in 15 mL of chloroform. The chloroform extract and 0.3 mL of acetic anhydride were mixed gently. The colour changes were noted after the addition of a drop of concentrated H_2SO_4 over a period of a few minutes. Observation was made to see pink colour.

Screening of Antimicrobial Activity of *U. lobata* Linn. (Kat-si-ne) Root

The plant of *Urena lobata* Linn. (Kat-si-ne) root was chosen to be studied and collected from Sittway University Campus. Sittway Township, Rakhine State, Myanmar. Antimicrobial activity of crude extracts from *U. lobata* Linn. (Kat-si-ne) root were screened by using agar well diffusion method, at Botany Department, Yangon University, Myanmar.

Meat extract (0.5 g), peptone (0.5 g) and sodium chloride (0.25 g) were mixed with distilled water and the solution was made up 100 mL with distilled water. The pH of this solution was adjusted at 7.2 with 0.1 M sodium hydroxide solution and 1.5 g of agar with cotton wool and then autoclaved at 121 ºC for 15 min. After cool

down to 40 ºC, one drop of suspended strain test organism was inoculated to the nutrient agar medium with the help of a sterilized disposable pipette near the burner. About 20 mL of medium was poured into the sterilized petri dish and left 10-50 min in order to set the agar. After that the agar wells were made with a 10 nm sterilized cork borer and the wells were filled with 0.1 mL of extract samples to be tested. And the plates were incubated at 37 ºC for 24 hrs. After incubation, the diameters of inhibition zones including 10 mm wells were measured.

Results and discussion

Phytochemical Investigation of Plant Samples by Standard Method

The investigation of the types of phytoconstituents present in root of Kat-si-ne was carried out according to procedure mentioned in Section. The results obtained are summarized in Table 1.

From this results, Kat-si-ne root showed the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, steroids, and terpenoids but cyanogenic glycosides, tannins and reducing sugars were absent. Figure 2 represented the photograph of phytochemical investigation of Kat-si-ne root by standard method.

Figure 2. Photograph of phytochemical investigation of Kat-si-ne root by standard method

Table 1. Results of Phytochemical Investigation of *U. lobata* L. (Kat-si-ne) root by Standard Method

No.	Test	Extract	Test reagent	Observation	Remark
1	Alkaloids	1%HCl	Mayer's reagent Wagner reagent Dragendorff's reagent Sodium picrate	White ppt Reddish brown ppt Brown ppt Yellow ppt	+ + + +
2	α-amino acids	H_2O	Ninhydrin reagent	Violet spot	+
3	Carbohydrates	H_2O	10 % α-napthol and Conc.H_2SO_4	Red ring	+
4	Cyanogenic glycosides	H_2O	Conc.H_2SO_4 and Sodium picrate	no colour change	-
5	Flavonoids	EtOH	Conc. HCl and Mg turning	Pink sol^n	+
6	Glycosides	H_2O	% lead acetate	White ppt	+
7	Phenolic compounds	H_2O	5 % $FeCl_3$ solution, $K_3Fe(CN)_6$	Deep blue colour	+
8	Reducing sugars	Dil H_2SO_4	NaOH and Benedict's solution	no colour change	-
9	Saponins	H_2O	Distilled Water	Frothing	+
10	Starch	H_2O	Iodine solution	Dark blue sol^n	+
11	Steroids	Pet-ether	Acetic anhydride & Conc.H_2SO_4	Green sol^n	+
12	Tannins	H_2O	2 % NaCl, gelatin	no colour change	-
13	Terpenoids	$CHCl_3$	Acetic anhydride & Conc.H_2SO_4	Pink sol^n	+

(+) = presence, (-) = absence

Separation and Isolation of Pure Organic Compound by Column Chromatographic Method

2.35 g of ethyl acetate crude extract was mixed with 1 g of silica gel. The mixture was allowed to evaporate with continuous agitation so that free flows dry silica gel of which the sample was uniformly adsorbed. The resulting powdered mixture was added to the column using a small long necked funnel. The top of the layer was wet with solvent that had previously been allowed to remain above the gel by opening the tap. Some adsorbed gel sticking on the inner wall was washed down with the solvent. A piece of cotton wool was placed between the solvent and the column gel. The tap was opened and the fractions were collected at the rate of one drop per four seconds. Gradient elution was performed successively with PE:EtOAc solvent systems in the ratios of 15:1, 7:1, 1:1 and 1:3 v/v. From this separation, a total

of 45 fractions were collected. Successive fractions obtained were combined on the basis of their behaviors on TLC. Finally, four main fractions F I to FIV were obtained. After removal of solvents, fractions F II (f_{16}-f_{24}) and F III (f_{28}-f_{35}) provided solid substances.

From fraction F III, 0.002 g (0.00067 %) of pure compound as a brown powder was provided, after purification by washing with PE:EtOAc (9:1 v/v) followed by crystallization from ethyl acetate.

Pure Compound

Figure 3. Appearance of isolated pure compound

Solvent System=PE:EtOAc (1:2v/v)

spraying agent = 5% H_2SO_4, Δ

R_f=0.45

Figure 4. Thin layer chromatogram of isolated pure compound

Separation and isolation of some organic compounds from ethylacetate crude extracts of Kat-si-ne root

Pure compound was obtained from ethylacetate crude extract of Kat-si-ne root as brown powder. Some physico-chemical properties were shown in Table 2. Pure compound was classified as a steroidal glycoside.

Table 2. Some Physico-chemical Properties of Isolated Pure Compound

Experiment	Compound	Remark
R_f	0.45	PE:EtOAc (1:1 v/v)
UV (254 and 365 nm)	Inactive	no conjugated double bond
1 % $FeCl_3$ sol^n test	no colour change	phenolic O-H absent
2, 4-DNP test	Yellow ppt.	C=O present
10 % $KMnO_4$	decolourized	C=C present
Liebermann Burchard test	blue	Steroid
5 % H_2SO_4, Δ	Purple on TLC	Steroid
10 % Lead acetate	White ppt.	Glycoside

Screening of Antimicrobial Activity of Different Crude Extracts by Agar Well Diffusion Method

Screening of antimicrobial activity of five extracts were tested on six strains of microorganisms such as *Bacillus subtilis, Staphylococcus aureus, Candida albican, Malassezia furfur, Escherichia coli* and *Salmonella typhi* by agar well diffusion method. This method is based on the zone diameter in millimeter (mm) of agar well. The large inhibitry zone including the well diameter shows the degree of antimicrobial activity.

The results of antimicrobial activity were shown in Table 3. According to these results, ethanol extract showed on Escherichia coli (14 mm), *Malassezia furfur* (16 mm) and other extract did not show antimicrobial activity on tested microorganisms. From these results ethanol extract of Kat-si-ne root may be used in the treatment of bacterial and fungal infections owing to its effective antibacterial and antifungal infection in fig.5.

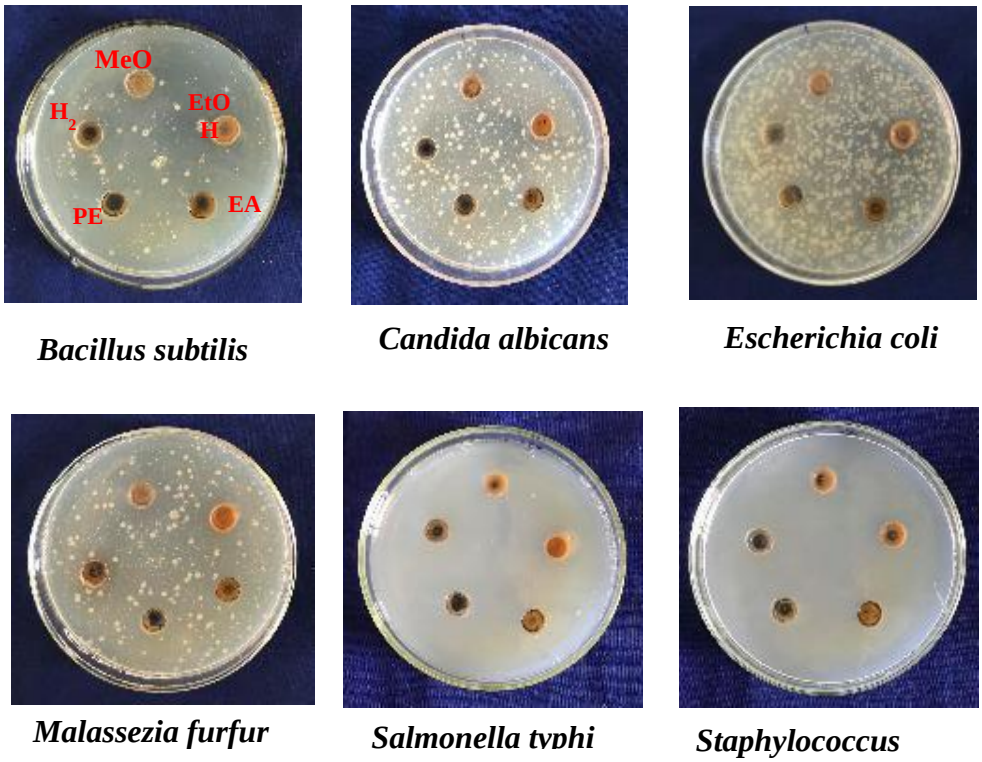

Figure 5. Inhibition zones of different extracts from Kat-si-ne root on six microorganisms by agar well diffusion method

MeOH = Methanol

EtOH = Ethanol

EtOAc = Ethyl acetate

PE = Petroleum ether

H_2O = Watery

Table 3. Inhibition Zone Diameters of Crude Extracts on Different Species of Organisms by Agar Well Diffusion Method

No.	Types of Organisms	Diameter of Inhibition Zone (mm)				
		MEOH	EtOH	EtOAc	PE	H_2O
1.	*Bacillus subtilis*	-	-	-	-	-
2.	*Candida albicans*	-	-	-	-	-
3.	*Escherichia coli*	-	14	-	-	-
4.	*Malassezia furfur*	-	16	-	-	-
5.	*Salmonella typhi*	-	-	-	-	-
6.	*Staphylococcus aureus*	-	-	-	-	-

10 -12 mm = weak activity, 13 - 18 mm = high activity,
>18 mm = very high activity (Well size = 10 mm)

Conclusion

U. lobata L. (Kat-si-ne) root is medicinal plant which is grown in Sittway University Campus. Preliminary phytochemical investigation tests on Kat-si-ne revealed that the presence of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, saponins, starch, steroids and terpenoids. But tannins, cyanogenic glycosides and reducing sugars were absent in the sample.

On silica gel column chromatographic separation, pure compound was isolated from Kat-si-ne root. A steroidal glycoside compound (0.002%, 0.00067 g) as brown powder was isolated.

The antibacterial activity of five crude extracts (methanol, ethanol, ethylacetate, petroleum ether and watery) of Kat-si-ne was screened by using agar well diffusion method. According to these results, only ethanol extract showed antimicrobial activity.

Acknowledgements

We are greately intebed to reactor and Pro-Reactors from Dagon University for their great kindness to do this conference. We also wish to mention our sincere thanks to Dr Tin Moe Aye (Professor and Head) and Dr Yee Yee Thu (Professor), Department of Botany, Dagon University, for their strong efforts to complete 4th Myanmar-Korea Conference on Plant Tissue Culture and Genetics hosted by Jeonbuk National University (Korea).

References

Adeloye, O. A., A. D. Akinpelu, O. A. Ogundainiand C. Obafemi. (2007). "Studies on Antimicrobial, Antioxidant and Phytochemical Analysis of *Urena lobata* Leaves Extract". *J. Phys. Nat. Sci.,* **1**(2),1-9

Chintamunnee, V.& M. F.Mahomoodally. (2012). "Herbal Medicine Commonly Used Against Infectious Diseases in the Tropical Island of Mauritius," *Journal of Herbal Medicine.*, **2**, 113–125

Gurib-Fakim, A.(2006). "Medicinal Plants: Traditions of Yesterday and Drugs of Tomorrow," *Molecular Aspects of Medicine.,* **27** (1), 1–93

Islam, H., K. M. Hasanur Rahman, S. Rahman and M.Rahmatullah.(2015). "Preliminary Antihyperglycemic, Antinociceptive Activity, Phytochemical Analysis and Toxicity Studies

on Leaves of *Urena lobata* L."*J Chem Pharm Res.*, **7**(4), 559-63
Mathappan, R., S. P. Umachigi,and V. V. Prasanth.(2013). "Wound Healing Activity of the Methanolic Extract of *U. lobata L.*" *Int J Pharm Chem Sci*., **2**(2), 793-800
Robinson, T. (1983). "*The Organic Constituents of Higher Plants".* North America: 5th Ed., Cordus Press, 285-286
Sajem, A. L. and K.Gosai. (2006). "Traditional Use of Medicinal Plants by the Jaintia Tribes in North Cachar Hills district of Assam". Northeast India, *J Ethnobiol Ethnomed*., **2,** 33
Trease, G. E. and W. C.Evans. (1978). *Phytochemistry*: "*Introduction and General Methods".* Pharmocognosy. London: Bailliere Tindall, 227-247
Vogel, A.I. (1996). "*A Test Book of Practical Organic Chemistry".* London: Longmans Green & Co. Ltd., 454