

**Detection of cyanogenic glycoside in *Acalypha indica* Linn.
(Kyaung-hsay-pin)**

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Medicinal plants and herbal drugs are being increasingly utilized for health purpose throughout the world. Also in Myanmar, many of the traditional remedies are accepted and used by a large segment of the population. Generally, people believe that herbal medicines are effective and have no toxic effect. Kyaung-yo-thay (or) Kyaung-hsay-pin is one of the well-known Myanmar medicinal plants used for bronchodilating and mucolytic activities. Its botanical name has been identified to be *Acalypha indica* L. This plant was reported in some literatures to contain cyanogenic glycoside. In order to prove the presence of toxic cyanogenic glycoside in Kyaung-hsay-pin, a highly sensitive color reaction test for cyanogenic glycoside was carried out on the various fresh and dried parts of the plant specimens. It was found that a significantly higher amount of cyanogenic glycoside was present in fresh specimens of plants as compared to the smaller amount present in dried specimens. Expressed juice of leaves was also tested for its acute toxicity administering orally to mice and rats. The results were compiled and discussed.

INTRODUCTION

A medicinal plant known as Kyaung-yo-thay (or) Kyaung-hsay-pin has been known to have possibly toxic effect when the fresh plant expressed juice was taken orally for medicinal purpose [1]. Studies [2, 3] have identified Kyaung-hsay-pin to be *Acalypha indica* Linn., and that the presence of cyanogenic glycoside in this plant has been mentioned as the possible cause of toxic effect. *Acalypha indica* Linn. (Euphorbiaceae) grows widely throughout the whole Myanmar and has been widely used for medicinal purposes [4]. Roots has been used as cathartics, and leaves have been used for scabies, snake bite and, as mild laxative in constipation [4]. The whole plant is reputed to have activities like anthelmintic, expectorant, emetic, anodyne and hypnotic. Fresh or dry plant is claimed to be effective for gastrointestinal disease and has been used as a substitute for

ipecacuanha [5]. Some people used expressed juice of fresh leaves and decoaction of dry leaves for bronchodilating activity. Since the plant is well known for its usefulness in spite of its likely toxicity, there is a need to explore the true toxic effect of its constituents so that its therapeutic status as a medicine will become well established.

Objectives

- To detect cyanogenic glycoside in fresh and dried specimen of Kyaung-hsay-pin
- To test its toxic effect on mice and rats.

Myanmar Name : Kyaung-hsay-pin (or)
Kyaung-yo-thay

Botanical Name : *Acalypha indica* Linn.

Family : Euphorbiaceae

Species : Indica

Genus : *Acalypha*

English Name : Indica *Acalypha*



Fig. 1. *Acalypha indica* Linn.

MATERIALS AND METHODS

Plant specimen was procured from the local traditional medicine shops. Both the freshly collected samples and the air-dried samples were subjected to chemical characterization and toxicity on mice and rats.

1. Chemical detection

Detection of cyanogenic glycoside from tested samples was carried out referring to the methods stated as follows.

Method I [Guignard test]

This test [6] was carried out by putting 2-5 g of plant material in a test tube and sufficient amount of distilled water was added to moisten the sample. Filter paper soaked in sodium picrate was inserted above the plant sample, and the test tube stoppered and warmed at 35°C for 3 hours. The presence of cyanide was noted by a change in colour of the yellow picrate test paper to brown.

Method II

The extracts of fresh and dried specimens were tested by Thin Layer Chromatography using precoated silica gel glass plate. TLC system for cyanogenic glycoside was n-butanol:ethanol:water(40:11:10) and detected on UV radiation at 254 nm [7].

2. Acute toxicity test

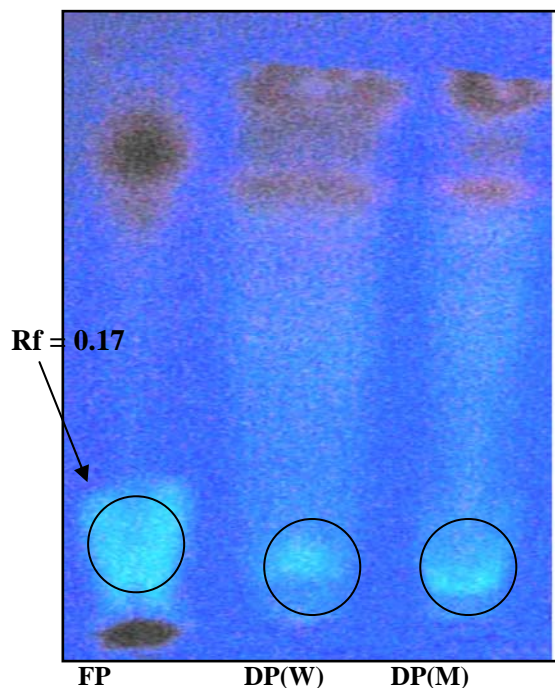
A total of 50 albino mice of ddy strain weighing 30~35 gm were issued from Laboratory Animal Services Division. They were fasted overnight but water was allowed *ad libitum*. Animals were divided into 5 groups of 10 each. Four drug dose levels (40, 50, 60 and 80 ml/Kg) were administered orally. One group received only distilled water, which was served as negative control group. The mice were then continuously observed for their mortality and behavioural responses for 4 hours and there after once daily until the 14th day. The death rates were observed and noted. LD50 was calculated by the method of Litchfield and Wilcoxon, 1948 [8].

RESULTS

Cyanogenic glycoside detection by Method 1 indicated the presence of cyanide compound by the development of a brown color. Fresh leaves and stems showed a more intense deep-brown color than fresh root specimens. Similarly, dried leaves and stems showed a deeper color development than roots.

It was observed that, weight by weight, the content of cyanogenic glycoside was higher in the fresh specimen than the dried specimen as noted from the deep brown color developed in the tests. Similarly, fresh and dried specimen of the whole plant also indicated that cyanogenic glycoside content was higher in the fresh specimen than in the dried specimen.

In the second method (Method II), expressed juice and methanol extract of dried plant were tested by TLC method. Rf value of fluorescence compound indicated cyanogenic glycoside in expressed juice was 0.17. Thin layer chromatogram is shown in Fig. 2.

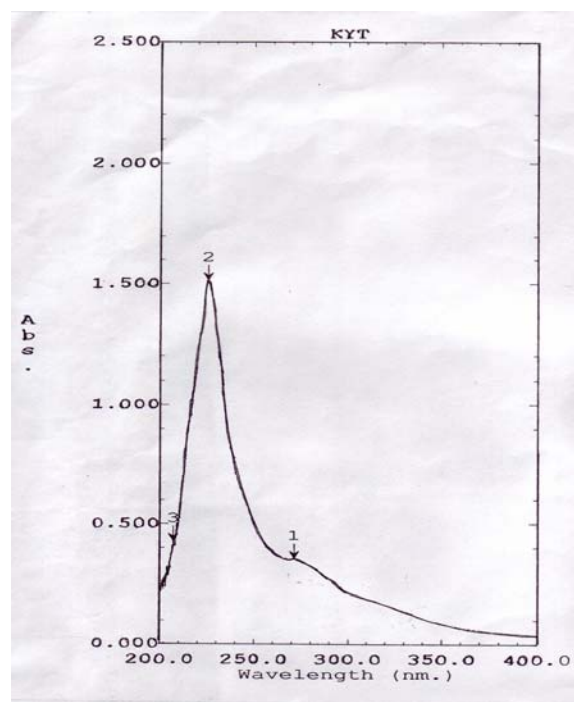


FP = Fresh plant juice
 DP(W) = Watery extract of dry plant
 DP(M) = Methanol extract of dry plant

Fig. 2. Thin Layer Chromatogram of Kyaung-hsay-pin extracts

The compound shown in Rf = 0.17 was the scraped off from the TLC plate and reconstituted in methanol. This was further scanned in UV radiation within the wavelength range of 200-400nm. Determination of wave length by UV spectrophotometer (DU 650) showed a UV spectrum at 223nm and a shoulder at 258nm (Fig. 3).

In acute toxicity test, behavioural changes observed after the oral administration of the fresh juice included vomiting, loss of locomotor activities and eventual death within 24 hrs. No significant changes were seen in the organs when observed by naked eye. The estimated LD50 value for the fresh juice was found to be 79 (56.8–109.8) ml/kg in mice when administered orally (Table 1).



Peak No	Wavelength (nm)	Abs.
1	258	0.355
2	223	1.5128
3	206	0.4268

Fig. 3. UV spectrum of cyanogenic glycoside

Table 1. Results of acute toxicity tests of fresh juice of *Acalypha indica* Linn. on albino mice and rats

Times to No.	human dose	Dosage ml/kg (1ml= 0.07g)	Mice		Rat	
			No. of death observed	% death	No. of death observed	% death
1	200 times	80 ml/kg	6/10	60%	not done	Not done
2	150 times	60 ml/kg	4/10	40%	not done	Not done
3	125 times	50 ml/kg	2/10	20%	4/5	80%
4	100 times	40 ml/kg	1/10	10%	not done	Not done

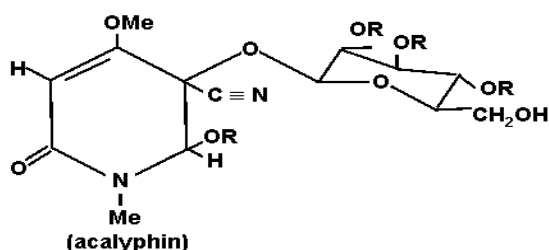
Five rats were administered with 50 ml/kg of fresh juice orally and were observed for 2 weeks; 4 rats died within 24 hours.

Detection of cyanide was also carried out in blood collected from cardiac chamber of the rat which died after administration of tested expressed juice. Blood specimen was acidified with sulphuric acid and release of hydrogen cyanide gas was detected by the

same picric paper method [9]. Yellow picric paper changing to brown, indicated the presence of cyanide in the test blood sample.

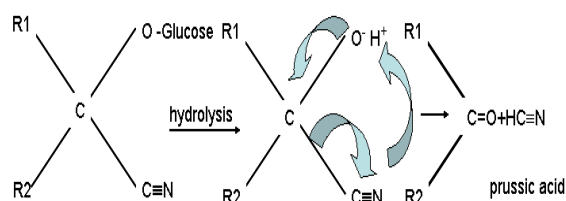
DISCUSSION

The presence of cyanogenic glycoside compounds in Kyaung-hsay-pin was preliminary detected by colour reaction. Trace amounts of fluorescence compound was collected and determined for its UV spectrum. Although the authentic sample was not available, acalyphin of the Kyaung-hsay-pin was identified by UV spectral data reported in the literature [10]. A peak at 223 nm and shoulder at 258nm of the isolated compound was observed to be very close with those of acalyphin at 223nm and 255nm. Therefore the cyanogenic glycoside of Kyaung-hsay-pin was identified as acalyphin. Its structure is shown below.



Living plant tissues of *Acalypha indica* Linn. contain cyanogenic glycoside and (β-D glycoside) in separate cells. When plant tissues are damaged, by chopping or grounding, enzymes ultimately come in contact with the cyanogenic glycoside that is released and result in the formation of prussic acid (HCN) [11].

The production of HCN from cyanogenic glycoside can be explained by the following mechanism.



$\text{C} \equiv \text{N}$ is a weak conjugated base, that is very stable. SP character donation power of the electron pair on carbon atom became very low. The combination of H^+ and: $\text{C} \equiv \text{N}$ anion leads to $\text{HC} \equiv \text{N}$. Accordingly, reaction with cyanogenic glycoside compound present in Kyaung-hsay-pin will certainly occur. The present study proved that Kyaung-hsay-pin, used as folklore medicine in Myanmar contain toxic cyanogenic glycoside compound. It had caused death in tested animals at the dose of 125 times of human dose (50 ml/kg) in 20% of mice and 80% of rats. It was assumed to be the cause of death since a significant amount of cyanide was detected in the rat blood from the cardiac chamber.

Once cyanide gets into the blood, it is readily distributed throughout the body. It is extremely toxic to all animals. In cells, cyanide reacts with cytochrome oxidase (an enzyme involved oxygen) to form a stable, inactive complex. As a result, the cyanide ion inhibits the release of oxygen from the hemoglobin of blood to individual cells. Without oxygen, cellular respiration ceases and cells die rapidly due to hypoxia [11]. Moreover, *Acalypha indica* Linn. was found to induce haemolysis in glucose-6-phosphate dehydrogenase deficiency [12].

CONCLUSION

The present study had proven that Kyaung-hsay-pin contains cyanogenic glycoside which can produce toxic cyanide compound. Expressed juice of fresh leaves and stem can cause death of mice at the dose of (80 ml/kg) but the extract of dried specimens at the same dose did not cause death in animals, since the cyanogenic glycoside content is more in fresh plants than in dried specimens. Consequently fresh specimen of Kyaung-hsay-pin is more toxic than dried specimens according to the chemical and toxicity data.

Finally, it was recommended that cyanogenic glycoside and related toxic effect should be seriously taken into consideration

whenever Kyaung-hsay-pin is used as folklore medicine or for any other edible purposes.

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