Phenylpropanoids HPTLC fingerprint of Edelweiss plants and extracts used in cosmetics

Keywords

Leontopodium spp., composition, flavonoids, caffeoyl esters, leontopodic acid, glycerin formulation, cosmetic

Introduction

Edelweiss (*Leontopodium* spp., *Astereracea*) is a famous plant with white and hairy inflorescent leaves. The genus comprises 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 for their anti-oxidative and radical scavenger properties in cosmetic preparations [1].

Scope

This HPTLC method is suitable for identification of phenolic compounds, flavonoids and caffeoyl esters, of Edelweiss in plants of various species and in ingredients (powdered or glycerin extracts) used in cosmetic products.

Required devices

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

Plants and extracts samples

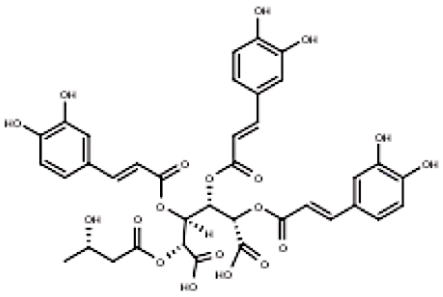
Powdered plant samples were provided by Stephan Schwaiger (Leopold-Franzens-University, Innsbruck, Austria).
Powdered extract was provided by EXTRASYNTHESE (France).
One glycerin sample coming from plant cell culture (PCC) (Majestem®) was provided by SEDERMA (France), and the other glycerin sample was purchased by CAMAG from another supplier.

Samples preparation

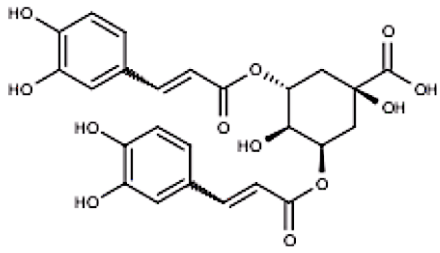
Powdered samples: 500 mg of powdered sample are suspended in 5 mL of methanol and sonicated for 10 min. The suspension is centrifuged for 5 min, and the supernatant is used as test solution.
Powdered dry extracts: 125 mg of powdered sample are suspended in 5 mL of methanol and sonicated for 10 min. The 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 ultrapure water, 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 ultrapure water. The supernatant is added at the top of the cartridge and washed with 10 mL of ultrapure water. The elution is achieved with 4 mL of ethanol. The collected sample solution is fill up to 5 mL with ethanol and used as test solution [2].

Standards

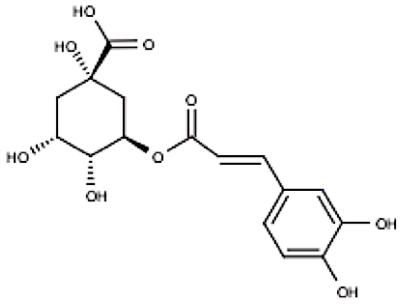
Standard solutions of chlorogenic acid, apigenin, and luteolin are prepared at 1 mg/mL in methanol. Leontopodic acids A and B, cynarin and 3,5-dicaffeoylquinic acid are prepared at 0.75 mg/mL in methanol. Luteolin-4-o-glucoside and luteoline-7-o-glucoside are prepared at 1 mg/mL in methanol.
Standards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



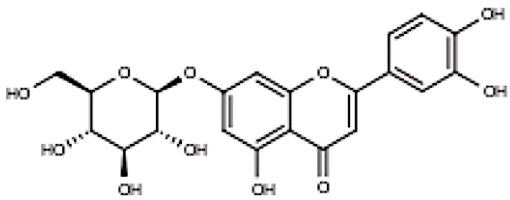
Leontopodic acid A - # 6032S



3,5-Dicaffeoyl quinic acid - # 4946S



Chlorogenic acid - # 4991S



Luteoline 7-glucoside - # 1126S

Chromatographic conditions

Stationary phase	HPTLC Si 60 F_{254}, 20 x 10 cm (Merck)
Sample application	2.0 µL of each *Test solution* and *Standard solution* are applied as 8 mm bands, track distance 11.4 mm, 8 mm from lower edge of plate. First application position is 20 mm from the left edge of the plate
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/**w**)
Plate drying	Drying 5 min in the ADC 2
Documentation	With the TLC Visualizer under white light and under UV 366 nm (after derivatization)
Derivatization	Reagent name: Natural product reagent Preparation: 1 g of diphenylborinic acid aminoethylester is dissolved in 200 mL of ethyl acetate. Use: heat the plate for 3 min at 100°C, and then dip the plate while still hot into the reagent (speed 3, time 0)

Results

A set of standards present in Edelweiss was proposed by EXTRASYNTHESE in order to characterize the extracts used in cosmetic products (Fig. 1).

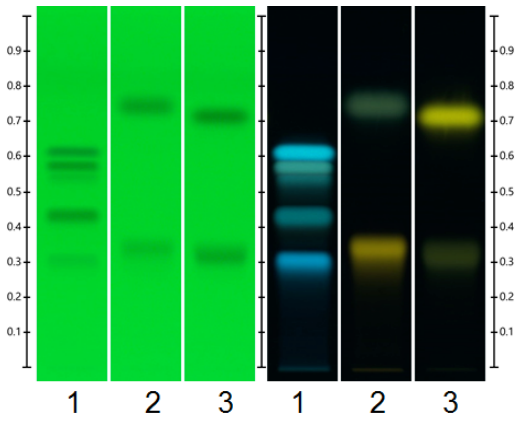


Fig. 1 Left: Plate under UV at 254 nm prior to derivatization, right: Plate under UV 366 nm after derivatization (NP). Track 1: chlorogenic acid, cynarin, leontopodic acid B, leontopodic acid A, 3,5-diccaffeoylquinic acid (with increasing R_F), track 2: luteolin-7-o-glucoside, apigenin (with increasing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

The set of standards was then simultaneously analyzed with dry powder of Edelweiss material in order to check the specificity of those markers for different species of *Leontopodium* (Fig. 2).

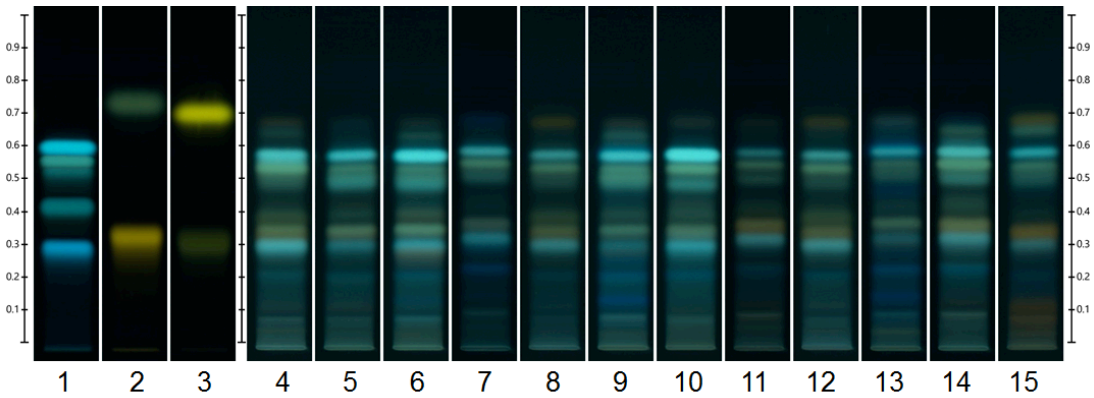


Fig. 2 Plate under UV 366 nm after derivatization (NP). Track 1: chlorogenic acid, cynarin, leontopodic acid B, leontopodic acid A, 3,5-diccaffeoylquinic acid (with increasing R_F), track 2: luteolin-7-o-glucoside, apigenin (with increasing R_F), track 3: luteoline-4-o-glucoside, luteolin (with increasing R_F), tracks 4-15: Edelweiss species, powdered (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)

In order to check the suitability of our method for real cosmetic ingredients two samples formulated in glycerin – PCC sample Majestem® from SEDERMA on track 4-5, and the other supplier sample purchased by CAMAG on track 6-7 - were tested together with one dry extract (Fig. 3).
The intensity of the profile observed for the glycerin sample purchased by CAMAG (track 6-7) inform about the high dilution of the edelweiss extract in glycerin.

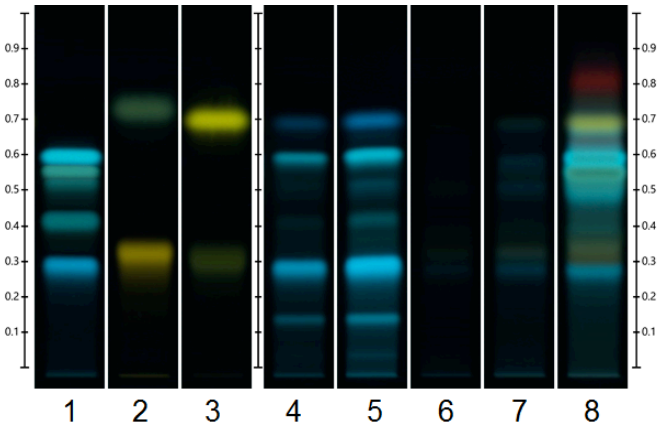


Fig. 3 Plate under UV 366 nm after derivatization (NP). Track 1: chlorogenic acid, cynarin, leontopodic acid B, leontopodic acid A, 3,5-diccaffeoylquinic acid (with increasing R_F), track 2: luteolin-7-o-glucoside, apigenin (with increasing R_F), track 3: luteoline-4-o-glucoside, luteolin (with increasing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

Conclusion

A general method, applicable to raw materials or extracts, has been developed to detect the major phenolic markers (flavonoids and caffeoyl esters) specific to *Leontopodium spp*.
Implementation of the method on commercial ingredient based on Edelweiss allows to assess quality and to differentiate of different grades and sources of products.
The study is a first attempt to compare various *Leontopodium spp*. by HPTLC and specially to outline the caffeoyl esters fraction: 3,5-dicaffeoyl quinic acid, leontopodic acids A and B.

Contacts

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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 extract preparation method provided by SEDERMA

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