Rapid detection of residues of cardenolides of Nerium oleander (linn.) by high-performance thin-layer chromatography (HPTLC) in autopsied samples

Praveen U Sanganalmath[1], Yogaraje Gowda C V[2], Gowtham M D[3]
Nayak V G[4] & Mohan B M[5]
[1,2,3] Scientific Assistant, [4]Scientific Officer, [5]Director
Forensic Science Laboratory, Bangalore – 560 068, Karnataka. INDIA
Email: praveen_usp@yahoo.com, cvyoga@rediffmail.com

ABSTRACT

Nerium oleander (common oleander) is an evergreen shrub of Apocyanaceae family cultivated worldwide as an ornamental plant. All parts of the plant are toxic and contain a mixture of very toxic cardiac glycosides of cardenolides, which inhibit plasmalemmal Na^+, K^+ ATPase pump. A number of techniques are used to determine the cardenolides of *Nerium oleander* in various biological matrices. A survey of literature has revealed that the use of HPTLC for the detection of oleander glycosides is very scanty. In this paper, a simple, precise and rapid high-performance thin-layer chromatography (HPTLC) method for separation and identification of cardenolides of *Nerium oleander* is reported. The cardenolides present in the aerial parts of the plant and residues available in the autopsied samples sent in cases of poisoning; are extracted into chloroform and sampled by using Accelerated Solvent Extractor (ASE). The efficacy of separation and detection of cardenolides are studied by using ten mobile phases and nine chromogenic reagents. Densitometric scanning was performed at 275nm and no interferences were observed in the UV spectra. Both the separation and detection is spectacular in benzene: acetone (7:3) as mobile phase and with Keddle reagent. The UV and fluorescent characteristics are evaluated for each of the spot

Correspondence Author: Dr. B. M. Mohan, Director, Forensic Science Laboratory, Madivala, Bangalore 560 068, Karnataka, INDIA. Email: drbmmohan@yahoo.co.in. Telefax: 91-80- 2553 2910.

developed in all the mobile phases and the chromogenic reagents used. The spots so developed are purified and their $_1H^1$-NMR are recorded. The structure of the component present in the spot is similar to those cardenolides present in *N. oleander*.

The method has specific advantage that the separation achieved is free from interferences from both the plant and forensic matrix and can thus become highly reliable and as an alternative to other chromatographic techniques.

Key words: cardenolides, autopsied samples, ASE, densitometric scanning

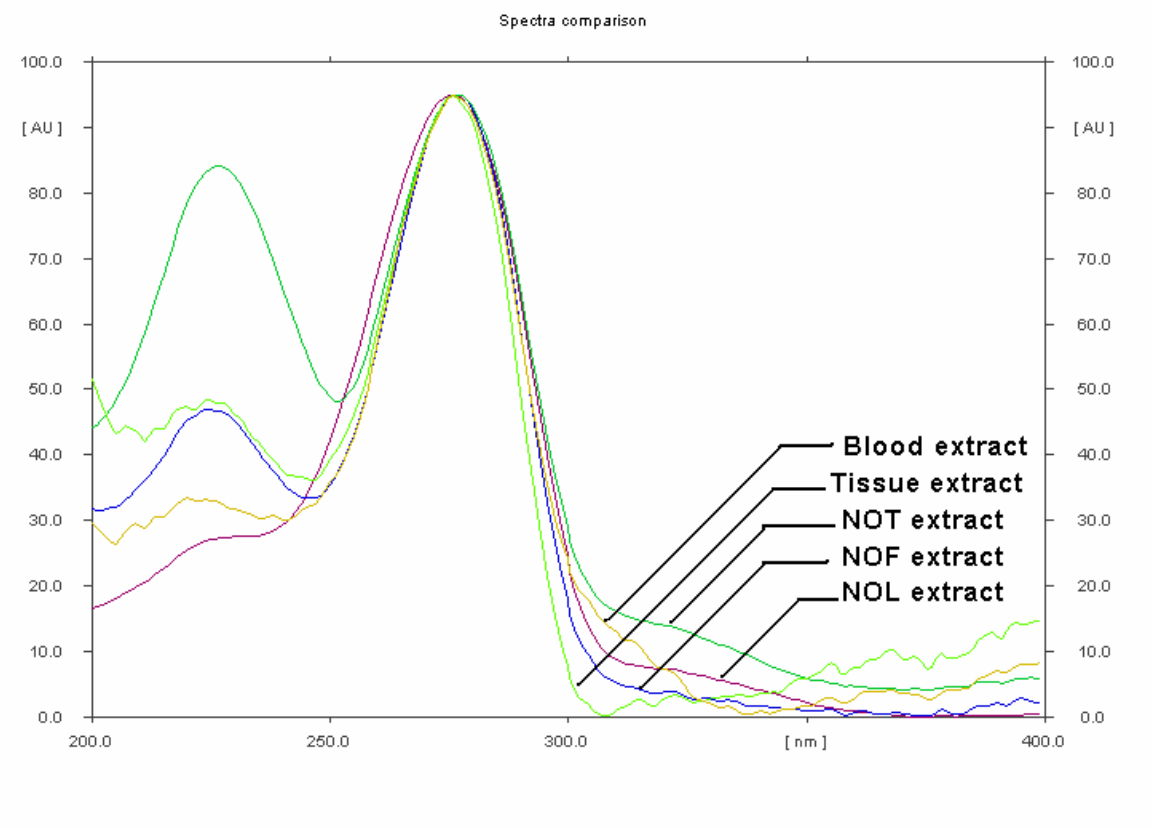

Fig. Overlay spectra of plant material extracts and extracts of autopsied samples.

NOL – Nerium oleander leaf, NOF – Nerium oleander flower, NOT – Nerium oleander twig.

Table
Optimized mobile phases and chromogenic reagents.

Sl. No	Mobile phase	hRf values	Chromogenic reagent*	Color of the spot and Stability
1	Benzene: Acetone (7:3)	14, 28, 41	Keddle reagent	Violet-red spots, stable for 5 minutes
2	Benzene: Ethanol (9:1)	20, 27, 38	p-anisaldehyde reagent	Heat for 10 mins at 110^0C, blue spots, stable for 20-30 mins.
3	Chloroform: Acetone: Acetic acid (8.5: 1: 0.5)	18, 31, 49	TCA-H_2O_2 reagent	Heat for 5-10 mins at 110^0C, blue fluorescence at 366nm.
4	Dichloromethane: Methanol (9.5:0.5)	35, 42, 52	p-toluene sulfonic acid reagent	Heat for 5 mins at 110^0 C, yellow spots, different fluorescent characters under 366 nm.
5	Ethyl acetate: Isopropanol: Water (7:2.5:0.5)	50, 63, 78	Aluminium chloride reagent	Heat for 5-10 mins at 110^0C, yellow spots, blue fluorescence at 366nm.
6	Chloroform: Acetone (8:2)	09, 19, 32	Antimony (III) chloride reagent	Yellow spots, dry the plate, different fluorescent characters under 366 nm.
7	Ethyl acetate: Methanol: Ammonia (8.5: 1: 0.5)	39, 52, 59	Phosphoric acid reagent	Yellow spots, heat for 5-10 mins at 110^0C, light reddish brown spots and bright blue fluorescence at 366nm.
8	Chloroform: Acetonitrile: Methanol (7:2.5:0.5)	38, 48, 59	Orcinol reagent	Yellow spot, different fluorescent characters under 366 nm.
9	Hexane: Ethyl acetate: Acetic acid (3:6.5:0.5)	07, 17, 28	Vanillin – Sulphuric acid reagent	Brown color spots.
10	Toluene: Ethyl acetate: Acetic acid (6:3.5:0.5)	04, 09, 19		

* All the chromogenic reagents were tested against the plates developed in each of the mobile phases.