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APPLICATION NOTE

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HPTLC fingerprint of Edelweiss plants and extracts used as ingredients in cosmeceuticals

A-104.1

Keywords

Leontopodium spp., leontopodic acid, glycerin formulation, cosmetics

Introduction

Edelweiss (*Leontopodium* spp., Astereracea) is a famous plant with white and hairy inflorescent leaves. Among the known species of this genus are two European species *L. alpinum* Cass. and *L. nivale*, and 41 species native to Asia. Wild Edelweiss is protected by law, but it is cultivated in large numbers. Extracts of the aerial parts are used in cosmetic formulations because of their anti-oxidative and radical scavenging properties [1].

Scope

This HPTLC method is suitable for the identification of phenolic compounds, flavonoids, and caffeoyl esters, extracted from various Edelweiss species. The presence or absence of Edelweiss ingredients (dry powdered or glycerin extracts) used in cosmetic formulations can be tested as well.

Required devices

Automatic TLC Sampler (ATS 4), Automated Development Chamber (ADC 2), TLC Visualizer 2, Chromatogram Immersion Device 3, TLC Plate Heater 3, and visionCATS software.

Samples

Powdered plant samples were provided by Stephan Schwaiger (Leopold-Franzens-University, Innsbruck, Austria).
One powdered dry extract was provided by EXTRASYNTHESE (France).
One glycerin sample originating from plant cells (PCC) (Majestem®) was provided by SEDERMA (France), and one glycerin sample was purchased from another supplier.

Samples preparation

Powdered samples: 500 mg of powdered sample are suspended in 5 mL of methanol and sonicated for 10 min. The suspension is centrifuged for 5 min, and the supernatant is used as test solution.
Powdered dry extracts: 125 mg of powdered sample are suspended in 5 mL of methanol and sonicated for 10 min. The suspension is centrifuged for 5 min, and the supernatant is used as test solution.
Glycerin samples: 1 g of glycerin sample is extracted with 4 mL of water (HPLC grade), stirred vigorously and centrifuged for 15 min. The supernatant is then prepared by SPE with Oasis HLB cartridges (Waters). The cartridge is attached to a vacuum manifold, conditioned with 5 mL ethanol, and then equilibrated with 5 mL of water (HPLC grade). The supernatant is added at the top of the cartridge and washed with 10 mL of water (HPLC grade). The sample is eluted with 4 mL of ethanol, filled up to 5 mL with ethanol and used as test solution [2].

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

Standards

Standard solutions of chlorogenic acid, apigenin, and luteolin are prepared at 1 mg/mL in methanol. Leontopodic acids A and B, cynarin, and 3,5-dicaffeoylquinic acid are prepared at 0.75 mg/mL in methanol. Luteolin-4-o-glucoside and luteoline-7-o-glucoside are prepared at 1 mg/mL in methanol. *Standards were provided by EXTRASYNTHESE (France).*

Chromatography

Stationary phase	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck)
Sample application	Bandwise application with ATS 4, 15 tracks, band length 8 mm, track distance 11.4 mm, distance from left edge 20 mm, distance from lower edge 8 mm, application volume 2.0 µL.
Development	In the ADC 2 with chamber saturation (with filter paper) 20 min and after conditioning at 33% relative humidity for 10 min using a saturated solution of magnesium chloride
Developing solvent	Butyl acetate, formic acid water 28:10:0.3 (*v/v/v*)
Plate drying	Drying 5 min in the ADC 2
Documentation	With the TLC Visualizer under white light and UV 366 nm (after derivatization)
Derivatization	Reagent name: Natural product reagent Reagent preparation: 1 g of diphenylborinic acid aminoethylester is dissolved in 200 mL ethyl acetate Reagent use: the plate is heated for 3 min at 100°C on the TLC Plate Heater, immersed into the reagent while still hot using the Chromatogram Immersion Device, immersion time 0 s, immersion speed 3 cm/s.

Results

Based on literature [1], a set of substances present in Edelweiss was proposed by EXTRASYNTHESE in order to characterize the extracts used in cosmetic formulations (Fig. 1).

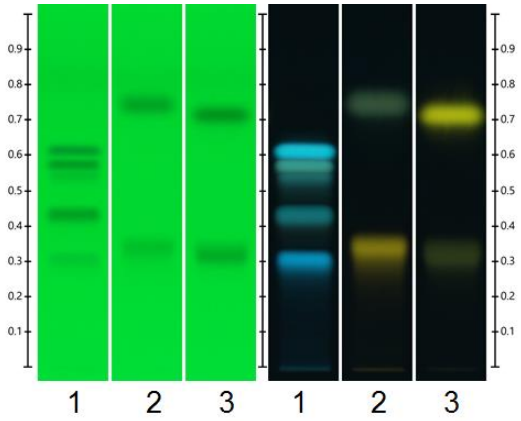

Fig. 1 Left: Plate under UV 254 nm prior to derivatization, right: plate under UV 366 nm after derivatization (NP). Track 1: chlorogenic acid, cynarin, leontopodic acid B, leontopodic acid A, 3,5-diccaffeoylquinic acid (with increasing R_F), track 2: luteolin-7-o-glucoside, apigenin (with increasing R_F), track 3: luteoline-4-o-glucoside, luteolin (with increasing R_F)

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The substances were simultaneously analyzed with powdered samples of Edelweiss in order to check the specificity of those markers for the different *Leontopodium* species (Fig. 2).

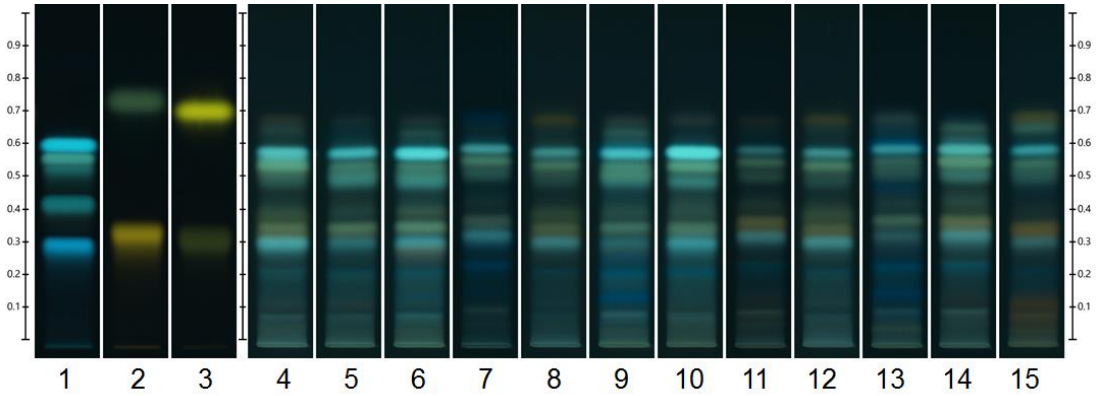

Fig. 2 Plate under UV 366 nm after derivatization (NP). Track 1: chlorogenic acid, cynarin, leontopodic acid B, leontopodic acid A, 3,5-diccaffeoylquinic acid (with increasing R_F), track 2: luteolin-7-o-glucoside, apigenin (with increasing R_F), track 3: luteoline-4-o-glucoside, luteolin (with increasing R_F), tracks 4-15: powdered samples of different Edelweiss species (from left to right: L. nivale ssp. alpinium, L. andersonii, L. artemisiifolium, L. calocephalum, L. campestre, L. dedekensii, L. franchetti, L. himalayanum, L. leontopodioides, L. sinense, L. souliei, L. strachey)

As real cosmetic ingredients two samples formulated in glycerin (PCC sample Majestem® from SEDERMA and one purchased from another supplier) were tested together with one powdered dry extract (Fig. 3).
The profile of the purchased glycerin sample (tracks 6-7) has shown a very low content of edelweiss extract (less intense zones).

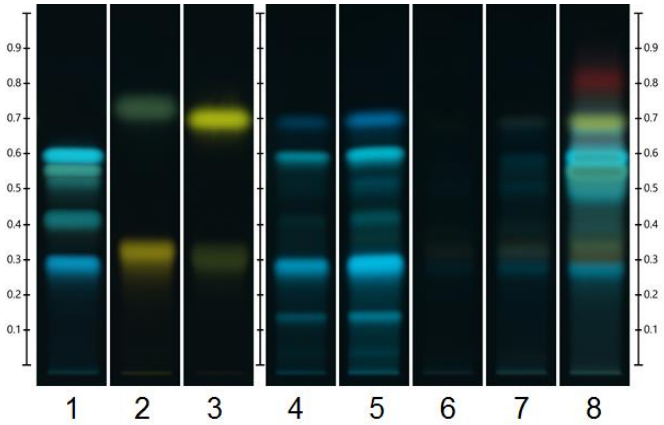

Fig. 3 Plate under UV 366 nm after derivatization (NP). Track 1: chlorogenic acid, cynarin, leontopodic acid B, leontopodic acid A, 3,5-diccaffeoylquinic acid (with increasing R_F), track 2: luteolin-7-o-glucoside, apigenin (with increasing R_F), track 3: luteoline-4-o-glucoside, luteolin (with increasing R_F), tracks 4-5: glycerin PCC samples (Majestem® from SEDERMA) (application volume: 2 and 5 µL), tracks 6-7: purchased glycerin sample (application volume: 2 and 5 µL), track 8: powdered dry extract (EXTRASYNTHESE).

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Conclusion

A method applicable to raw materials and extracts has been developed to detect the major phenolic markers (flavonoids and caffeoyl esters) specific to *Leontopodium* spp. The set of standards of caffeoyl esters (3,5-dicaffeoyl quinic acid, leontopodic acids A and B) is suitable for the identification of *Leontopodium* spp. With this method the quality of Edelweiss raw materials and ingredients can be assessed. Furthermore different sources and grades of cosmetic ingredients and finished products can be differentiated.

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Literature

[1] S. Schwaiger, C. Seger, B. Wiesbauer, P. Schneider, E.P. Ellmerer, S. Sturm., H. Stuppner. *Phytochemical Analysis* 17 (2006) 291–298

[2] Glycerin extraction procedure provided by SEDERMA