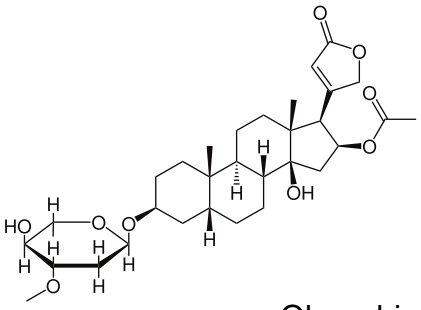
APPLICATION NOTE

Identification of oleander (*Nerium oleander* L.) and detection of oleandrin, a toxic cardiac glycoside

A-134.1



Oleandrin

Keywords

Nerium oleander L., oleandrin, COVID-19, HPTLC, quantification

Introduction

On 18 August 2020 the American Herbal Products Association (AHPA), the American Botanical Council (ABC) and other organizations issued warning letters to consumers of the herbal supplements and the general public alerting about the potential risk of ingesting any part of the oleander plant, and its constituent oleandrin. This concern was raised because of a discussion about an oleander extract as a promising treatment for COVID-19 that received significant media attention [1-3]. According to AHPA, “oleandrin is a cardiac glycoside found in the oleander plant. Oleander is a highly toxic plant, and death has been associated with oral ingestion of even small amounts of oleander leaf.” [1].

Scope

This HPTLC method rapidly identifies oleander (*Nerium oleander* L.), discriminates the different plant parts, and detects the presence of the toxic cardiac glycoside oleandrin with certainty.

Recommended devices

Automatic TLC Sampler (ATS 4), Automatic Developing Chamber (ADC 2), TLC Scanner 4, Derivatizer, TLC Visualizer, *visionCATS*

Samples

0.5 g of dried and finely powdered sample are mixed with 5.0 mL of methanol, sonicated for 10 min and then centrifuged. The supernatant is used as test solution.

NOTE: The presented results are to be regarded as examples only!

Please contact CAMAG for more application notes and products!

Standards

Chlorogenic acid, hyperoside and isoquercitrin are prepared at 0.5 mg/mL in methanol. Oleandrin is prepared in methanol at 0.25 mg/mL.

Chromatography

Stationary phase	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck)
Sample application	2 µL of each standard and sample solution are applied with ATS 4 as 8.0 mm bands, 15 tracks, track distance 11.4 mm, distance from left edge 20.0 mm, distance from lower edge 8.0 mm
Developing solvent	Ethyl acetate, formic acid, and water 80:10:10 *(v/v/v)*
Development	In the ADC 2 with chamber saturation (20 min). Plates were conditioned at 33% relative humidity for 10 min using a saturated solution of magnesium chloride ($MgCl_2$).
Developing distance	70 mm (from the lower edge)
Plate drying	Drying 5 min in the ADC 2
Derivatization A	Natural Products reagent (NP) Preparation: 1 g of 2-aminoethyl diphenylborinate are dissolved in 100 mL of methanol
Derivatization B	Anisaldehyde – sulfuric acid reagent (AS) Preparation: Slowly and carefully 170 mL of ice-cooled methanol are mixed with 20 mL of acetic acid and 10 mL of sulfuric acid. The mixture is allowed to cool to room temperature, then 1 mL of anisaldehyde (p-methoxy benzaldehyde) is added. Use: The plate is heated at 100°C for 3 min, cooled down, and then derivatized with AS reagent (Derivatizer: 3 mL, green nozzle, spraying level 3), dried for 2 min. Images are recorded. Subsequently the plate is derivatized with NP reagent (Derivatizer: 3 mL, blue nozzle, spraying level 3), heated at 100°C for 3 min, and then cooled down. Images are recorded.
Densitometry	Densitometric analysis of oleandrin is performed after derivatization with NP plus AS reagent in absorbance mode at 403 nm with the TLC Scanner, slit dimension 5.0 x 0.2 mm, scanning speed 20 mm/s.

Results

Fingerprints of oleander leaf (different stages), flower, stem, and twig

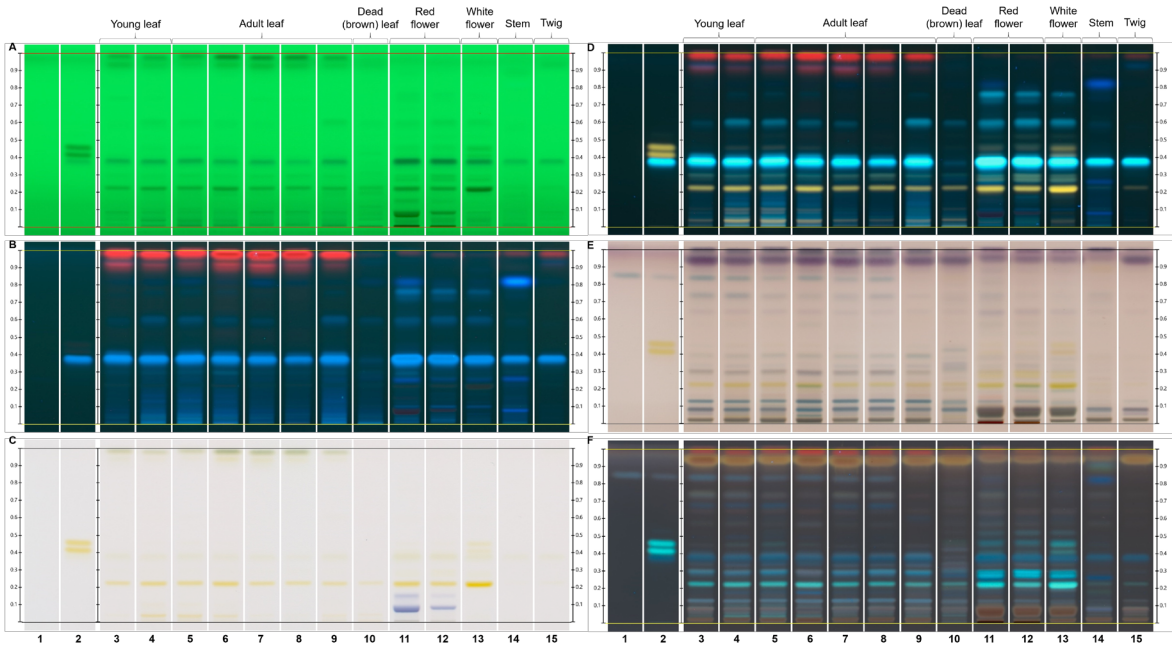
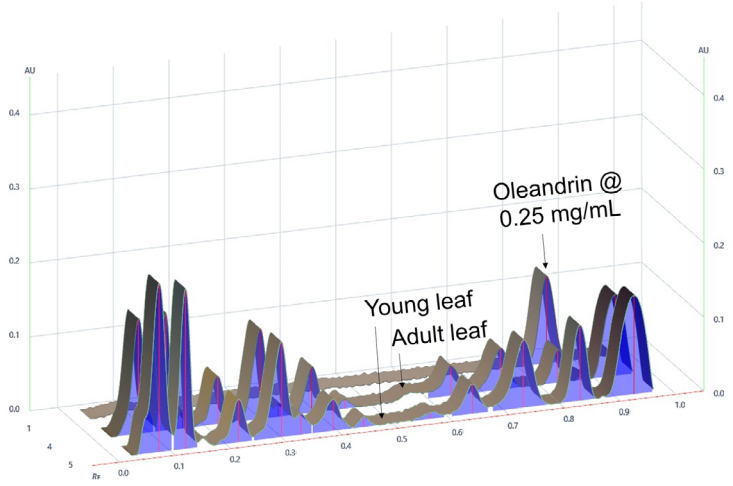


Figure 1: HPTLC fingerprints under UV 254 nm (A), under UV 366 nm (B) prior to derivatization, under white light after derivatization with NP reagent (C), under UV 366 nm after derivatization with NP reagent (D), under white light after derivatization with NP plus AS reagent (E), and UV 366 nm after derivatization with NP plus AS reagent (F). Track 1: oleandrin 0.25 mg/mL; track 2: chlorogenic acid, hyperoside, isoquercitrin (0.5 mg/mL) and oleandrin (0.1 mg/mL) (with increasing R_F)

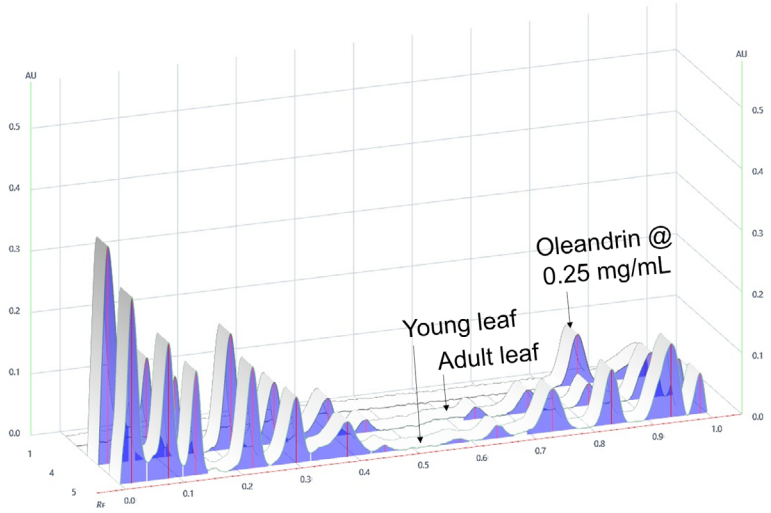
Under white light after both derivatizations (detection F, Figure 1), only the young and adult leaf samples show a detectable zone at the position of oleandrin. One flower sample (track 11) shows traces of oleandrin. Under white light after derivatization with NP reagent (detection C, Figure 1), the red flowers show two purple zones in the lower third of the chromatogram, absent in the white flower and other parts of oleander. Both types of flowers show a distinctive flavonoid fingerprint (detection D). The stem and the twig samples show nearly no yellow zones due to flavonoids in detection D.

The zone due to oleandrin can be detected after derivatization with NP plus AS reagent at 403 nm by scanning densitometry and in the generated peak profiles from the white light image.

Peak profiles from image (PPI) captured under white light after derivatization with NP plus AS reagent of oleandrin standard solution and oleander leaf test solutions



Peak profiles from scanning densitometry (PPSD) at 403 nm after NP plus AS reagent of oleandrin standard solution and oleander leaf test solutions



Literature

[1] American Herbal Products Association (AHPA). AHPA statement on oleandrin and *Nerium oleander*. 18.08.2020. Available at: http://www.ahpa.org/News/LatestNews/TabId/96/ArtMID/1179/ArticleID/1468/AHPA-Statement-on-oleandrin-and-Nerium-oleander.aspx. Accessed on 24.08.2020.

[2] The American Botanical Council (ABC). American Botanical Council warns consumers about high toxicity of oleander. Herbal science group emphasizes that consumers should NOT attempt to produce oleander-based home-remedies or self-medicate with dangerous plant. 18.08.2020. Available at: http://cms.herbalgram.org/press/2020/ABC-Warns-High-Toxicity-Oleander.html?ts=1598295352&signature=666022163b958e6713c4155cab457fc2. Accessed on 24.08.2020

[3] The New York Times. Drug Pitched to Trump for Covid-19 Comes From a Deadly Plant. 20.08.2020. Available at: https://www.nytimes.com/2020/08/20/health/covid-oleandrin-trump-mypillow.html. Accessed on 24.08.2020.