CEYLON J. MED. SCI., Vol. 17 No. 2 (December 1968)

Toxic Principles of *Gloriosa superba*

by

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The incidence of poisoning by tubers of *Gloriosa superba* L. (Liliaceae) is frequent in the rural areas of Ceylon, (where the plant grows wild), due to the resemblance of these tubers to the edible yams of *Ipomea batatas* (sweet potato). Ingestion of the poisonous tubers is most frequently accidental or suicidal. A non-fatal case of alopecia after poisoning by *G. superba* has been reported from Ceylon (Gooneratne, 1966).

Clewer, Green and Tutin (1915) attributed the poisonous effect of *G. superba* to the alkaloid colchicine which they isolated from tubers collected in Ceylon. Besides colchicine they found an unidentified substance, m. p. 267° and a basic substance, m.p. 177-178°. These authors also isolated benzoic acid, salicylic acid, 2-hydroxy-6-methoxy benzoic acid, choline, dextrose, palmitic and a mixture of unsaturated acids, a hydrocarbon, m.p. 63-65°, a mixture of phytosterols and a mixture of phytosterolins.

Bryan and Lauter (1951) found 0.043% colchicine per dry weight of tubers of *G. rothchildiana*, a related species grown in America as an ornamental plant. Subbaratnam (1952) isolated colchicine from *G. superba* of Indian origin. He also isolated gloriosine, m.p. 248-250° which he thought was a new alkaloid but was later shown to be identical with the alkaloid, N-formyl-desacetylcolchicine or substance B, already isolated by Santavy and Bartek (1952) from *Colchicum autumnale* and from *G. superba* grown in Czechoslovakia and the Netherlands.

Colchicine, substance B, substance C (2-demethyl-colchicine) and lumicolchicine were isolated by Santavy, Kincl & Shinde (1957) from tubers of *G. superba* collected in Poona, India. Maturova, Lang, Reichstein and Santavy (1959) analysed tubers of *G. superba* growing in Africa and they found a similarity between the alkaloidal constituents of the Indian and African tubers but a variation in the proportion of alkaloids (Table 1.) Maturova *et al.* (1959) were also able to isolate very small quantities of three basic substances which did not contain a tropolone ring. Santavy *et al.* (1957) have reported that the tubers of *G. superba* grown in European hot houses contain about four times the colchicine found in the Indian tubers. Subbaratnam (1953) reported that the tender tubers of *G. superba* do not contain colchicine but only gloriosine. It has also been reported that the flowers contain only a trace of colchicine (Santavy *et al.*, 1957).

In this investigation the mature and tender tubers, the seeds and the flowers of *G. superba* were examined for their alkaloidal constituents, using thin layer chromatography, and an attempt made to isolate as many of the alkaloids as possible.

MATERIALS AND METHODS

Fresh tubers of *G. superba* were collected in the Kandy district during the months June-July. The tubers were sliced thinly, dried at 55° and then ground to a coarse powder.

2.3 kg of powdered mature tubers were extracted twice with 6 litres of 95% ethanol at 26° and twice with 6 litres of 90% ethanol at boiling point. The combined extracts were evaporated under reduced pressure. Aqueous sodium sulphate (400 ml of 20%) was added to the oily residue and the resinous material was extracted with petroleum ether followed by ether. Citric acid was added to the remaining aqueous solution to adjust the pH to 4 and the solution was extracted six times with 300 ml chloroform each time (Neutral-Phenolic extract). Ammonia was then added to the aqueous solution to adjust the pH to 9 and the solution extracted six times with 300 ml chloroform each time (Basic extract). The ether extracts which did not contain any alkaloids were discarded. Dried tender tubers (495 g), dried flowers (28 g) and dried seeds (1 g) were similarly treated. The tender tubers were whitish in appearance and were 1.5 - 4 cm in length whereas the length of the mature tubers varied between 8 and 23 cm.

RESULTS

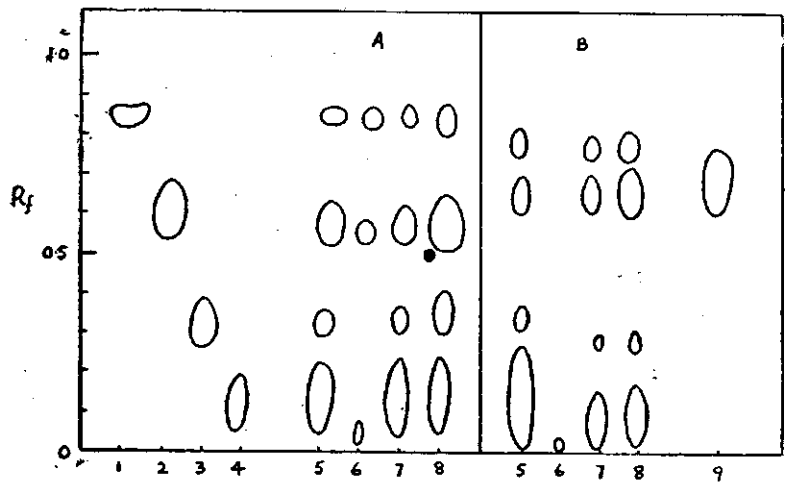

FIG. 1

FIG.—1. Thin layer chromatography on alumina of chloroform extracts of *G. superba* developed in chloroform: acetone: diethylamine (5:4:1). A. Neutral phenolic extract; B. Basic extract; 1. β-lumicolchicine; 2. Colchicine; 3. Substance B; 4. Substance C; 5. mature tubers; 6. seeds; 7. flowers; 8. tender tubers; 9. Demecolin.

Thin layer chromatography on alumina (Aluminiumoxid G-Merck) was used to monitor all the extracts. The solvent systems used were: a) chloroform : acetone: diethylamine, 5: 4: 1. b) chloroform: 100% ethanol, 95:5. The plates were sprayed with Dragendorff's reagent to detect the alkaloids.

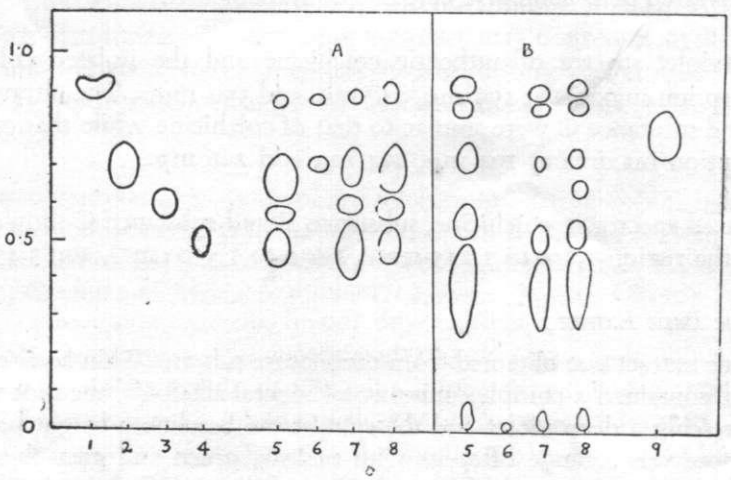

FIG. 2

FIG. 2 As Fig. 1 but using chloroform: 100% ethanol (95:5) as solvent system.

Alkaloids in the Neutral-Phenolic Extract

4.5 g of neutral-phenolic extract from the mature tubers was added to a column of 135 g neutral alumina (Merck) and the alkaloids eluted according to the method of Maturova *et al*, (1959), The ether-chloroform (2:1) fraction, yielded 10 mg of an alkaloid, m.p. 178-179° which crystallised from ethyl acetate-ether. It was identified as β-lumicolchicine from the melting point, mixed melting point with an authentic sample, ultraviolet spectrum and Rf values in 2 solvent systems. It gave an intense orange colour with concentrated sulphuric acid.

From the chloroform fractions, 570 mg of colchicine were isolated. It recrystallised from ethyl acetate-ether in rosettes of colourless needles. It had a melting point of 155-157° and the mixed melting point with authentic colchicine did not show any depression. Ultraviolet and infrared spectra and Rf values of the alkaloid were identical with those of authentic colchicine. It gave a positive Zeisel reaction.

The chloroform fractions also yielded 60 mg of substance B (N-formyl-desacetylcolchicine) which crystallised out of ethyl acetate-ether in pale yellow rectangular plates with a melting point of 259-261°. A mixed melting point with an authentic sample, ultraviolet and infrared spectra, elemental analysis and Rf values further confirmed its identity.

Analysis :	Found :—	C,	65.44;	H,	6.02;	N,	3.64 %
	Calculated :—	C,	65.34;	H,	5.93;	N,	3.82 %

The chloroform-methanol fractions could not be crystallised and were therefore acetylated according to the method of Santavy and Bartek (1952). The acetate (110 mg) crystallised from ethyl acetate-ether in fine pale yellow needles, with a melting point of 224-225°. After hydrolysis it gave a dark olive green colour with ferric chloride. The ultraviolet and infrared spectra and Rf values further indicated that this substance was the acetyl derivative of 2-demethyl colchicine (Substance C.)

The ultraviolet spectra of authentic colchicine and the isolated colchicine showed identical absorption maxima at 196 mμ, 247 mμ. and 350 mμ. The ultraviolet spectra of substance B and substance C were similar to that of colchicine while that of lumicolchicine showed absorption maxima at 194 mμ, 224 mμ and 266 mμ.

The infrared spectra of colchicine, substance B and substance C showed three absorption bands in the region 1,250 to 1,245 cm^{-1}, 1,630 to 1,530 cm^{-1}, and 3,450 to 3,250 cm^{-1}

Alkaloids in the Basic Extract

1.45 g basic extract was obtained from the mature tubers. Thin layer chromatography showed that it contained a complex mixture of several alkaloids, the spot with the highest Rf value fluorescing a deep violet and the spot at the baseline a bright blue in UV light. In between there was a range of spots with yellow, green and grey fluorescence. With Dragendorff's reagent it was possible to detect at least six alkaloids in the basic extract. The basic extract was separated on a column of 43.5 g of neutral alumina. The eluates contained very small quantities of the alkaloids which could not be obtained in a crystalline form. These alkaloids did not give any colour reaction with ferric chloride.

Tender Tubers, Seeds and Flowers

Thin layer chromatography indicated that the tender tubers contained the same alkaloids as the mature tubers. The seeds showed the presence of colchicine, lumicolchicine and a trace of substance C but there was no evidence of substance B. There was also no evidence of alkaloids in the basic extract of the seeds. The flowers contained colchicine, lumicolchicine, substance B, substance C and the same mixture of alkaloids in the basic extract, as was detected in the tubers (Figs 1. and 2).

TABLE 1. PERCENTAGE OF THE CHIEF ALKALOIDS ISOLATED FROM *G. superba* TUBERS.

COUNTRY OF ORIGIN	DRYWEIGHT (g)	ALKALOIDS ISOLATED IN mg			REFERENCE
		COLCHICINE	SUBSTANCE B	SUBSTANCE C	
Czechoslovakia	215	505 (0.23%)	32 (0.015%)	48 (0.022%)	Santavy and Bartek (1952)
Netherlands	521	946 (0.181%)	15 (0.003%)	46 (0.01%)	Santavy and Bartek (1952)
India	1360	748 (0.05%)	430 (0.03%)	130 (0.009%)	Santavy *et al.* (1957)
Africa	1000	1200 (0.12%)	115 (0.0115%)	47 (0.0047%)	Maturova *et al.* (1959)
Ceylon	2300	570 (0.025%)	60 (0.003%)	110 (0.005%)	Present communication

DISCUSSION

The occurrence of colchicine as the principal alkaloid in the tubers of *Gloriosa superba* is consistent with previous observations. However the colchicine content was remarkably low in the tubers analysed, compared to that found in the tubers of European and African origin (Table 1). Low values have also been reported by Subbaratnam (1953) and Santavy *et al.* (1957) in the Indian tubers. These values however may not represent the true alkaloid content of the tubers as there may be significant loss during the process of extraction. In fact, preliminary recovery experiments suggest that the loss during extraction may be even as high as 90%.

The quantity of substance C (2-demethylcolchicine) relative to colchicine is higher in the Indian and Ceylon tubers (1:5) than in the European (1:14) and African (1:25) tubers. Relatively more substance B (N-formyl-desacetylcolchicine) has also been found in the tubers from India, Ceylon and Africa.

Jayatilaka, Balasubramaniam, Dunuwille and Bibile (1967) found that the LD 50 for rats was 10 g/kg for fresh tubers of *G. superba* and 5 mg/kg for colchicine. But the amount of colchicine present in 10 g fresh tubers according to our estimation (Table 1) is 0.6 mg. If, on the other hand, the loss during extraction is as much as 90% the colchicine content of 10 g fresh tuber would in fact be 6 mg. This agrees well with the figure of 5.0 mg/kg obtained as the LD 50 for pure colchicine. The amount of fresh tuber that is consumed accidentally or suicidally is approximately 125 g which would contain about 75 mg colchicine if the loss during extraction is taken to be about 90%. This quantity of colchicine is sufficient to cause death; for example, Nickolls (1965) has suggested that the fatal dose may be about 60 mg even though smaller amounts have also caused death.

It was interesting to find that unlike the Indian tender tubers (Subbaratnam, 1953), the Ceylon tender tubers contained colchicine. The Ceylon flowers contained colchicine, as well as the other alkaloids that were found in the tubers while the seeds showed the absence of substance B.

SUMMARY

Examination of the tubers of *Gloriosa superba* L. which commonly cause poisoning in the rural areas of Ceylon, revealed colchicine to be the chief alkaloid. Three other alkaloids, namely N-formyl-desacetylcolchicine, 2-demethylcolchicine and lumicolchicine were also isolated and identified. Several other alkaloids were present in small amounts. The alkaloid content has been compared with that of tubers grown in Europe, Africa and India. Colchicine was also found to be present in the tender tubers, seeds and flowers of *G. superba*.

ACKNOWLEDGEMENTS

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ACKNOWLEDGEMENTS

We are grateful to Professor F. Santavy and Professor Finn Sandberg for gifts of lumicolchicine, substance B, substance C and demecolin. We thank Dr. Seetha Rodrigo, Dr.

N. G. Baptist and Mrs. M. E. Fernandopulle for their assistance in determining infrared and ultraviolet spectra and for the elemental analysis. We are deeply indebted to Dr. R. O. B. Wijesekera for his advice throughout this investigation.

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