

An Investigation of Some Chemical constituents and Some Biological Activity of Leaves and Tuber of *Trichosanthes tricuspidata* Lour. (Kyi-ah)

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ABSTRACT

Recent time, the trend of medicinal system of world is shifting from synthetic to herbal medicine. In Myanmar, *Trichosanthes tricuspidata* Lour. known as Kyi-ah is also used as traditional medicine. The aim of the present research work is to investigate the some chemical constituents and some biological activities of Kyi-ah. The leaves and tuber samples were selected and collected from Sit-Kwin Village, Danuphyu Township area. Phytochemical screening of ethanolic aqueous extracts revealed the presence of alkaloids, α -amino acids, carbohydrates, glycosides, flavonoids, phenolic compounds, reducing sugars, saponins, steroid and tannins. Starch was absent in leaves sample but present in tuber sample. Nutritional values (moisture, protein, fiber, ash content, carbohydrates content and energy values) for leaves and tuber samples were determined by recommended methods. Some elements such as potassium, calcium and sulphur were present significant quantity for both samples. The antimicrobial activities of selected samples were determined by Agar Well Diffusion method. All extracts (except PE), EtOH, EtOAc and H₂O extracts of both samples showed activity for six microorganisms. Among them water extract was more potent than others. Antioxidant activity of selected samples was tested by DPPH dot-blot method. Both samples also showed antioxidant activities.

Keywords: *Trichosanthes tricuspidata* Lour., phytochemical screening test, nutritional values, antimicrobial activity,

Introduction

The demand of medicinal plants is increasing day by day in both developing as well as developed countries as a result of recognition of the non-narcotic nature, lack of side effects and easily availability of many herbal drugs. Most often the medicinal plants are collected from the wild. There are numerous data on the uses of medicinal plants. Today due to resistance of different allopathic medicines natural source is preferred mainly to block the development of cancer in human. The therapeutic potential of various herbal plants have to be explored for its medicinal use (Duvey, 2012). This research work concerns with the information available on the potency of *Trichosanthes tricuspidata* because of its immense medicinal potential as it is a very important medicinal plant.

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Materials and Methods

In all analytical procedure of the experiments, recommended standard methods and techniques were applied. The chemicals were used from internationally established companies such as BDH, Kento, Merck, Hopkin, Williams and also locally from the

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Sample collection and identification

The samples were collected from Sit-kwin village, Danuphyu Township and identified as *Trichosanthe stricuspidata* Lour. (Kyi-ah) according to the aurtherized botanist at Department of Botany, MaubinUniversity. The collected samples were cleaned by washing with water and then dried at room temperature. The dried samples were ground into purely fine powder by using an electric grinder and then stored in air tight container to prevent moisture changes and other contaminations.

Scientific classification

Myanmar name	- Kyi-ah
Botanical name	- <i>Trichosanthe stricuspidata</i> Lour.
Family name	- Cucurbitaceae
English name	- Red Apple Gourd
Part of uses	- Leaves and Tuber



Figure 1. *Trichosanthe stricuspidata* Lour. (Kyi-ah) Leaves and Tuber

Phytochemical Investigation

Phytochemical tests of collected sample were carried out according to the reported methods to investigate the presence or absence of phytoconstituents such as alkaloids, α -amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, reducing sugar, saponins, starch, steroids, and tannins in *Trichosanthe stricuspidata* Lour.

Physicochemical Properties

Some physico chemical properties such as moisture contents, nutritional values: fat, protein, carbohydrate fiber were determined. In this work, the elemental contents in the leaves and tuber were determined by EDXRF technique at the West Yangon University.

Antimicrobial activity test

Antimicrobial activity of various crude extracts of the selected sample was tested by Agar Well Diffusion Methods performed at the Pharmaceutical and Food Research Department, Ministry of Industry (1), Yangon.

Antioxidant Activity by Dot-blot and DPPH staining

The straining of the silica plate was based on the procedure of Huang, *et al.*, (2005). The MeOH extracts of leaves and tuber samples (each 0.2 mL) were loaded on a 2 cm x 12 cm TLC plate (silica gel GF₂₅₄, Merck) in order of decreasing amount (400 µg (conc: 2 mg / mL) up to 12.5 µg (conc: 0.062 mg / mL)) along the now.

The sheet bearing the dye spots was placed upside down for 10 s in a 0.4 mm DPPH solution. Then, the excess solution was dried with a hair-dryer blowing cold air. The stained silica layer revealed a purple black grown with pale yellow spots at the location of the drops, which showed radical scavenger capacity. The intensity of the pale yellow color depends upon the amount and nature of radical scavenger present in the samples (Changet.al, 2007).

Results and Discussion

Preliminary Phytochemical Investigation of of *Trichosanthes tricuspidata* Lour. Green vegetables occupy an important place among the food crops as they provide adequate amounts of phytochemicals. The presence of secondary metabolites such as alkaloids, flavonoids, saponins, glycosides in the vegetable may contribute to their medicinal value .

The phytochemical constituents of selected samples revealed that the alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compound, reducing sugars, saponins, tannins were present in both leaves and tuber. Starch is absent in leaves sample but present in tuber sample. The results are shown in Table 1.

Physicochemical Properties

Some physicochemical properties such as moisture, crude fiber, crude fat, protein, ash carbohydrate were determined and energy value was calculated. The results are shown in Table 2. As result, it was found that crude fat and fiber were present as major nutritional in samples. Fiber is the insoluble and combustible organic residue which prevent and treating constipation, helping to lower the risk of hemorrhoids, diverticular disease and assisting in reducing blood pressure.

Table 1 . Results of Phytochemical Investigation of Leaves and Tuber of Kyi-ah

No	compounds	Extract	Reagent used	Observation	Remark	
					Leaves	Tuber
			Mayer's reagent	White ppt	+	+
1	Alkaloids	1%HCl	Dragendroff's reagent	Orange ppt	+	+
			Sodium picrate reagent	Yellow ppt	+	+
2	α -amino acids	H ₂ O	Ninhydrin reagent	Violet spot	+	+
3	Carbohydrates	H ₂ O	10% α -naphthol, conc: H ₂ SO ₄	Red ring	+	+
4	Flavonoids	EtOH	Mg ribbon, conc: HCl	Pink colour	+	+
5	Glycosides	H ₂ O	10% lead acetate solution	White ppt	+	+
6	Phenolic compounds	H ₂ O	5% FeCl ₂ , sol: K ₂ Fe(CN) ₆ sol:	Deep blue Colouration	+	+
7	Reducing sugars	H ₂ O	Fehling solution A and B	Brick-red ppt	+	+
8	Saponins	H ₂ O	Distilled water	Frothing	+	+
9	Starch	H ₂ O	I ₂ solution	Blue colour	+	+
10	Tannins	H ₂ O	5% FeCl ₃ solution	White ppt	+	+
11	Steroid	PE	Acetic anhydrous, conc: H ₂ SO ₄	Green ppt	+	+
12	Terpenoids	EtOAc	Acetic anhydrous, conc: H ₂ SO ₄	Pale green	+	+

(+) = presence

(-) = absence

(ppt) = precipitate

Table 2. Physicochemical Properties of Kyi-ah Leaves and Tuber

No.	Principle contents	Observed Values	
		Leaves	Tuber
1	Moisture (%)	7.87	12.98
2	Crude Fiber (%)	14.68	23.62
3	Crude Fat (PE extract) (%)	10.00	10.00
4	Protein (%)	14.08	7.40
5	Ash (%)	16.50	19.10
6	Carbohydrate (%)	36.87	26.90
7	Energy value (kcal/100g)	293.81	227.23

Qualitative Elemental Analysis

In this work heavy toxic metals and macronutrient mineral elements present in dried powdered of *Trichosanthes tricuspidata* Lour. (Kyi-ah) leaves and tuber were determined by EDXRF technique. The results are reported in Table.3. It can be seen that potassium, calcium, sulphur, zinc, iron and silicon are present in selected sample and calcium and potassium are the most predominant mineral elements. These elements played an important role for various metabolism and activity of enzyme. Some heavy toxic metals such as As, Cd, and Pb were not detected by EDXRF technique.

Based on CH balanced results of both samples, it can be seen that Kyi-ah leaves and tuber may be present as effective organic compounds.

Antimicrobial activity

Antimicrobial activities of PE, EtOH, EtOAc and H₂O extracts of Kyi-ah leaves and tuber have been preliminary screening on six microorganisms such as *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*, *Candida albicans* and *Escherichia coli* by Agar Well Diffusion Method. Water extracts of both samples show the most pronounced antimicrobial activity within the inhibition zonediameters ranged between 12-14 mm in leaves and 11-14 mm in tuber. Ethanol extract and ethylacetate have the lower antimicrobial activity with inhibition zone diameter ranged between 12-14 mm in leaves and tuber but Pet-ether extract showed antimicrobial activity on *Candida albicans* and *Escherichia coli* for both samples. The results are shown in Table 4. Figure.2., Table 5, and Figure 3.

Table 3. Results of Elemental Contents and CH Balance of Kyi-ah

No	Contents	Observed values(%)	
		Leaves)	Tuber
1	Si	3.845	ND
2	Ca	2.387	2.739
3	K	1.257	2.297
4	S	0.504	0.263
5	Fe	0.010	0.067
6	Mn	0.009	0.005
7	Sr	0.003	0.007
8	Ti	0.002	0.005
9	Zn	0.002	0.006
10	Cu	0.001	0.001
11	Ag	0.001	ND (not detected)
12	CH balance	91.979	94.607

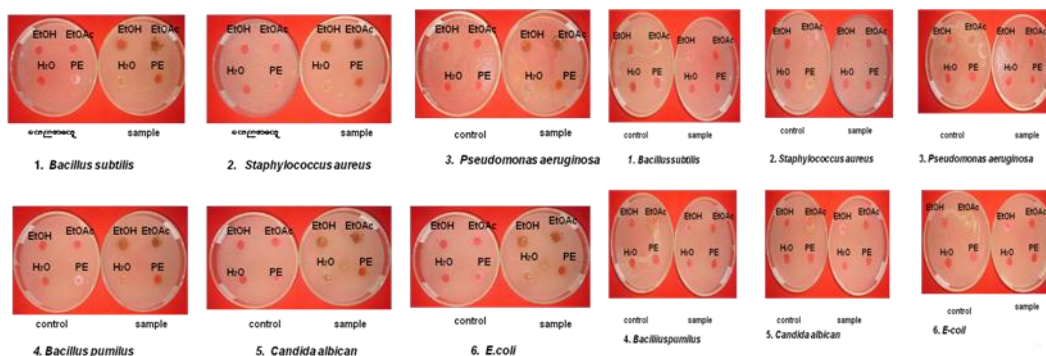


Figure 2. Antimicrobial screening of crude extracts of Kyi-ah Leaves

Table 4. Antimicrobial Activity of Kyi-ah Leaves by Agar Well Diffusion Method

No.	Extracts	Inhibition diameters					
		I	II	III	IV	V	VI
1	PE	-	-	-	-	11mm (+)	13 mm (+)
2	EtOH	13mm (+)	13 mm (+)	12 mm (+)	13 mm (+)	12 mm (+)	14 mm (+)
3	EtOAc	13 mm (+)	13 mm (+)	-	13 mm (+)	13 mm (+)	14 mm (+)
4	H ₂ O	13 mm (+)	13 mm (+)	12 mm (+)	13 mm (+)	12 mm (+)	14 mm (+)

Table 5. Antimicrobial Activity of Kyi-ah Tuber by Agar Well Diffusion Method

No.	Extracts	Inhibition diameters					
		I	II	III	IV	V	VI
1	PE	-	-	-	-	12 mm (+)	12 mm (+)
2	EtOH	13mm (+)	13 mm (+)	11 mm (+)	-	12 mm (+)	12 mm (+)
3	EtOAc	13 mm (+)	13 mm (+)	11 mm (+)	-	12 mm (+)	12 mm (+)
4	H ₂ O	14 mm (+)	13 mm (+)	11 mm (+)	13 mm (+)	13 mm (+)	13 mm (+)

Agar well-	Organisms
10 mm ~ 14 mm (+)	I - <i>Bacillus subtilis</i>
15 mm ~ 19 mm (++)	II - <i>Staphylococcus aureus</i>
20 mm above (+++)	III - <i>Pseudomonas aeruginosa</i>
	IV - <i>Bacillus pumilus</i>
	V - <i>Candida albican</i>
	VI - <i>E-coli</i>

Antioxidant activity

In the present work DPPH dot- blot method that measured the radical-scavenging activity of antioxidants against free radicals like the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenger capacity. The intensity of the pale yellow color depends upon the amount and nature of radical scavenger present in the samples. The conversion of yellow to white spots on the TLC plate depicts the antioxidant potential of different extracts of Kyi-ah Leaves and Tuber. The results are shown in Figure 4 and 5. According to these results Kyi-ah Leaves showed more antioxidant activity than Tuber.

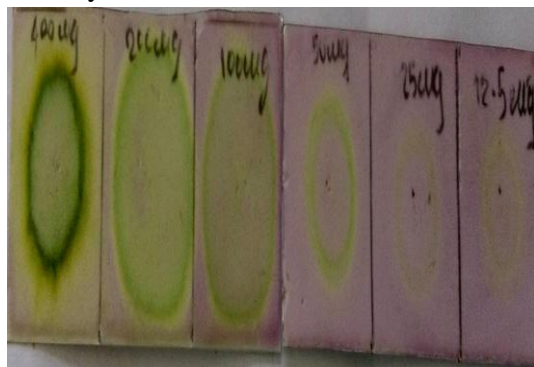


Figure 4. Screening of antioxidant activity of Kyi-ah Leaves

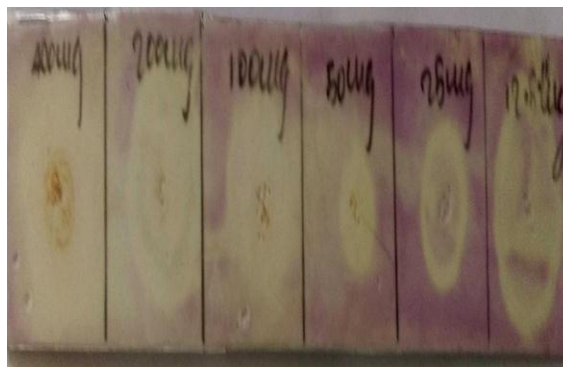


Figure 5. Screening of antioxidant activity of Kyi-ah Tuber

Conclusion

The aim of the present work is to investigate the physicochemical properties, some biological activities and antioxidant activity of *Trichosanthes cucurbitifolia* Lour. (Kyi-ah). From the overall assessment of present research work, the following inferences could be deduced. The preliminary phytochemical studies of selected samples by test tube method revealed generally the presence of flavonoids, alkaloids, α -amino acids, glycosides, carbohydrates, steroids, phenolic compounds, reducing sugars, saponins, tannins and terpenoids. Starch was absent in leaves but present in tuber.

Nutritional values of Kyi-ah samples were determined by AOAC method. The results for Kyi-ah leaves were found to be moisture (7.87 %), ash (16.50 %), protein (14.08 %), fiber (14.68 %), crude fat (10.00 %), carbohydrate (36.87 %). The energy value was 293.81 kcal/100g in leaves sample. For tuber sample, moisture (12.98 %), ash (19.10 %), protein (7.40 %), fiber (23.62 %), crude fat (10.00 %), carbohydrate (26.90 %). The energy value was 227.23 kcal/100g. Semi-quantitative elemental analysis by EDXRF also described that silicon, potassium, calcium, phosphorus, sulphur, zinc and iron were present and potassium was the highest content.

In vitro screening of antimicrobial activity was also detected by agar well diffusion method. All extracts (EtOAc, EtOH and H₂O extract) of Kyi-ah leaves and tuber showed antimicrobial activity on six strains of crude microorganisms (inhibition zone diameter, 10 ~ 20 mm). Petroleum ether extracts of both samples did not show activity against four microorganisms; *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus pumilus*. According to these results, water extracts of Kyi-ah showed the highest antimicrobial activity for six microorganisms (zone of inhibition ranging from 12-14 mm).

Moreover antifungal activity test for various crude extracts (PE, EtOAc, EtOH and H₂O extract) were carried out by Agar Well Diffusion Method at the Pharmaceutical and Food Research Department. From these results, all extracts of Taw-Kinpom fruits showed considerable high antifungal activity against *Candida albicans* and *Aspergillusniger*(zone of inhibition ranging from 15mm-19mm) . Therefore the selected sample, Kyi-ah leaves and tuber were suitable for consuming as veggies and also useful for traditional medicine because of their effective properties

Acknowledgements

I would like to express my sincere thanks to the experts who have contributed towards development of this paper. I would like to express my deepest appreciation and special thanks to DrMaungMaungNaingRector, Yadanarbon University, for his kind permission to perform this paper. andPro-rectors, DrSi SiKhin and DrTint Moe Thuzar, Yadanarbon University for their kind permission to submit this paper. I would also like to express my sincere gratitude to Professor DrHlaingHlaingMyat, Head of Department of Chemistry, Yadanarbon University and Professor DrHtayHtay Win for their kind encouragement and advice.

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