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Detection of cyanogenic glycosides in ethnomedicinal plants of Euphorbiaceae used by Tribes in Mizoram, India

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Abstract
Cyanogenic glycosides are natural plant toxins found in various plants, the majority of which are consumed by humans. Cyanide is formed after cyanogenic glycoside hydrolysis that occurs during the crushing of the edible plant material, either during consumption or during the processing of the plant. In this paper, we tested for cyanogenic glycosides using picrate impregnated filter paper in a number of ethnomedicinally important plants. We evaluate 19 species of euphorbiaceae, out of which 7 are cyanogenic. In Mizoram, people living in the urban region still relies heavily on ethnomedicinal plant, most of the times these plants are eaten boiled, cooked or raw, so it is important that we know the content of these plants. From the research we found several plants to contain cyanogenic glycosides which are eaten as a form of medication for various ailments.

Keywords: Cyanogenic glycosides, cyanide, Euphorbiaceae, Picrate impregnated filter paper

Introduction
Cyanogenic glycoside are plant toxins that are found in several plants, cyanide is formed after the hydrolysis of cyanogenic glycosides during crushing of the plant material during processing for consumption. There are approximately 25 known cyanogenic glycosides [1]. At least 2700 higher plant species have been shown to contain compounds which can produce hydrogen cyanide (HCN) or prussic acid [2], A German pharmacist reported the ability of the plant to produce HCN from a parent substance in 1803 [3]. Cyanogenic glycosides play pivotal roles in organization of chemical defence system in plants and in plant-insect interactions [4], and to fend of herbivores to some extent. Cyanogenic glycosides are present in the vacuoles in the case of plants [5]. Cyanogenic study and its distribution in plants have been studied around the world as it helps in chemo-taxonomic evaluation of genera [6, 7, 8]. This group of chemicals are also used in cancer research [9, 10]. In Mizoram many plants are eaten on claim of their ethnomedicinal properties without the knowledge of their chemical content, even though there are rare cases of a person getting ill because of consumption of the plants, it is important to know the exact kind of chemical content in the plants.

Materials and Methods
Picrate paper preparation: Strips of Whatman filter paper No.1 (5.0 X 1.5 cm) were immersed in a 0.05 M solution of aqueous picric acid that had been neutralized with sodium bicarbonate. The paper was then taken out, and left to dry at room temperature.

Detection of cyanogenic glycosides: Cyanogenic glycosides were detected using the technique of the picrate-impregnated paper according to Harbone [11].
Small portions of plant material crushed in a test tube with 2 ml of distilled water and a few drops of chloroform was added preferably less than 10 drops. The test tube was stoppered with a cork with the picrate filter paper attached to it, and the tube is sealed in order to prevent gas exchange. The test tube is than left at room temperature and observation is made periodically.
If there was a change in the colour of the picrate filter paper, it indicates the presents of cyanogenic glycosides in the plants.
If there was no release of HCN within 2 h, indicating a negative test, the tube was left at ambient temperature for 24h and 48h, so that it could be re-examined.
Within 2 hours: Presence of cyanogenic glycoside with respective hydrolytic enzyme and re considered cyanogenic in the field. If no colour change is observed within 2 hours, the tube is kept for further observation.

Within 48hrs: Cyanogenic glycoside spontaneously release HCN without enzyme hydrolysis and are considered not cyanogenic in the field as the synthesis is very slow. If no colour change is observed after 48 hours, the tube is kept for further observation.

No change in colour represents absence of Cyanogenic glycosides or either the synthesis is extremely slow to even consider its relevance in the study.

Results

Table 1: List of plants with cyanogenic glycoside content

Sl. No	Scientific name	Part Used/ Examined	Within 2 hrs	24-48hrs	After 48hrs
1	*Aporusa octandra*	Bark	-	-	-
2	*Baccaurea ramiflora*	Bark	-	-	-
		Leaves	-	-	-
3	*Bischofia javanica*	Leaves	-	-	-
4	*Claoxylon khasianum*	Roots	-	+	+
5	*Croton Caudatus*	Leaves	-	+	+
		Fruits	-	+	+
6	*Euphorbia royleana*	Latex	-	+	+
7	*Jathropa curcas*	Leaves	-	+	+
		Fruits	+	+	+
		Stems	-	-	-
8	*Macaranga denticulata*	Leaves	-	-	-
		Stems	-	-	-
9	*Macaranga indica*	Leaves	-	-	-
10	*Mallotus leucocarpus*	Roots	-	-	-
11	*Mallotus phillipensis*	Fruits	-	-	-
12	*Mallotus roxburghianus*	Twigs	-	-	-
		Leaves	-	+	+
13	*Manihot esculenta*	Roots	-	+	+
		Leaves	+	+	+
14	*Phyllanthus acidus*	Fruits	-	-	-
15	*Phyllanthus airy-shawii*	Leaves	-	-	-
16	*Phyllanthus Emblica*	Fruits	-	-	-
17	*Phyllanthus fraternus*	Whole plant	-	-	-
18	*Ricinus communis*	Fruit	+	+	+
		Leaves	-	+	+
19	*Securinega virosa*	Leaves	-	-	-

Out of the 19 species of plant screened, 7 species contained cyanogenic glycosides. Out of the 7 species, 3 species show cyanogenesis are cyanogenic in field and 4 species shows cyanogenesis but at a much slower rate to even considered to be relevant. After 48hrs, there is no change in colour of the paper in all samples.

Discussion

Cyanogenesis can thus serve as a plant defence against herbivores, but the effectiveness depends on many factors on both sides of the plant-animal interaction [12]. From the observation, although there is a number of plants containing cyanogenic glycosides, there have not been a report of people having negative reaction because of the plants they have eaten as medicines, we come to a conclusion that there is not enough chemical content in the plant that should made the factor significant when it comes to consumption of these plants and that the study of cyanogenic glycosides may be useful in chemotaxonomy [13, 14]. Leaf and fruits of *Croton caudatus* contains cyanogenic glycosides and the plant is used to in treatment of cancer by the locals while these group of chemicals are used on cancer research [15, 16].

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