



„Screening of active constituents in hemp roots via thinlayer chromatography“

Workshop „Industrial hemp – From cultivation to application“

8th May 2025

Jennifer Dutschke



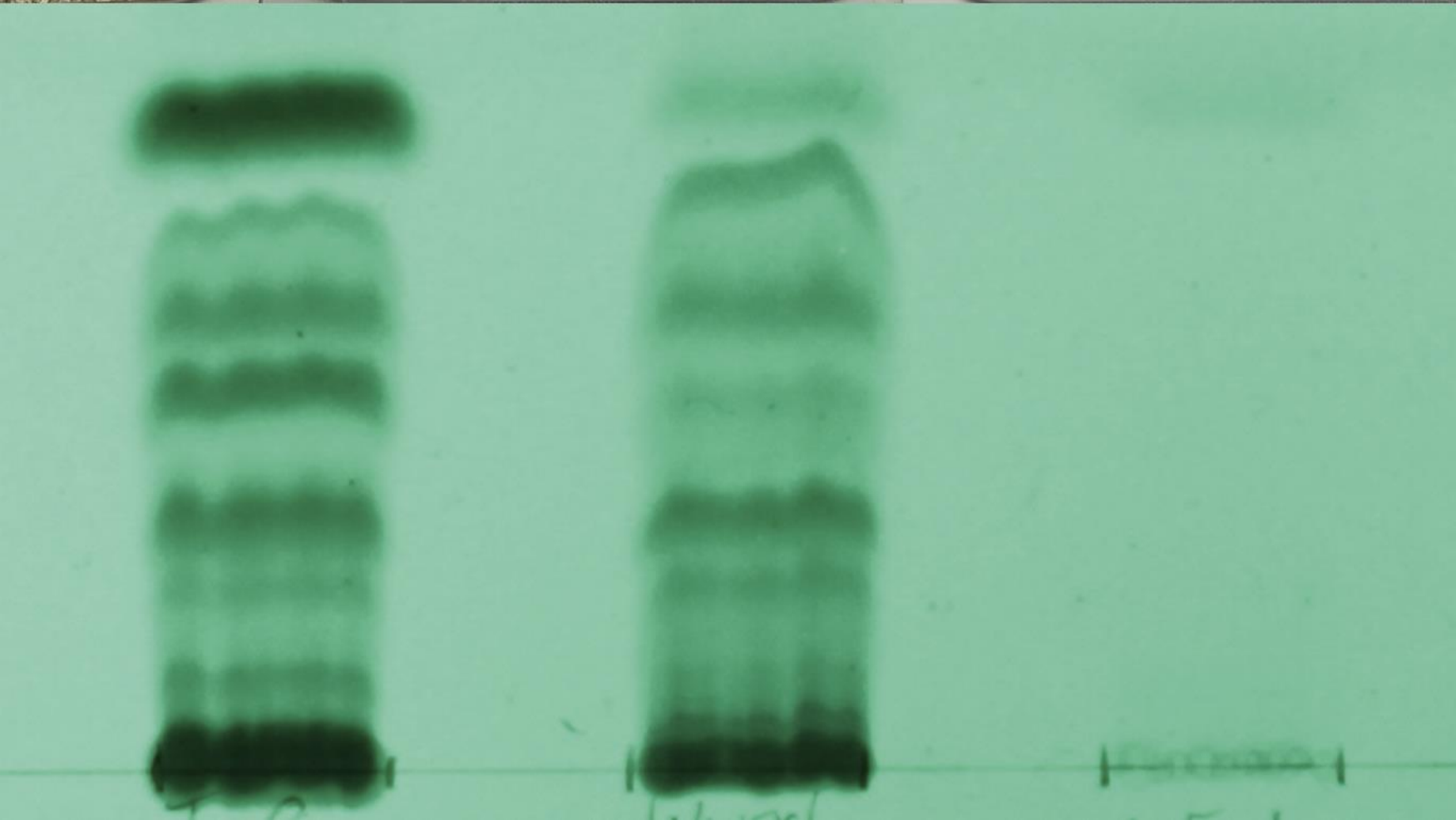
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Diese Maßnahme wird mitfinanziert durch
Steuermittel auf der Grundlage des vom
Sächsischen Landtag beschlossenen Haushaltes.



Table of contents



1. Hemp roots in literature
2. Screening results
3. Summary
4. Future Prospects



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ZIRKON

- Hemp roots known as traditional medicine for many centuries

- Historic use:

- Gout, arthritis, joint pain

- Skin burns

- Difficult child birth, postpartum hemorrhage

- 1970s: Several scientific papers about hemp roots

STEROIDS OF CANNABIS SATIVA ROOT

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(Received 12 July 1974)

Chemical Constituents of *Cannabis sativa* L. Root

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ASAAD N. MASOUD‡, MAYNARD W. QUIMBY, and PAUL L. SCHIFF, Jr.§

Abstract □ An extract of *Cannabis sativa* L. root contained two tetracyclic triterpenes, friedelin and epifriedelanol, a β -phenylethyl)-*p*-hydroxy-*trans*-cinnamamide. Six triterpenes were confirmed by preparation of derivative to authentic friedelin. Epifriedelanol was confirmed by mass spectrometry. The amide was confirmed by synthesis.

Keyphrases □ *Cannabis sativa* L.—isolation and chemical constituents □ Friedelin—isolated and chemical constituents □ Epifriedelanol—isolated and chemical constituents □ *N*-(*p*-Hydroxy- β -phenylethyl)-*p*-hydroxy-*trans*-cinnamamide

CANNABISATIVINE, A NEW ALKALOID FROM CANNABIS SATIVA L. ROOT

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(Received in USA 11 June 1975; received in UK for publication 1 July 1975)

Anhydrocannabisativine, a New Alkaloid from *Cannabis sativa* L.

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JOSEPH E. KNAPP ‡, PAUL L. SCHIFF, Jr. ‡, and DAVID J. SLATKIN ‡

Received February 7, 1977, from the *Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677, and the †Department of Pharmacognosy, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261. Accepted for publication March 30, 1977.

Abstract □ Ethanol extracts of the leaves and roots of a Mexican variant of *Cannabis sativa* L. (marijuana) afforded, after partitioning and chromatography, the new steroidal alkaloid, anhydrocannabisativine.

EXPERIMENTAL¹

Plant Material—Leaves and roots of a Mexican variant of *C. sativa* L. were used².

- Since ~2018 more research about hemp roots again
- Substance classes found:
 - Amino acids
 - Cannabinoids
 - Fatty acids
 - Lignans
 - Sterols
 - Mono-, di- and triterpenes

Original Papers

Thieme

Detection and Quantification of Cannabinoids in Extracts of *Cannabis sativa* Roots Using LC-MS/MS

2018

Authors

Waseem Gul^{1,2}, Shahbaz W. Gul¹, Suman Chandra², Hemant Lata², Elsayed A. Ibrahim^{2,3}, Mahmoud A. ElSohly^{1,2,4}

Secondary Metabolites Profiled in Cannabis Inflorescences, Leaves, Stem Barks, and Roots for Medicinal Purposes

2020

Dan Jin^{1,2}, Kaiping Dai², Zhen Xie² & Jie Chen^{1,3*}



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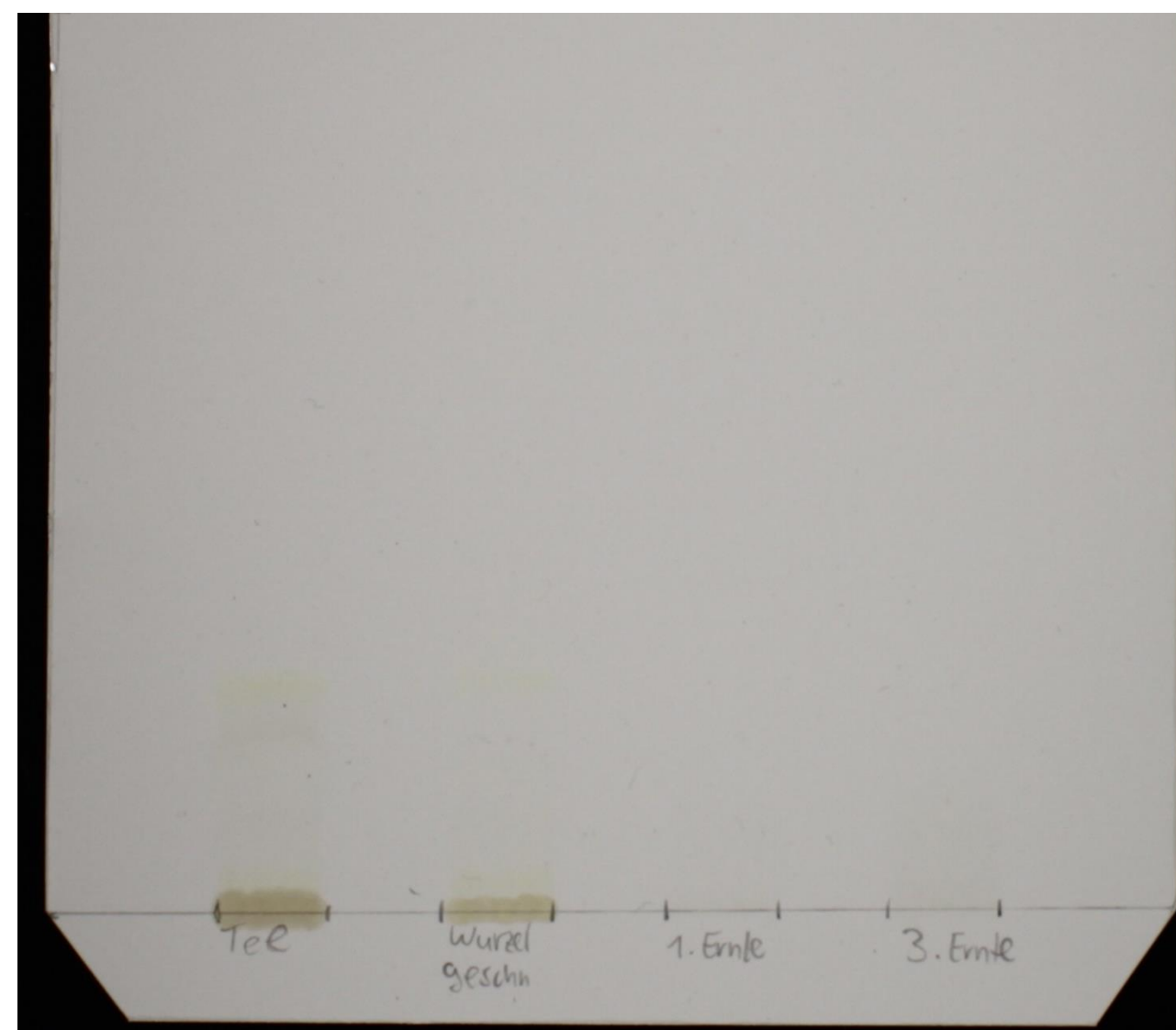
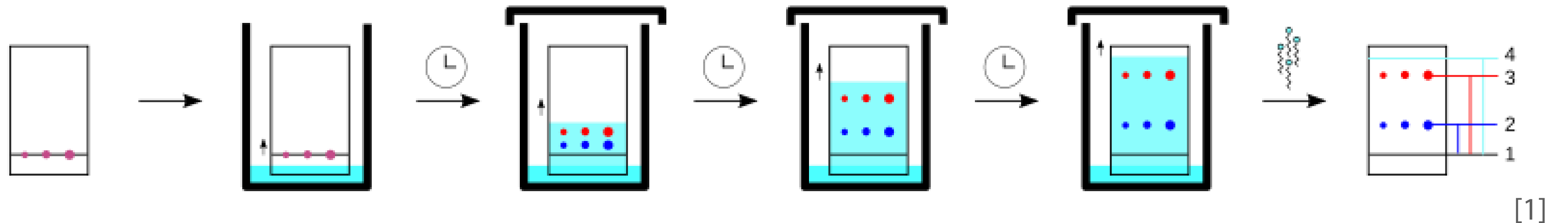


Chemical composition and antioxidant potential of *Cannabis sativa* L. roots

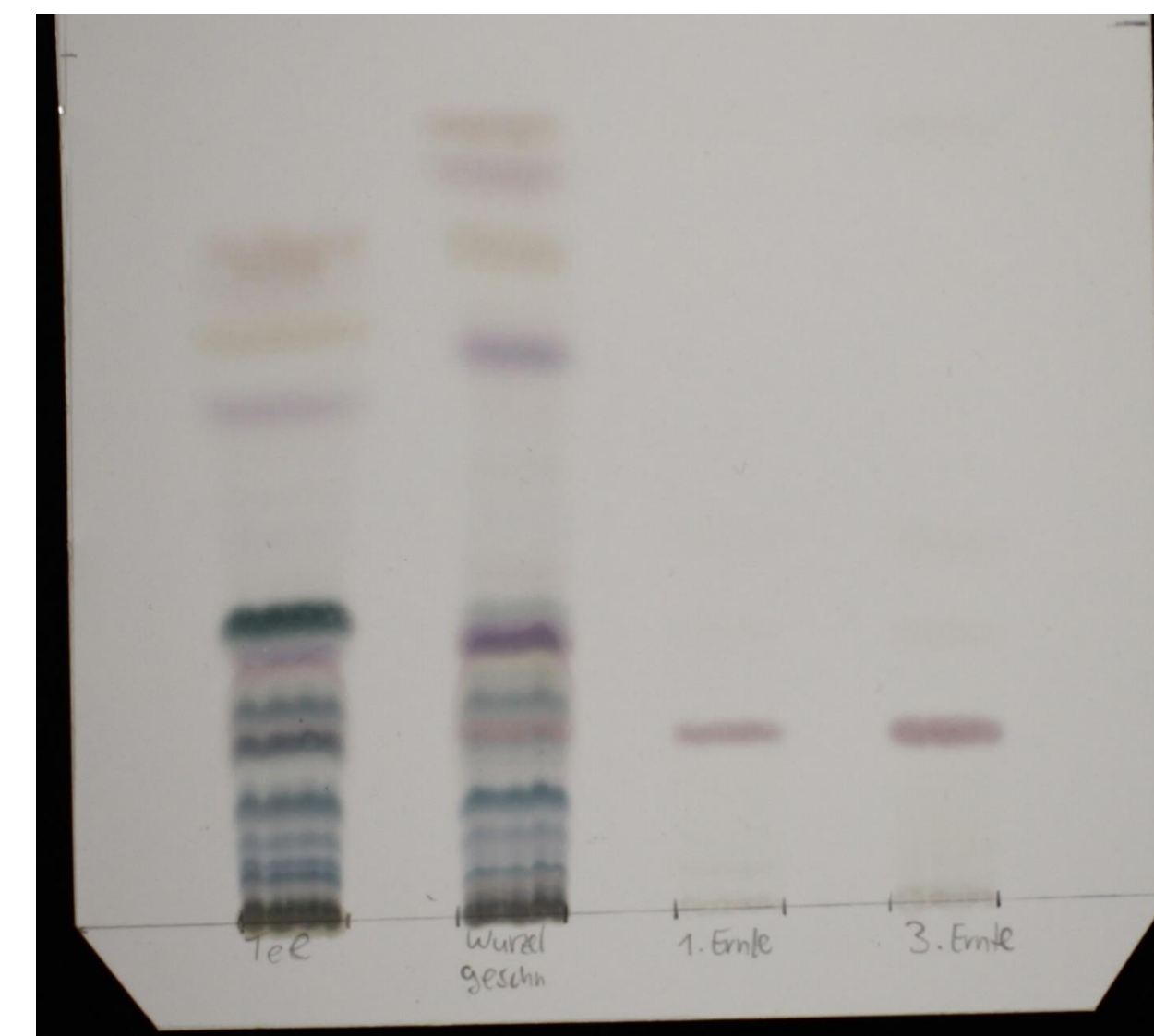
2021

Christoph Kornpointner^a, Aitor Sainz Martinez^b, Silvija Marinovic^a,
Christian Haselmair-Gosch^a, Polona Jamnik^c, Katharina Schröder^b, Christian Löffke^d,
Heidi Halbwirth^{a,*}

- Quick overview about substance groups via thin layer chromatography (TLC)
- TLC principle:



spraying with
anisaldehyde sulfuric
acid and heat to 110°C



Experimental details:

- tested substance groups
 - anthraglykosides
 - coumarins
 - alkaloids
 - phenols
 - flavonoids and phenolcarboxylic acids
 - essential oils
 - terpenes
 - valepotriates
 - saponins
 - bitter principles
- material
 - Shredded hemp roots (Fedora 17) of 2 mm particle size
 - Sample 1 (S1): 50 days old plants
 - Sample 2 (S2): 177 days old plants

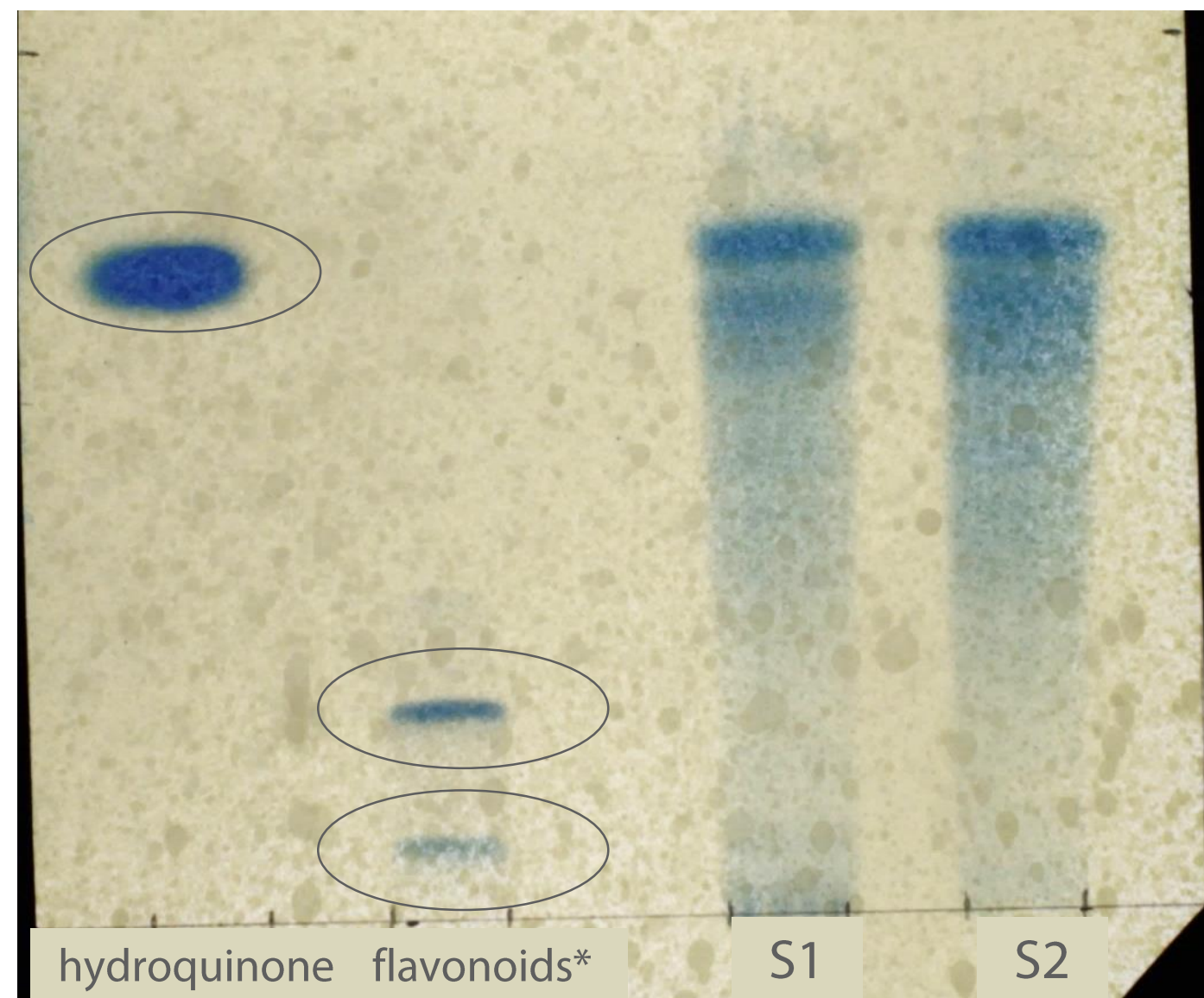


Plants and shredded roots sample 1



Plants and shredded roots sample 2

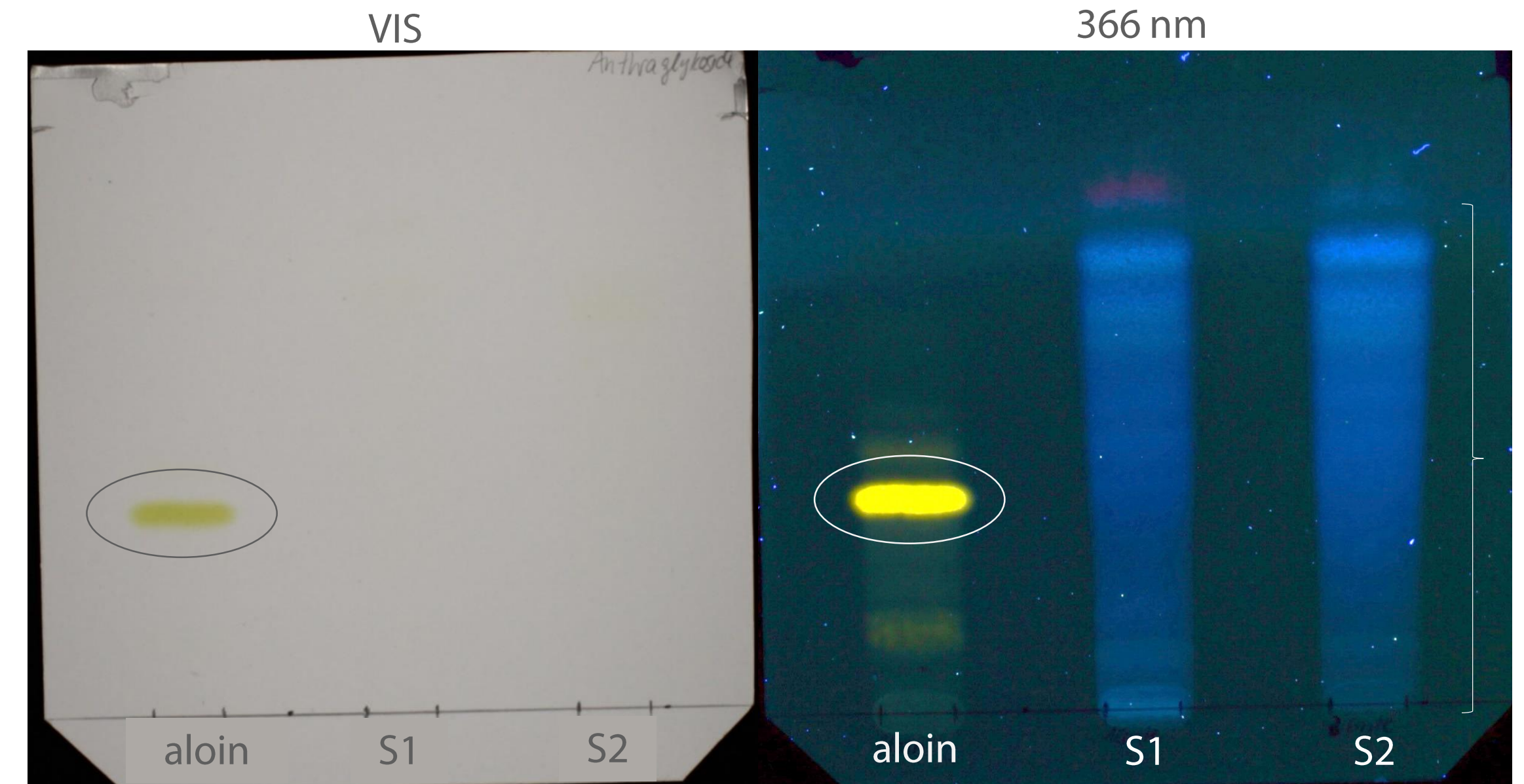
Phenols (MeOH, 60°C; EtOAc/MeOH/H₂O; Berlin Blue)



*flavonoids = rutin, chlorogenic acid, hyperoside

- Phenols show blue zones after detection
- Positive signal for hydroquinone and flavonoids;
1 band is missing at flavonoids
- Both hemp root samples show several blue bands → **many phenols**

Anthraglykosides (MeOH, 60°C; EtOAc/MeOH/H₂O; eth. KOH)

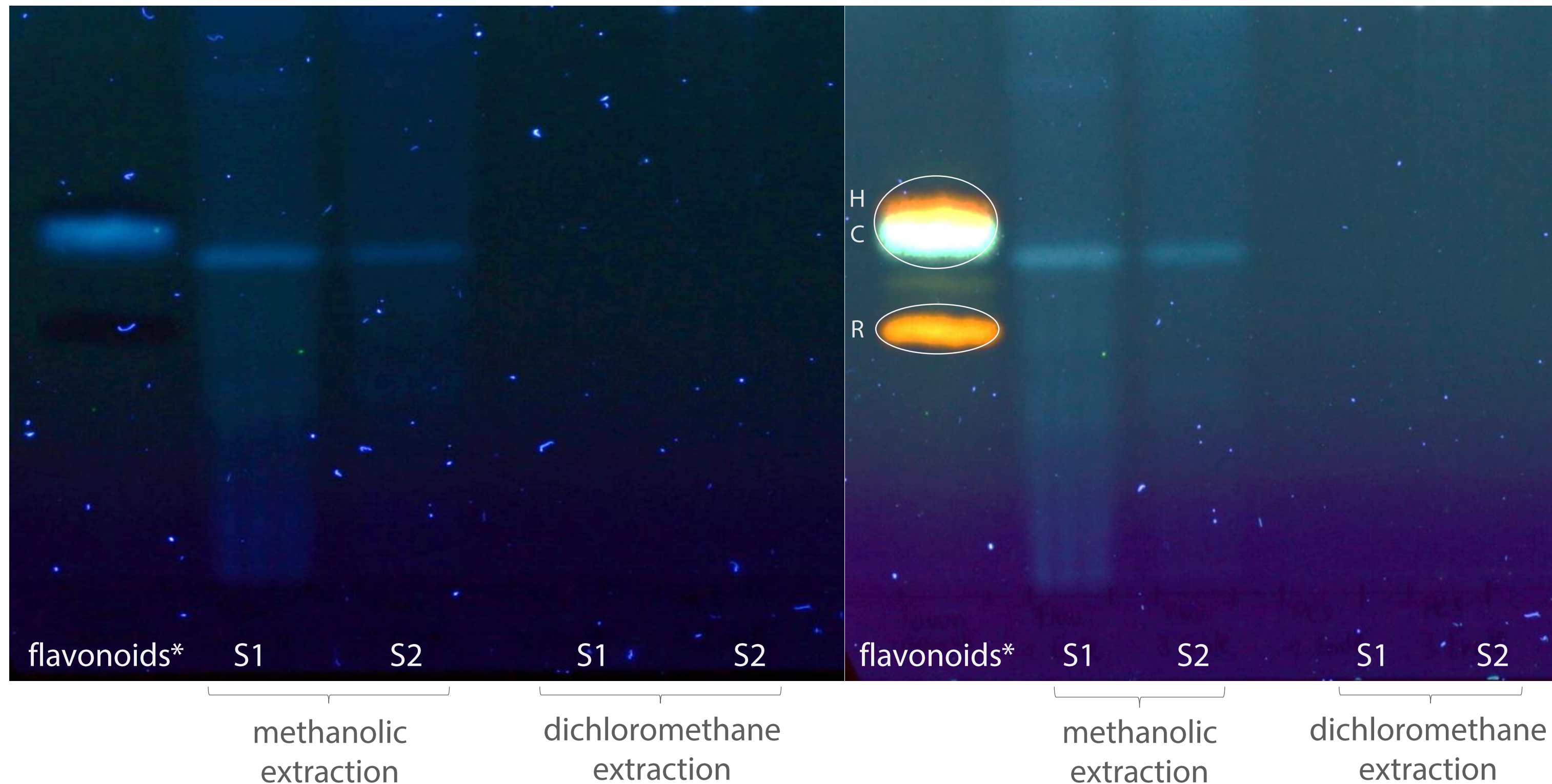


- After detection: anthraquinones red (VIS/366); anthrones, anthranole yellow (VIS/366)
- Positive signal for aloin (anthron)
- **Hemp root samples show no positive signal**
- Instead light blue fluorescence (366 nm) → **Coumarins or phenolcarboxylic acids**
→ **Coumarin TLC showed no positive signal**

Phenolcarboxylic acids (EtOAc/HCOOH/glacial acetic acid/H₂O; Natural substance A/PEG)

Before detection (366 nm)

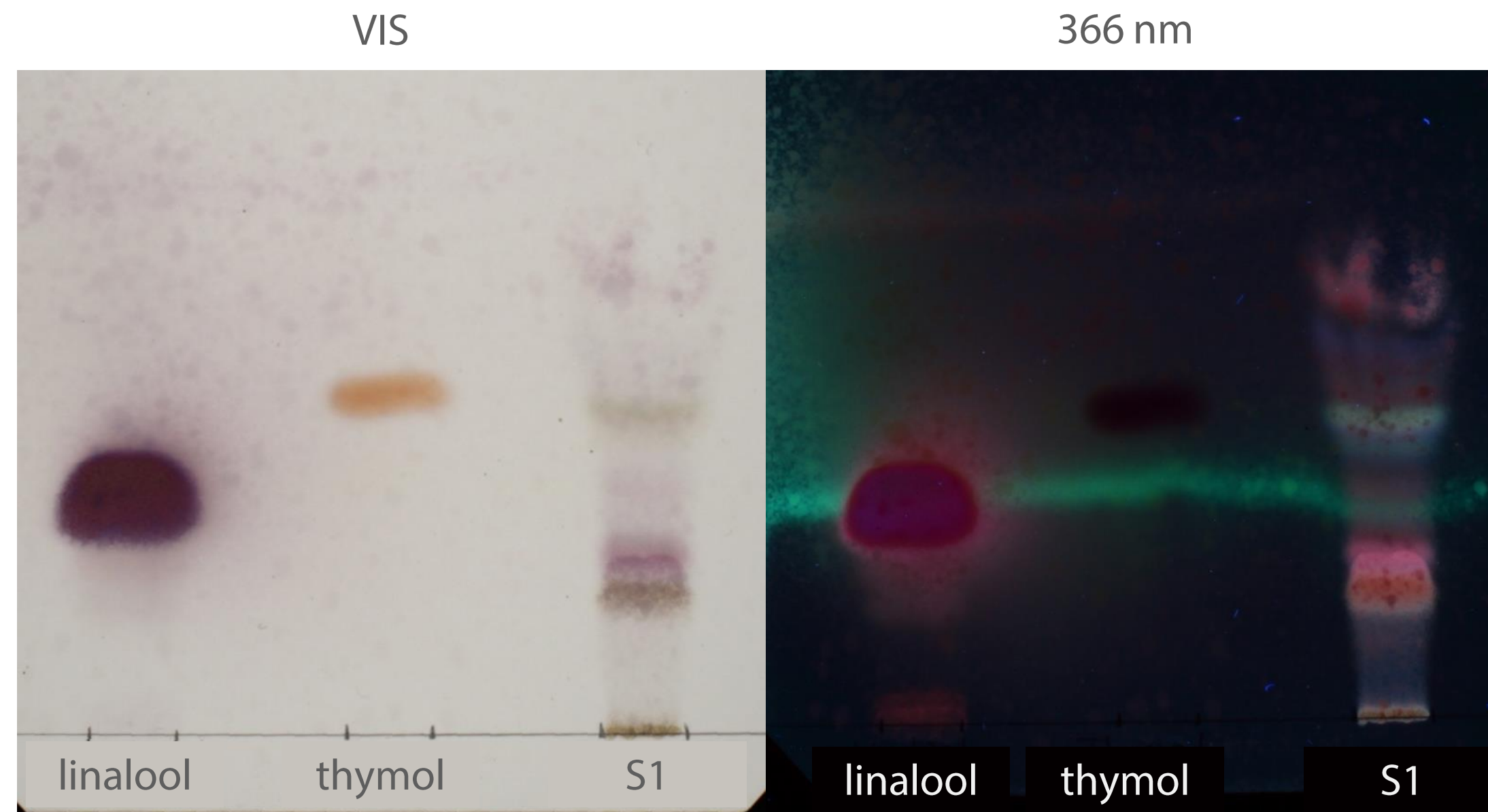
After detection (366 nm)



- After detection: intensive yellow, orange or green fluorescent zones (flavonoids); blue fluorescence (PCA)
- Positive signals for flavonoids (H, R, C)
- Light blue fluorescence only at **methanolic extracts** (both samples) → **phenolcarboxylic acids present**
- No fluorescence at dichloromethane extracts → **no phenolcarboxylic acids present**
- **Maybe only polar PCAs in hemp roots**

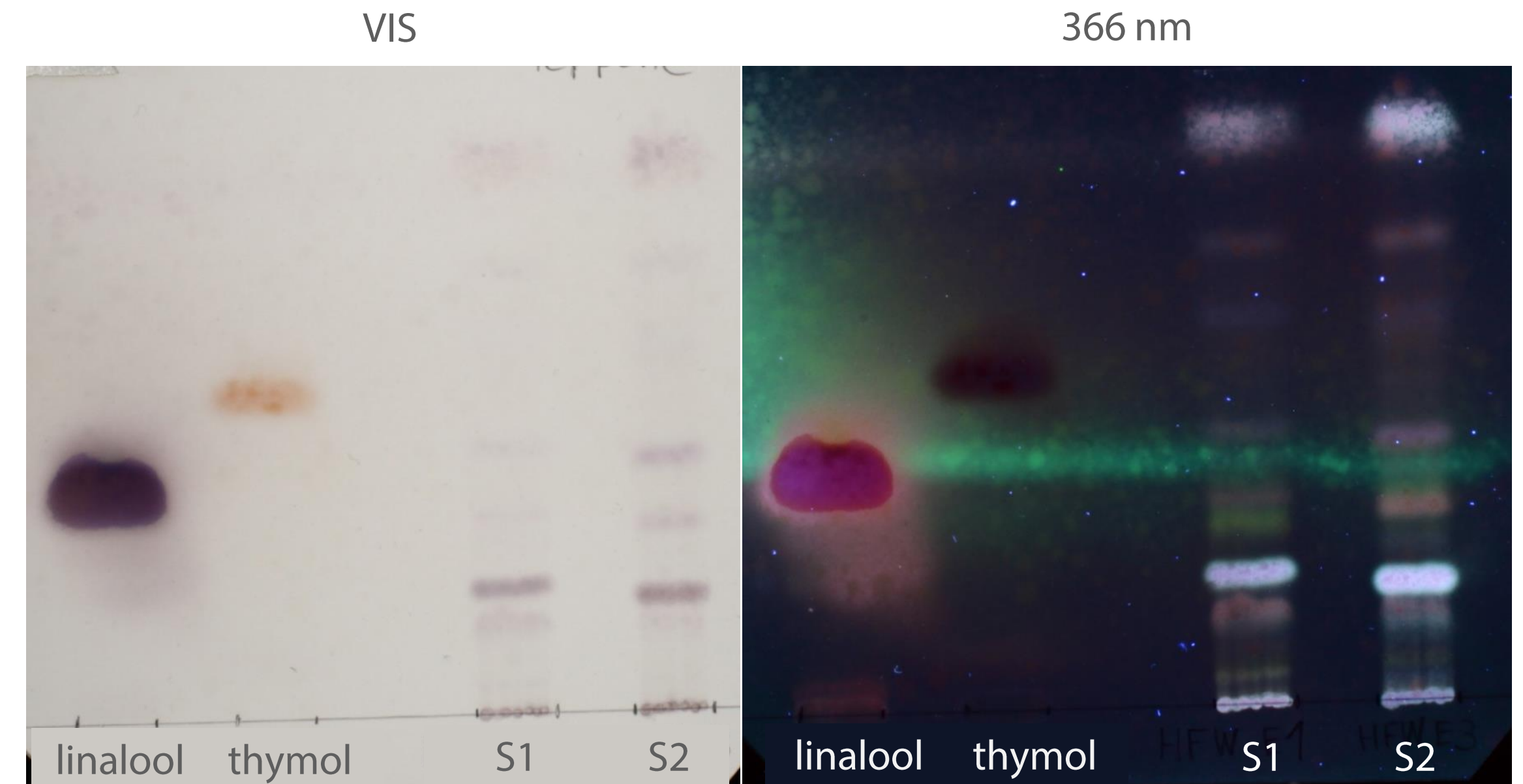
*flavonoids = rutin (R), chlorogenic acid (C), hyperoside (H)

Essential oils (Steam distillation, toluol/EtOAc, anisaldehyd-sulfuric acid)



- After detection: intensive blue, green, red or brown bands; most components show fluorescence
- Positive signal for linalool and thymol
- **Several bands for hemp root sample 1 → essential oil components present**

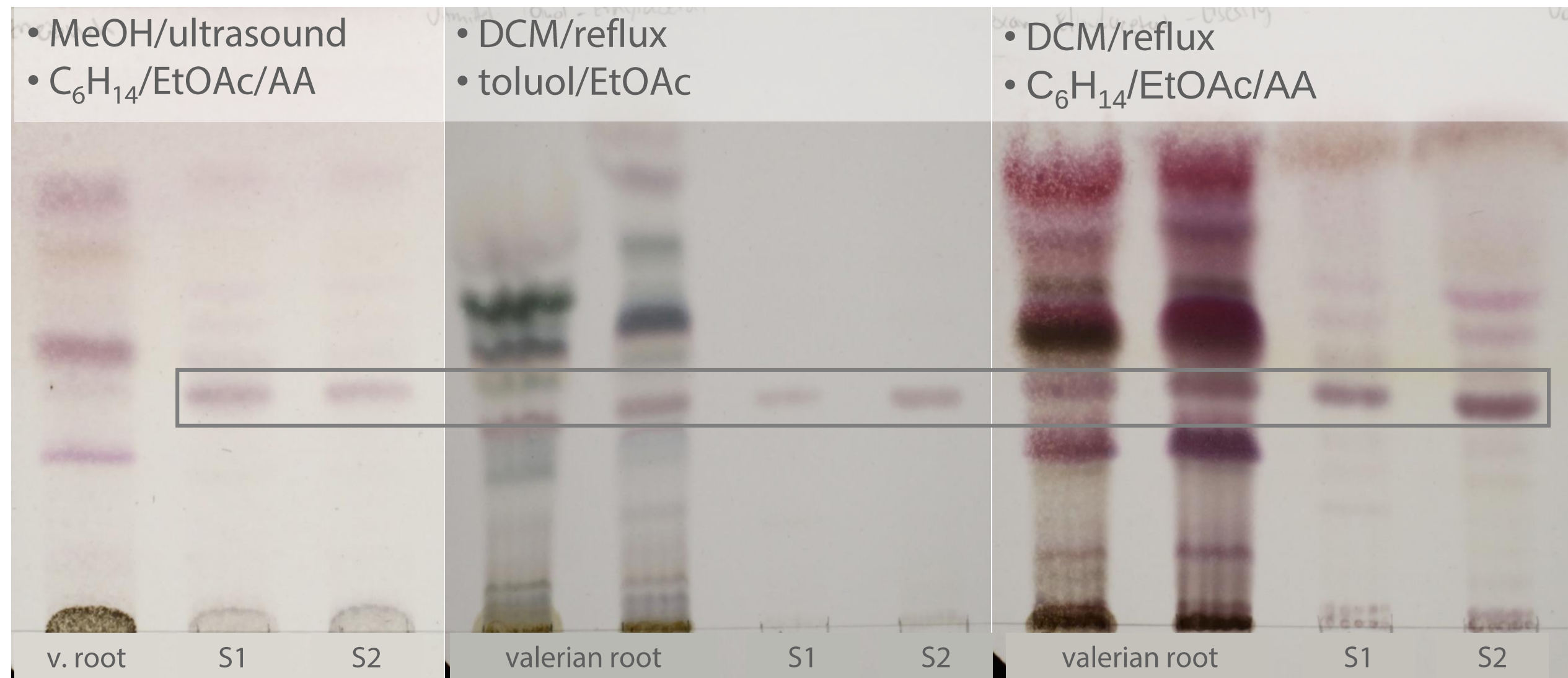
Terpenes (CH_2Cl_2 , reflux, 40°C; toluol/EtOAc; anisaldehyd-sulfuric acid)



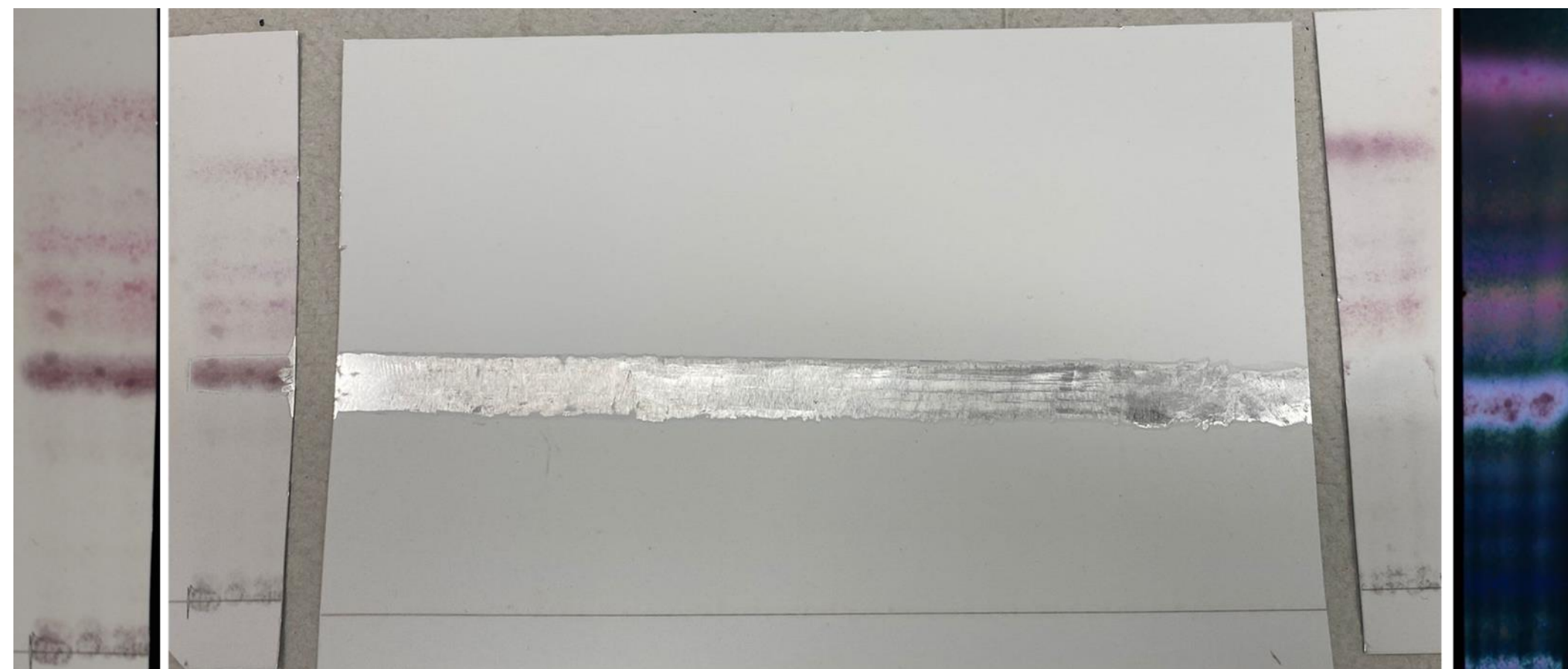
- After detection: blue-violet, red-red/violet bands in VIS (triterpenes)
- Positive signal for linalool and thymol
- **Several red-violet bands for S1 and S2 → terpenes present**

substance (group)	sample 1	sample 2	note
phenols	Yes	Yes	-
anthraglykosides	No	No	Indications of phenolcarboxylic acids
phenolcarboxylic acids	Yes	Yes	Only with methanolic extraction
essential oils	Yes	Pending	-
terpenes	Yes	Yes	-

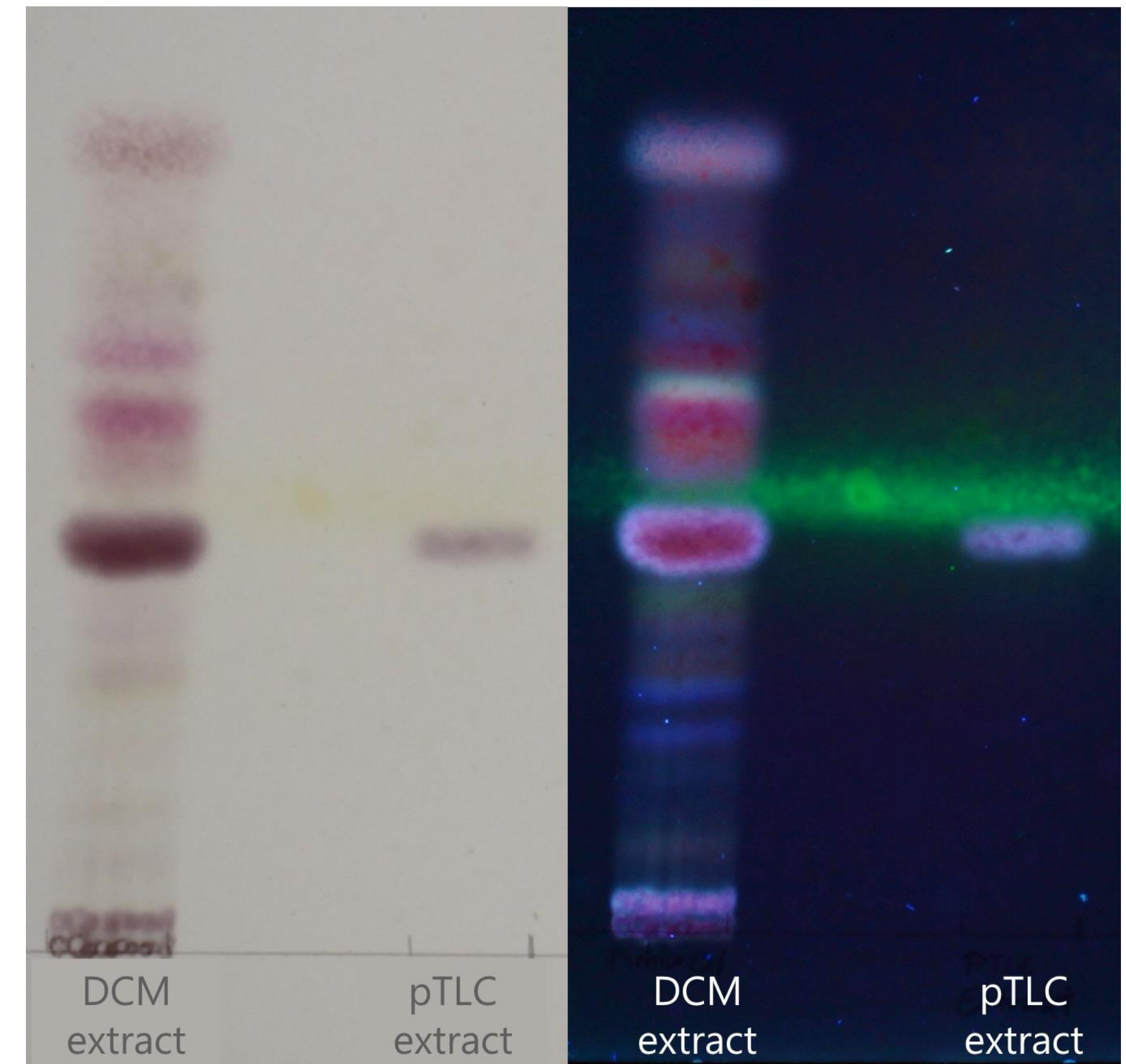
- Negative results for coumarins, alkaloids, flavonoids, valepotriates, saponins and bitter principles



Three tlcs with different extracts, tlc conditions and detection



Preparative thin layer chromatography (ptlc) with hemp root extract



TLC from crude extract and ptlc extract



Thank you for your attention!

If you have questions, feel free to contact us:

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ZIRKON

- [1] von Tlc_sequence.png: Quantockgoblinderivativework: Shakiestone(talk) -Diese Datei wurde von diesem Werk abgeleitet Tlcsequence.png;, Gemeinfrei, <https://commons.wikimedia.org/w/index.php?curid=18232566>
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Table 2

Yields of friedelin and epifriedelinol in the extracts of *C. sativa* (n = 3, mean ± SD).

Sample (Chemovar)	Harvest	Drying	Extraction	Yield _{Friedelin} /(mg/g DW)	Yield _{Epifriedelinol} ^a /(mg/g DW)
A (Futura 75)	07/ 2019	Air	EtOH	0.373 ± 0.012c	0.144 ± 0.009g
B (Futura 75)	08/ 2019	Air	EtOH	0.434 ± 0.038bc	0.205 ± 0.016e
C (Futura 75)	10/ 2019	30 h at 45°C	EtOH	0.100 ± 0.005d	0.059 ± 0.004h
D (Uso 31)	08/ 2019	Air	EtOH	0.422 ± 0.037bc	0.203 ± 0.016e
E (Felina 32)	08/ 2019	Air	EtOH <i>n</i> -hexane scCO ₂	0.709 ± 0.036a 0.698 ± 0.078a 0.548 ± 0.073b	0.188 ± 0.007ef 0.179 ± 0.024efg 0.148 ± 0.016fg

Mean values with different letters (a, b, c, etc.) within the same column are statistically different ($p < 0.05$).

No cannabinoids and flavonoids were found in the ethanolic extracts with HPLC analysis (Data not shown).

Abbreviations: EtOH: ethanol; scCO₂: supercritical carbon dioxide.

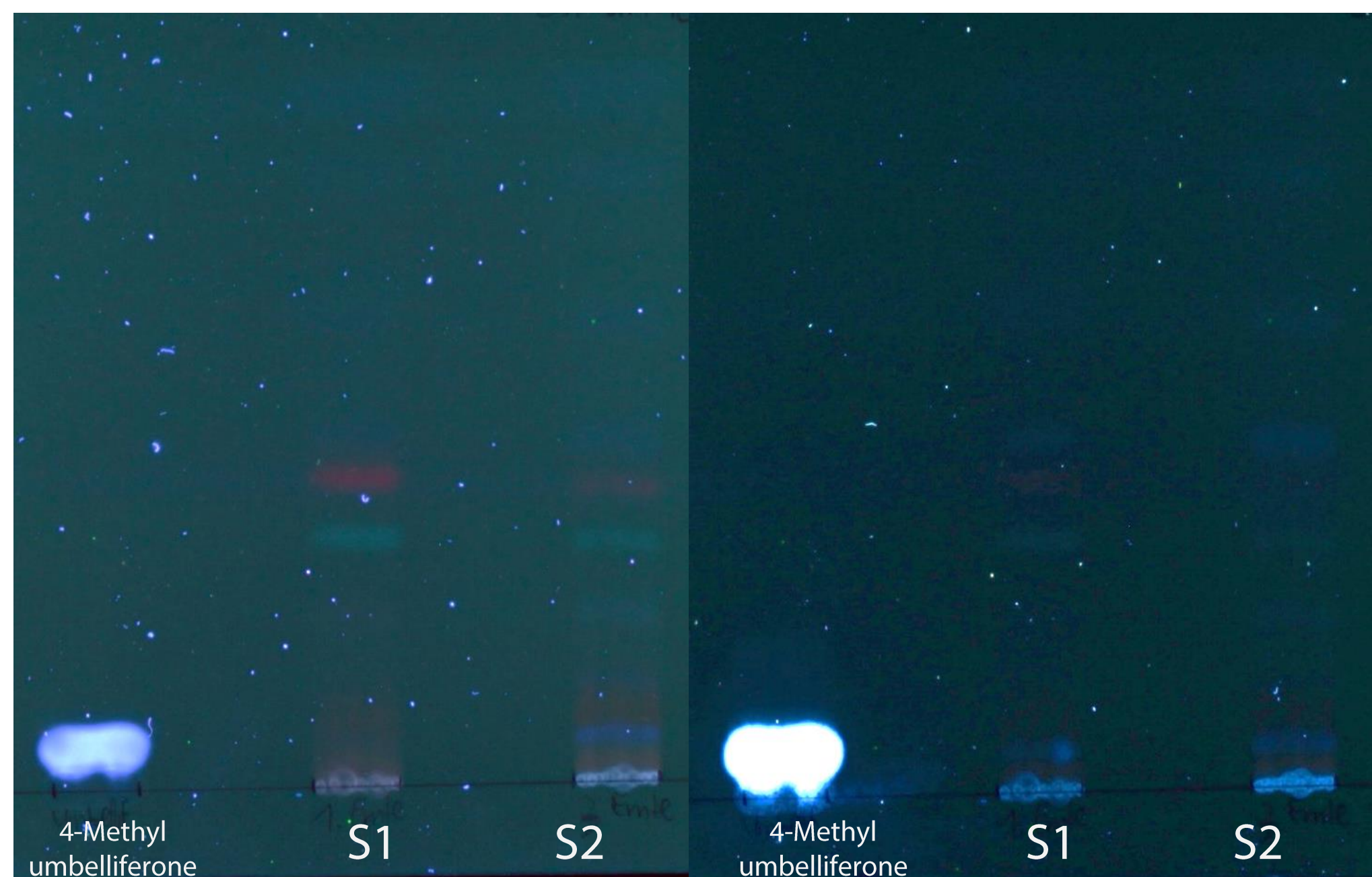
^a Calculated in friedelin equivalents.

From: Kornpointner, Christoph; Sainz Martinez, Aitor; Marinovic, Silvija; Haselmair-Gosch, Christian; Jamnik, Polona; Schröder, Katharina et al. (2021): Chemical composition and antioxidant potential of Cannabis sativa L. roots. In: *Industrial Crops and Products* 165. DOI: 10.1016/j.indcrop.2021.113422.

Coumarins (CH₂Cl₂, reflux, 40°C; toluol/EtOAc; eth. KOH)

Before detection

After detection

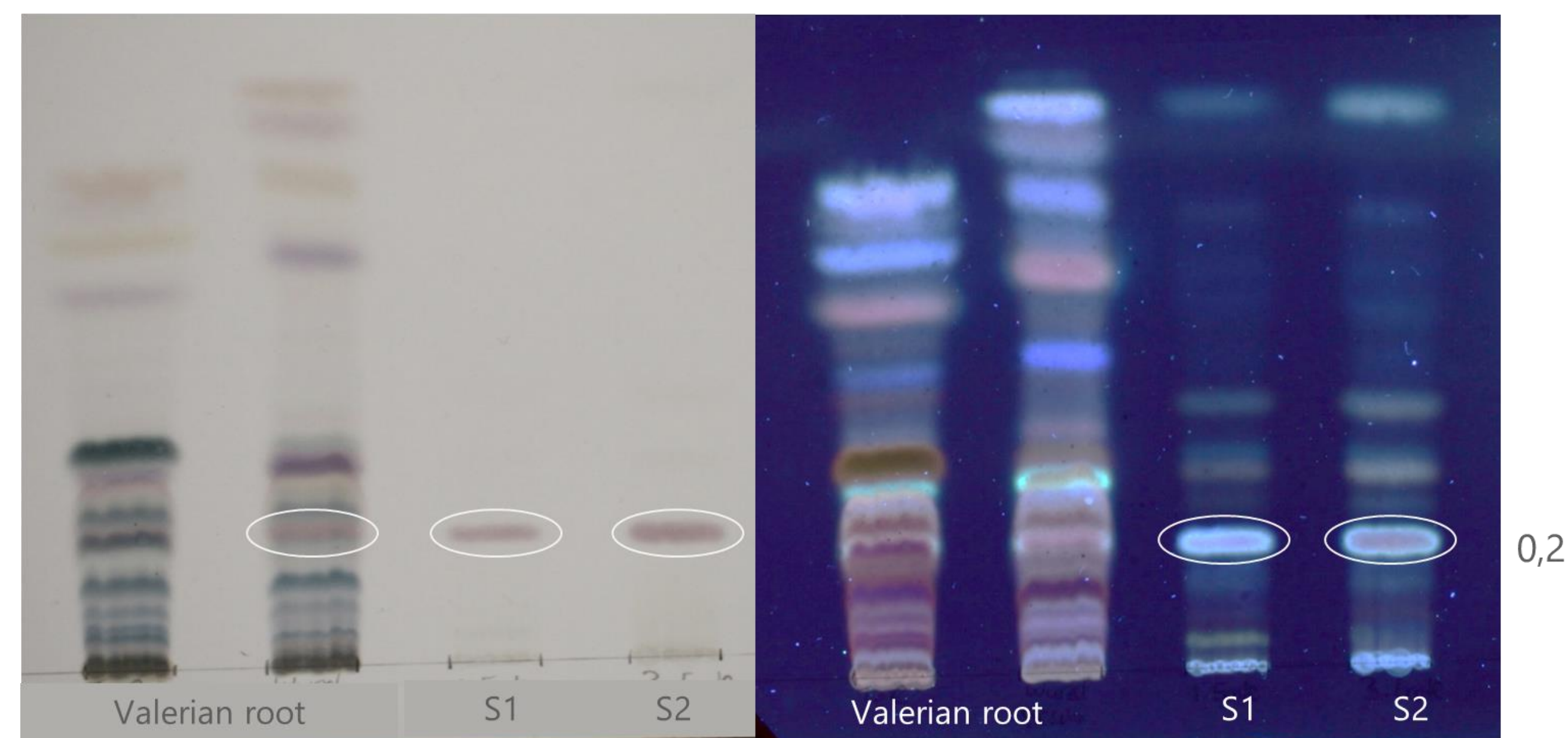


- After detection: more intense fluorescence (366 nm)
- Positive signal for 4-Methylumbelliferon
- **No positive signal for hemp root samples**

Valepotriates (CH₂Cl₂, reflux, 40°C; toluol/EtOAc; hydrochloric acid acetate)

VIS

366 nm



- After detection: blue bands (valtrate, IVHD valtrate, acevaltrate or brown bands (didrovaltrate)
- Positive signal for valerian root samples
- **No positive signal for valepotriates in S1 or S2**
- **Instead one violet band (R_f = 0,21)**, also present in valerian root samples
- Same band as in terpene tlc (same extraction, same tlc conditions, different detection)