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Note

Detection of *Cerbera odollam* by thin-layer chromatography

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Cerbera odollam is a poisonous plant peculiar to the State of Kerala, and grows in tidal forests and salt swamps on the coasts of India[1]. Although attempts have been made to isolate and characterise the toxic components of the fruit, little work has been done on the isolation from and identification of the toxic principles in the viscera. *C. odollam* is responsible for *ca.* 50% of the plant-poisoning cases and 10% of the total poisoning cases in Kerala, and it is usually detected in cases of suicide involving women and children of the villages in the coastal area; it is also used as an arrow poison in Southern and Eastern Africa[2].

The plant belongs to the order *Apocyanacea* and has fleshy lanceolate leaves, large white flowers and green fibrous fruits enclosing a kernel[3], which contains a non-poisonols oil[4] and toxic glycosides[1,5,6]. It has been reported to contain cerberin (which acts as a parasympathomimetic poison[7], but Chen and Steldt[8] reported that in the fresh fruit, they found not cerberin, but a digitalic compound similar to digitalose, *viz.*, cerberoside. Frerejacque[9] also observed that fresh almonds contain not cerberin, but a hetereoside rich in sugars, and cerberin is found only after the darkening of the kernals of *Cerbera*, which is brought about by the action of diastase on the nuts. The poisonous glycoside thevetin is said to be present in the latex[1,6]. Thus, there is ample evidence that the fruit contains more than one toxic component, a fact also established by us (see later).

At present, the only available method for detection of this plant poison is precipitation of the glycoside from the concentrated extract obtained in the Stas–Otto procedure by adding diethyl ether in excess; the precipitate so obtained is subjected to certain colour reactions, *e.g.*, addition of concentrated sulphuric acid, which produces an intense crimson colour[3]. When visceral samples have been preserved in sodium chloride, this method is laborious and time-consuming, 2 or 3 days being required to complete an analysis. We describe here a simple method for isolating the poisonous components from viscera and detecting them by thin-layer chromatography (TLC).

EXPERIMENTAL

Extraction and purification procedure

The well macerated sample of viscera was extracted with ethanol–ethyl acetate (1:1) (Merck reagents). After separation of the phases by centrifugation, the upper layer was evaporated to dryness on a water bath, the residue was extracted with ethyl

acetate, and this extract was evaporated to dryness. The residue was washed well with light petroleum to remove any co-extracted fact, then dried and dissolved in 0.5 ml of ethanol; this solution was submitted to TLC. The kernels of fresh, ripe *C. odollam* were similarly treated, the final solution in ethanol being used as standard.

Chromatography

Glass plates (20 × 10 cm) were coated with a 250-μm layer of a slurry prepared from 25 g of silica gel G and 50 ml of water; the layers were dried in air and then activaled at 120° for 30 min before use. An aliquot of the test solution was applied 1 cm above the bottom edge of the plate by means of a capillary tube, and a portion of extract from the fresh fruit was similarly applied. After drying, the chromatogram was developed for 30 min by the ascending technique at 25° in a solvent-saturated glass chamber. The plate was then dried at room temperature. The best separation was attained with chloroform–methanol (48:2) as mobile phase; increasing the amount of methanol and decreasing the amount of chloroform led to poor discrimination.

To locate the spots, the dry plate was sprayed with concentrated sulphuric acid and then heated at *ca.* 100° for 3 min; purple to brown spots were obtained.

Toxicity test for isolated compounds

Portions of the extract from the fruit were applied on two plates in as narrow and even bands as possible, and the two chromatograms were developed simultaneously in the same tank, as described above, and dried. One was then sprayed with concentrated acid and heated in an electric oven at 100° for 3 min to locate the bands. The corresponding zones, together with 0.5 cm on either side, were scraped from the untreated plate, and the components were eluted from the gel with successive small portions of ethanol. The liquid from each elution was centrifuged at 1200 *g* for 15 min, and the combined supernatant solutions were evaporated to dryness on a water bath at 50°. An aqueous solution of each residue was injected into the dorsal lymph sac of a frog. Frogs receiving the extracts from the two inner bands (R_F 0.57 and 0.80, respectively) exhibited toxic symptopms and died in *ca.* 30 min; the extracts from the other two bands were non-toxic.

TABLE I

R_F VALUES OF GLYCOSIDES OF *Cerbera odollam*

Component No.	*Control sample*	*Viscera samples*			
		1	*2*	*3*	*4*
1	0.46	0.46	0.46	0.47	0.46
2	0.57	0.56	0.56	0.56	0.56
3	0.80	0.78	0.79	0.78	0.78
4	0.97	0.97	0.97	0.97	0.97

RESULTS AND DISCUSSION

TLC is a versatile method for separating various complex mixtures, and it has often been applied to difficult separations, including those of steroid glycosides[10–15]. In this work, we found that defatted *C. odollam* gave four spots; the R_F values are shown in Table I.

The proposed technique of extraction and detection was successfully applied in several cases of poisoning by *C. odollam*. The components of *C. odollam* were extracted from different autopsy tissues (stomach, intestine, liver, kidney and spleen) by the proposed method, and detection was made by qualitative TLC.

In an analytical method the sensitivity, the accuracy and the time needed for the whole procedure are of prime importance. Our method is sensitive and accurate and involves only few steps which will facilitate the analysis and detection of glycosides in visceral samples reducing the time involved from 2-days to less than 2 h.

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