IDENTIFICATION OF WILD AND CULTIVATED SPECIES OF *PASSIFLORA* IN SRI LANKA AND SCREENING OF THESE PLANTS FOR CYANOGENIC GLYCOSIDES

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ABSTRACT

Collection of forty accessions of the genus *Passiflora* were made from several localities in Sri Lanka, and keys were constructed for identification of species and cultivars. Leaves of the *Passiflora* collections were screened for cyanogenic glycosides using the alkaline picrate paper technique. Results of this survey show that there are considerable variations in the cyanogenic glycoside content of the different species. Distribution of cyanogenic glycosides in different plant parts such as leaves, stems, tendrils floral parts, fruits and roots of the most extensively cultivated form of *P. edulis f. flavicarpa* was studied. Seeds, juice and pollen grains were relatively free of the HCN releasing enzymes and their substrates; all the other plant parts contained relatively larger amounts of the glycosides compared to mature parts. Quantitative analysis of cyanogenic glycosides in leaves from eight species using the alkaline titration method showed that *P. quadrangularis* (138 ppm) contained more cyanogenic glycosides than *Manihot*-var. Mu 51 (110 ppm) while *P. edulis f. flavicarpa* (61 ppm) and *P. edulis f. edulis* (8 ppm) had moderate and small quantities of glycosides.

KEY WORDS; Cyanogenic glycosides, HCN content, *Passiflora*

INTRODUCTION

The family Passifloraceae consists mainly of vines. Some species produce ornamental and show flowers and edible fruits. About twenty genera with 600 spp. have been identified and the family is native to the tropics and subtropics of the New World. *Passiflora edulis* and *P. quadrangularis*, popularly known as passion fruits are widely cultivated in the tropics. The exact date of introduction of these species to Sri Lanka is not known. Account of its cultivation in Peradeniya is reported by Buell (1955). Of the two commercially important varieties *P. edulis f. edulis* (purple fruited form) and *P. edulis f. flavicarpa* (yellow fruited form), the former is more productive at higher elevations. The pulp is used in the preparation of cordials, jams and jellies.

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Beside the fruit pulp, the leaf is consumed to a limited extent as a vegetable in Sri Lanka. A recent survey carried out by the Medical Research Institute of Sri Lanka, shows that the leaves of passion fruit have greater nutritive value than those of spinach (*Basella* spp.) and Thampala (*Amaranthus* spp.) and are similar to most of the other leafy vegetables. Purseglove (1975) and Franklin and Ruth (1975) reported that leaves and unripe fruits of *P. foetida* and the leaves and roots of *P. quadrangularis* contain cyanogenic glycosides. The fleshy tuberous root of *P. quadrangularis* is usually regarded as poisonous, although they are said to be eaten in Jamaica as a substitute for yams. Several human diseases such as goitre and tropical ataxic neuropathy resulting from excessive consumption of cassava have been attributed to chronic intake of cyanogenic glycosides found in tubers (Ermans and Delange, 1973: Wilson, 1973).

There has been no report in the literature on the examination of cyanogenic glycosides present in *Passiflora* species both wild and cultivated in Sri Lanka. The present study was conducted to identify cyanogenic glycosides containing *Passiflora* species found in Sri Lanka. This paper also describes a grouping and arrangement of these species according to their morphological features.

MATERIALS AND METHODS

A total of forty samples of *Passiflora* were procured from Badulla, Dunhinda, Gannoruwa, Hemmathagama, Irriyagama, Kandapola, Muruthalawa, Nuwara Eliya and Peradeniya covering the districts of Badulla, Kandy, Kegalle and Nuwara Eliya.

Based mainly on morphological characters, collections were identified with the help of Bailey's Horticultural Manual (1949), other taxonomic literature (Abeywickrama, 1959; Alston, 1931; Purseglove, 1975) and also by comparing them with those at the National Herbarium, Peradeniya.

The mature and tender leaves of all species collected were screened for cyanogenic glycosides by using the alkaline picrate paper method (Harborne, 1973). This was followed by detailed examination of the distribution of cyanogenic glycosides in the different parts of the plant of the popularly cultivated form of *P. edulis f. flavicarpa*. The plant parts that were examined included leaves, stems, tendrils, roots, flower buds, pericarp,

mesocarp and juice of fruits. The floral parts, bracts, sepals, petals, anthers, ovary, stigma, and pollen were also subjected to screening for cyanogenic glycosides.

Alkaline picrate papers were prepared as follows: Whatman No. 1 filter papers soaked in 5% picric acid solution was air-dried and then resoaked in 10% sodium carbonate (Na_2CO_3). Papers were once more air-dried and cut into strips of 9×1.5 cm dimension and used for HCN detection. The botanical sample (1 g) was cut into small pieces or crushed (seeds) and inserted separately into 10×2.5 cm specimen tubes. Chloroform (15 ml) was then added and sodium picrate paper suspended in the specimen tube with the help of a stopper without touching the sample. *Manihot esculento* (var. Mu 51) sample was included as a standard. Each test was replicated three times.

The rate of colour change was scored at 5 minute intervals during the first hour and then at hourly interval during the subsequent two hours. At the completion of 3 hours, the samples were classified as having high, medium, low, very low and non detectable amounts based on the rate of appearance of brown colour reaction on the paper.

Further studies were carried out to detect HCN content for some of the species and cultivars using the alkaline titration method (Hoowitz, 1975). Twenty grams of three terminal leaves from each species were cut into small pieces and inserted separately into distillation flasks. Water (200 ml) was added and allowed to stand for 2—4 hours in order to set free all the bound hydrocyanic acid. During this operation, the flask was connected with the distillation apparatus. Steam distillation was carried out and distillate (200 ml) was collected in 0.25% NaOH. The distillate was diluted to a volume of 250 ml. To 100 ml of the above distillate 8 ml 6N NH_4OH and 2 ml 5% KI solution were added and titrated with 0.02 N $AgNO_3$ using a microburette (1 ml of 0.02 N $AgNO_3$ correspond to 1.08 mg of hydrocyanic acid). Each test was replicated three times.

RESULTS AND DISCUSSION

The forty specimens collected from Badulla, Kandy, Kegalle and Nuwara Eliya districts were identified as the following species/varieties based on morphological characters: *Passiflora caerulea* L., *P. edulis f. edulis*,

P. edulis f. flavicarpa, hybrid of *P. edulis f. edulis* × *P. edulis f. flavicarpa*, *P. foetida*, *P. ligularis* Juss., *P. mollissima* (HBK) Bailey (Syn. *Tacsonia mollissima* HBK), *P. quadrangularis* L., *P. stipulata* Aubl., *P. suberosa* L. *and P. vanvolexmi* Lem. (Syn, *P. antioquiensis* Karst). The keys prepared for identification of these species and varieties in the present study are given in Tables 1 and 2, respectively.

No fruits are produced by the ornamental spp. *P. caerulea* L. This may be attributed to the fact that it was introduced and propagated by cuttings resulting in self sterile clonal material or to the improper adaptation under climatic conditions in mid-country wet zone. *P. stipulata*, *P. suberosa*, *P. foetida* and *P. edulis f. edulis* are naturalized species found growing wild in Sri Lanka and the fruits of *P. stipulata* and *P. suberosa* are not edible. The following three species *P. ligularis*, *P. mollissima* and *P. vanvolexmii* were introduced to Sri Lanka and are found growing only at higher elevations. The species *P. edulis f. edulis* and *P. quadrangularis* are edible and cultivated in home gardens as fruit crops. The two commercially important varieties are *P. edulis f. flavicarpa* and hybrid of *P. edulis f. edulis* × *P. edulis f. flavicarpa* known as the Rahangala hybrid.

Screening large number of samples for their cyanogenic glycosides required a simple but sensitive method. The alkaline picrate paper method was chosen for the preliminary quantitative survey and HCN concentrations in the range 30—50 microgram/g could be detected.

Fresh tender and mature leaf samples of eleven plants were screened for cyanogenic glycosides. The colour of the paper gradually changed from yellow (original) through shades of reddish brown to dark brown depending on the amount of HCN released from the samples. Rate of colour change is proportional to the amount of cyanogenic glycosides present. The samples were classified as high, medium, and not detectable (Table 3). The following spp. were found to be free of cyanogenic glycosides: *P. edulis f. edulis*, *P. ligularis* and *P. stipulata*. On the other hand, *P. quadrangularis*, *P. foetida* and *P. caerulea* contained relatively high amounts of glycosides. *Passiflora quadrangularis* had rather high amounts as compared to the *Manihot* cultivar used for comparison. Rate of release of HCN from mature leaves was lower than that of tender leaves in all the species examined suggesting that enzyme activity or amount of glycosides decreases with age.

The distribution of cyanogenic glycoside in different parts of the *P. edulis f. flavicarpa* is shown in Table 4 while amounts detected in the floral parts are given in Table 5.

Data show that tender parts contain high quantities whilst seed and juice are relatively free of the cyanogenic glycosides. The roots showed a high degree of cyanogenesis but their release was considerably low. It was also found from this study that pollen grains are free of cyanogenic glycosides while the ovary and corona were high in cyanogenic glycosides.

Tender leaves of the 8 species which can be grown in mid-country wet zone were quantitatively analysed, using the alkaline titration method. The end point in titration was indicated by a fair but persistent turbidity, which may be easily recognized especially against a black background. The amount of hydrocyanic acid was calculated in parts per million (ppm) and results are given in Table 6.

The data indicate that the concentration of cyanogenic glycosides are rather low in *P. edulis f. edulis* (8 ppm) and high in *P. quadrangularis* (138 ppm). The hybrid variety "Rahangala" (47 ppm) and *P. edulis f. flavicarpa* (61 ppm) can be categorised as containing moderate amounts of cyanogenic glycosides.

Within the genus *Passiflora* the variations found among the species with regard to occurrence of cyanogenic glycosides indicate the possibility that this character could be used as a chemotaxonomic marker. The genetic transmission of this trait to hybrids may have some value in breeding programme.

Chemical structure of the cyanogenic glycosides found in *Passiflora* species remain to be characterized. Only about 15 such compounds have been isolated and identified, from other plant sources (Conn, 1980; Harborne, 1973). The most common cyanogenic glycosides are amygdalin (almond), durrin (sorghum), linamarin (cassava), lotaustralin (clover) and prunasin (*Prunus* seeds).

Findings reported in this study may also be useful in incompatibility studies of the genus *Passiflora*. The role of cyanogenic glycoside in incompatibility between peach scions and almond root stocks has shown that incompatible cultivars contained more glycosides than compatible cultivars (Gur and Blum, 1973).

CONCLUSION

This study, confirmed the existence of cyanogenic glycosides in the species and cultivars of *Passiflora* cultivated in Sri Lanka. Although HCN itself is one of the most powerful poisons known, the toxicity of the glycosides present in *Passiflora* may be doubtful. In general most food preparation procedures appear to bring together enzymes and substrate by cell rupture, followed by elimination of the liberated HCN by volatilization.

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REFERENCES

Abeywickrama, B. A. 1959. Check list of the flowering plants of Ceylon 2: 199.

Alston, A. H. G. 1931. Supplement to Trimen's Handbook to the Flora of Ceylon. Dulau & Co. Ltd., London. pp. 132—133.

Bailey, L. H 1949. Passifloraceae. *In* Manual of cultivated plants. pp. 689 — 691. The Macmillan Company, New York.

Buell, E. P. 1955. Training and pruning the passion vine. Tropical Agriculturist 111: 18—27.

Conn, E. E. 1980. Cyanogenic Compounds. Ann. Rev. of Plant Physiol. 31: 433—453.

Coursey, D. G. 1973. Cassava as food: toxicity and technology. *In* Chronic cassava toxicity: Proceeding of an interdisciplinary workshop, London. Int. Develop. Res. Centre Mongr. IDRC — OICe: 27—36.

Ermans, A. M. and F. Delange. 1973. Mechanism of the goitrogenic action of cassava. *In* Chronic cassava toxicity: Proceeding of an interdisciplinary workshop, London. Int. Develop. Res. Centre Mongr. IDRC—OICe: 153—157.

Franklin, W. M. and M. R. Ruth. 1975. Tropical leaves that are poisonous. *In* Edible leaves of tropics. pp 93—102. Published jointly by the Agency for International Development, Dept. of State and the Agriculture Res. Service, U. S. Dept. of Agriculture, Puerto Rico.

Gur, A. and A. Blum. 1973. The role of cyanogenic glycoside in incompatibility between perch scions and almond rootstocks. Hort. Res. 13: 1—10.

Harborne, J. B. 1973. Phytochemical methods. Chapman and Hall, London. 278 p.

Hoowitz, William. 1975. Alkaline Titration method. *In* Official Methods of A. O. A. C. 12th Edition. pp. 481—482. A. O. A. C. P. O. Box 540, Benjamin, Washington DC.

Purseglove, J. W. 1975. Passifloraceae. *In* Tropical crops Dicotyledons pp. 420—429. Longman Group Limited, London.

Wilson, J. 1973. Cyanide and human disease. *In* Chronic cassava toxicity: Proceeding of an interdisciplinary workshop, London. Int. Develop. Res. Centre Mongr. 121—125.

Table 1. Key to indentification of *Passiflora* species now growing under cultivation or in the wild state in Sri Lanka

(A) Leaves: Never Lobed.

(B) Floral bracts free, stems and branches strongly 4—angled or winged. Fruits, oblong greenish yellow, 20 — 30 cm long.

(*1*) *P. quadrangularis*

(BB) Floral bracts connate to about the middle. Stems terete. Fruits, ovoid, 7.5 cm long, orange — brown or purplish when ripe.

(*2*) *P. ligularis*

(AA) Leaves: Usually Lobed

(B) Receptacle or tube elongated, 3.75 cm long.

C) Flowers oblong (about 15 — 20 cm), slender peduncles; bright red.

(*3*) *P. vanvolexemii*

CC) Flowers on relatively short peduncles; pink coloured.

(*4*) *P. mollissima*

(BB) Receptacle or tube short

C) Leaves: 5 lobed. Outer rays of floral corona distinctly shorter than petals. Fruits, not produced under Sri Lankan conditions.

(*5*) *P. caerulea*

CC) Leaves: usually 3 lobed, fruits. produced

D) Leaves: serrate and shining above.

(*6*) *P. edulis*

DD) Leaves: entire

E) Leaves: hairy 3 lobed, lobes not equal. Floral bract hairy and finely divided.

(*7*) *P. foetida*

EE) Leaves: not pubescent or hairy, 3 lobed, lobes approx. equal.

(*8*) *P. stipulata*

EEE) Leaves: lobed or sometimes not lobed. Fruits, small ($\frac{1}{2}$ — 1 cm), green turning dark purple on maturation.

(*9*) *P. suberosa*

Table 2. Key to identification of cultivars of *P. edulis*

(A) Tendrils, stems and petioles purple coloured.
  (B) Fruits: Yellow coloured
    P. edulis f. flavicarpa
  (BB) Fruits: Pink coloured
    P. edulis (Hybrid)
(AA) Tendrils, stems and petioles green coloured
  (B) Fruits: deep purple coloured
    P. edulis f. edulis

Table 3. Relative content of cyanogenic compounds in tender and mature leaves of wild and cultivated *Passiflora* and *Manihot* (Var Mu 51)

Species		*Time (minutes)*							*Degree of cyanogenesis*
		10	*20*	*30*	*40*	*50*	*60*	*180*	
P. caerulea	T	***	***	****					High
	M	**	***	***	***	***	****		
P. edulis f. edulis	T	—	—	—	—	—	—	—	Not detectable
	M	—	—	—	—	—	—	—	
P. edulis f. flavicarpa	T	**	**	***	***	***	****		Medium
	M	—	×	*	*	*	**	**	
Hybrid of *P. edulis f. edulis* × *P. edulis. f. flavicarpa*	T	**	***	***	***	***	***	****	Medium
	M	×	*	*	*	***	***	***	
P. foetida	T	***	***	****					High
	M	**	***	***	***	***	***	****	
P. ligularis	T	—	—	—	—	—	—	—	Not detectable
	M	—	—	—	—	—	—	—	
P. mollissima Bailey (Syn *Tacsonia mollissima*)	T	×	*	**	**	***	***	***	Medium
	M	×	*	*	*	*	*	**	
P. quadrangularis	T	****							High
	M	×	*	**	***	***	***	***	
P. stipulata	T	—	—	—	—	—	—	—	Not detectable
	M	—	—	—	—	—	—	—	
P. suberosa	T	**	***	***	****				Medium
	M	×	**	***	***	****			
P. vanvolexemmi	T	**	***	***	****				Medium
	M	×	*	**	**	**	**	***	
Manihot (Var. Mu 51)	T	***	****						High
	M	***	****						

T — Tender leaves M — Mature leaves

1. **** Dark Brown; 2. *** Reddish Brown; 3. ** ½ of the paper is Reddish Brown;
4. * ¼ of the paper is Reddish Brown; 5. × ⅛ of the paper is Orangish Brown;
6. — Yellow colour or not detectable.

Table 4. Relative distribution of cyanogenic compound in different parts of *P. edulis f. flavicarpa*

Plant parts	*Time (minutes)*							*Degree of cyanogenesis*
	10	*20*	*30*	*40*	*50*	*60*	*180*	
Young leaves	**	**	***	***	***	****		High
Mature leaves	—	×	*	*	*	**	**	Low
Yellowing leaves	—	—	—	—	—	—	×	Very low
Young stems	*	**	***	***	****			High
Old stems	—	×	*	*	*	*	**	Very low
Young tendrils	*	**	***	***	***	***	****	High
Old tendrils	—	—	×	×	×	×	×	Very low
Young flowering buds	×	*	**	**	***	***	***	Medium
Flowering buds before opening	×	*	**	**	***	***	***	Medium
Roots	—	×	×	*	*	**	****	High
Immature fruits (3 days after pollination)	*	**	**	***	***	***	****	High
Immature fruits–pericarp	×	×	*	*	**	**	***	Medium
Immature fruits–mesocarp	—	×	*	*	**	**	***	Medium
Mature fruits – pericarp	—	—	×	×	×	×	×	Very low
Mature fruits – mesocarp	—	—	×	×	×	×	×	Very low
Mature fruits – juice	—	—	—	—	—	—	—	Not detectable
Mature fruits – seeds	—	—	—	—	—	—	×	Extremely minute

1. **** Dark brown; 2. *** Reddish Brown; 3. ** ½ of the paper is Reddish Brown;
4. * ¼ of the paper is Reddish Brown; 5. × ⅛ of the paper is Orangish Brown;
6. — Yellow colour or not detectable.

Table 5. Relative distribution of cyanogenic compounds in the floral parts of *P. edulis f flavicarpa*

Floral parts	*Time (minutes)*							*Degree of cyanogenesis*
	10	*20*	*30*	*40*	*50*	*60*	*180*	
Bracts	×	×	×	*	*	*	***	Medium
Sepals	×	×	×	*	*	*	***	Medium
Petals	×	×	*	*	*	*	***	Medium
Corona	×	*	*	**	**	**	****	High
Anthers	×	×	*	*	*	*	***	Medium
Pollen	—	—	—	—	—	—	—	Not detectable
Ovary	*	**	***	***	****			High
Stigma	×	×	×	*	*	*	***	Medium

1. **** Dark Brown; 2. *** Reddish Brown; 3. ** ½ of the paper is Reddish Brown:
4. * ¼ of the paper is Reddish Brown; 5. × ⅛ of the paper is Orangish Brown;
6. — Yellow colour or not detectable.

Table 6. Amount of hydrocyanic acid and level of toxicity in species of *Passiflora* and *Manihot esculenta*

Species	*HCN content*	*Toxicity**
P. caerulea	62	Moderately poisonous
P. edulis f. edulis	08	Innocuous
P. edulis f. flavicarpa	61	Moderately poisonous
Hybrid of *P. edulis f. edulis* × *P. edulis f. flavicarpa*	47	Moderately poisonous
P. foetida	103	Dangerously poisonous
P. quadrangularis	138	Dangerously poisonous
P. stipulata	10	Innocuous
P. suberosa	73	Moderately poisonous
M. esculenta (Var. MU—51)	110	Dangerously poisonous

* As a rough guide to HCN toxicity the flollowing categorisation was adopted (Coursey, 1973):

Innocuous	—	less than 50 ppm HCN
Moderately poisonous	—	50—100 ppm HCN
Dangerously poisonous	—	over 100 ppm HCN