

Eigenständigkeitserklärung

Die Eigenständigkeitserklärung wurde aus der elektronischen Version dieser Dissertation entfernt.

Lebenslauf und Publikationsverzeichnis

Der Lebenslauf wurde aus der elektronischen Version dieser Dissertation entfernt.

Publikationen*Peer reviewed Publikationen*

- 1 S. Gerschler, S. Guenther, and C. Schulze, ‘Antibiofilm Activity of Sundew Species against Multidrug-Resistant Escherichia coli Strains’, *IJMS*, vol. 23, no. 22, p. 13720, Nov. 2022, doi: 10.3390/ijms232213720.
- 2 S. Gerschler, N. Neumann, N. Schultze, S. Guenther, and C. Schulze, ‘Quality parameters for the medicinal plant *Drosera rotundifolia* L.: A new approach with established techniques’, *Archiv der Pharmazie*, vol. 357, no. 1, p. 2300436, Jan. 2024, doi: 10.1002/ardp.202300436.
- 3 J. Rockstroh, S. Gerschler, N. Schultze, C. Schulze, M. Wurster, K. Methling, S. Guenther, M. Lalk, ‘A Targeted Approach for the Metabolome Analysis of *E. coli* Biofilms’, *Helvetica Chimica Acta*, vol. 107, no. 6, p. e202300240, Jun. 2024, doi: 10.1002/hlca.202300240.
- 4 S. Gerschler, S. Maaß, P. Gerth, L. Schulig, T. Wildgrube, J. Rockstroh, M. Wurster, K. Methling, D. Becher, M. Lalk, C. Schulze, S. Guenther, N. Schultze, ‘*Drosera rotundifolia* L. as *E. coli* biofilm inhibitor: Insights into the mechanism of action using proteomics/metabolomics and toxicity studies’, *Biofilm*, vol. 9, p. 100268, Jun. 2025, doi: 10.1016/j.bioflm.2025.100268.

Non peer reviewed Publikationen

- 1 S. Gerschler, ‘Sonnentau – Medizin aus dem Moor’, *Zeitschrift für Komplementärmedizin*, vol. 14, no. 03, pp. 60–63, Mar. 2022, doi: 10.1055/a-1817-6641.

Danksagung

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Sundew herb
Droserae herba

DEFINITION

The whole or fragmented, dried aerial and underground parts of *Drosera rotundifolia* L.

Content: minimum 4 per cent of total flavonoids, expressed as hyperoside (C₂₁H₂₀O₁₂; M_r464.4) (dried drug);
minimum 2.0 per cent of flavonoid glycosides, expressed as hyperoside (C₂₁H₂₀O₁₂; M_r464.4) (dried drug);
minimum 0.2 per cent and maximum 1 per cent of naphthoquinones, expressed as plumbagin (C₁₁H₈O₃; M_r188.2) (dried drug)

CHARACTERS

Bitter taste.

IDENTIFICATION

- A. Weakly developed, only a few centimeters long, fibrous roots. From a squat rosette, usually upright-standing, 1- 7 cm long leaves arise. The genus-typical trap leaves show a near-round shape, with a diameter of 0.5 to 1.8 cm. These are each covered with about 200, up to 4 mm long, protruding, red, glandular, hairy tentacles, which are clearly longer at the edge than in the middle of the leaf. The surface of the leaf bottom shows no covering hair. The petiole inserts laterally into the lamina. At the ground of the leaf petioles, 4-6 mm long, flat, fringy stipules can be found. Up to 30 cm high, unilateral wraps with up to 25 white, 1 cm large, closed or open flowers, with 5 - 6 mm large petals may be present. The petal surface shows no covering hair. 4 mm large sepals may be present. The stylus is divided in two. Ovoid fruit capsules with numerous, 1.5 mm long, spindle-shaped, brown-black seeds, may be present.
- B. Microscopic examination. Place 2-3 drops of the solution containing chloral hydrate on a glass slide. Add a tiny amount of the powdered plant to the liquid and place a cover glass over the preparation. Keep the preparation on low flame for a short time and then examine under the microscope. The powder has the following diagnostic features (Figure 1): Partially red colored, but also colorless tentacles can be seen, where the dye has dissolved out [B]. They consist of a stalk and an oval head. The head has radially elongated epidermal cells with weakly thickened outer walls, and below them a layer of isodiametric, and a layer of tangentially elongated parenchyma cells [A]. Internally, there are several elongate, broad tracheids with spirally thickened walls. Glandular hairs usually with two, rarely with three or four terminal secretory cells are present on the outer walls [A, E, F, I]. The drug contains two types of glandular hairs: Type 1 without stem cells [Aa and Ia lateral view; Ib top view] and Type 2 with two stem cells in two rows [Ea and Fb lateral view; Fa top view]. Both types are found on the undersides of calyxes and leaves and Type 1 is additionally present on the tentacle stalks [Ia, Ib]. Fragments of leaf blades with epidermal cells having curved, thickened outer walls can be observed [C]. The epidermal cells have elongated, straight lateral walls on one side and the other side of the leaf underside. The mesophyll usually consists of 4 to 5 layers of spongy parenchymal cells with irregularly waved lateral walls. There are stomata present, surrounded by 6 to 7 subsidiary cells [D, G]. Sclerenchyma fibers are attached to vessels with ring-shaped and spiral thickenings. Additionally, fragments of the vascular tissue of leaf veins were visible, sometimes along with cells of the medullary rays with thickened, dotted walls. The conducting tissues of the stems show ring and spiral thickenings and are often interspersed with sclerenchyma fibers. Spiny pollen grains are also visible.

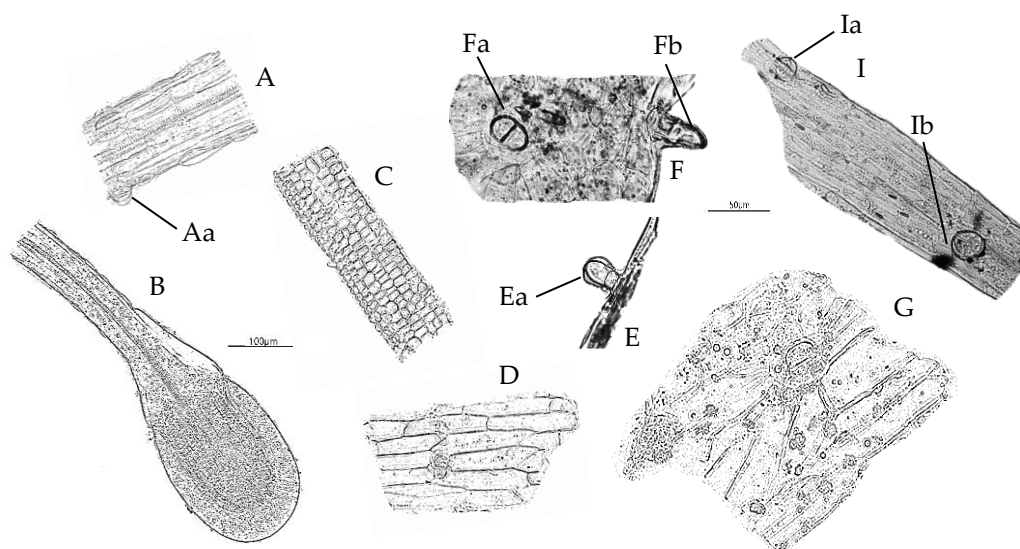


Figure 1. – Illustration for identification test B of powdered herbal drug of sundew herb with root.

C. Thin-layer chromatography.

Test solution. To 0.5 g of the powdered herbal drug (500) add 5 mL of ethanol. Sonicate for 15 min in a water-bath at 50 °C, then centrifuge for 5 min, fill the supernatant into a flask and repeat the processes twice with the residue, combine the supernatants into the same flask and dilute to 50 mL with ethanol.

Reference solution. Dissolve 5.0 mg of quercetin, 5.0 mg of hyperoside and 5.0 mg of isoquercitrine in ethanol and dilute to 50 mL with the same solvent.

Plate: TLC silica gel plate (5-40 µm) [or TLC silica gel plate (2-10 µm)].

Mobile phase. anhydrous formic acid, water, ethyl methyl ketone, ethyl acetate and tert-butyl methyl ether (10:5:10:70:10 v/v/v/v)

Application. 10 µL [or 3 µL] as bands of 8 mm [or 4 mm].

Development. 15 cm [or 8 cm] from the lower edge of the plate.

Drying. in air at room temperature for 5 min.

Detection. Spray the plate with a 10 g/L solution of diphenylboric acid aminoethyl ester in methanol, heat at 100-105 °C for 5 min and then spray with a 50 g/L solution of macrogol 400 in methanol. Allow the plate to dry in air for about 1 min and examine in ultraviolet light at 366 nm.

System suitability. reference solution:

the chromatogram shows in the middle third 2 distinct zones which may not be touching. The lower zone (hyperoside) and the upper zone (isoquercitrine) shows a yellow or orange fluorescence.

Results. see below the sequence of zones present in the chromatograms obtained with reference solution and the test solution. Furthermore, other faint yellow, orange or blue fluorescent zones may be present in the chromatogram obtained with the test solution.

Top of the plate	
Quercetin: a yellow or orange fluorescent zone _____ Isoquercitrine: a yellow or orange fluorescent zone Hyperoside: a yellow or orange fluorescent zone _____	a red fluorescent zone (Chlorophyll) a yellow or orange fluorescent zone (Quercetin) _____ an intense yellow or orange fluorescent zone (2''-O-galloylhyperoside) a yellow or orange fluorescent zone (Hyperoside) _____
Reference solution	Test solution

TESTS

Foreign matter. Take 5 grams of the dried plant (50 °C, laboratory oven, 24 h) and disperse it evenly in a thin layer. Inspect the sample for any foreign particles either with the naked eye or using a 6x magnifying lens. Separate the foreign matter, weigh it, and determine the percentage of foreign matter present. Maximum 10 per cent of fragments of moss and maximum 3 per cent of other foreign matter are allowed.

Loss on drying. Heat a silicate crucible to 100-105 °C for 2 hours and then cool it in a desiccator for 30 minutes and weigh the crucible. Then fill 1.00 g of the powdered and sieved (pore size 500 µm) plant into the crucible and dry the crucible for 2 hours at 100-105 °C in a laboratory oven. Then weigh the crucible and determine the percentage of loss of drying. A maximum of 10.0 percent is permitted.

Total ash. Heat a silicate crucible to redness for 30 minutes and then cool in a desiccator for 30 minutes. Then weigh 1.00 g of powdered and sieved (pore size 500 μm) plant, add to the crucible and heat it first at 100 to 105 $^{\circ}\text{C}$ for 2 h in a laboratory furnace and then at 600 $^{\circ}\text{C} \pm 25$ $^{\circ}\text{C}$ in a muffle furnace for two hours. After that, allow the crucible to cool in a desiccator for 30 min and weigh the crucible. Repeat the heat process until constant mass. A maximum of 5.0 percent is permitted.

Ash insoluble in hydrochloric acid. To the ash obtained by the Test "Total ash", add 15 mL deionized water (DI) and 10 mL hydrochloric acid and cover with a watch glass. Then boil the mixture for 10 minutes and cool at room temperature. Filter the residue through an ashless filter and wash the filter with hot water until the filtrate is neutral. Then dry the residue including the filter for 1 h at 100 to 105 $^{\circ}\text{C}$ in a laboratory oven and then heat the residue with the filter to 600 $^{\circ}\text{C} \pm 25$ $^{\circ}\text{C}$. Cool the obtained ash in a desiccator for 30 min. Then weigh the ash. Reheat the ash to 600 $^{\circ}\text{C} \pm 25$ $^{\circ}\text{C}$ until the difference between two successive weightings does not exceed 1 mg. Determine the percentage of the residue. A maximum of 2.5 percent is permitted.

***Drosera intermedia* HAYNE.**

Thin-layer chromatography.

Test solution. To 0.5 g of the powdered herbal drug (500) add 10 mL of n-hexane. Sonicate for 15 min in a water-bath at 50 $^{\circ}\text{C}$, then centrifuge for 5 min, fill the supernatant into a evaporating dish and repeat the processes twice with the residue, combine the supernatants into the same evaporating dish, evaporate the solvent and dissolve the residue with 1 mL n-hexane.

Reference solution. Dissolve 5 mg of plumbagin in methanol and dilute to 5.0 mL with the same solvent.

Plate: TLC silica gel plate (5-40 μm) [or TLC silica gel plate (2-10 μm)].

Mobile phase. tert-butyl methyl ether and n-hexane (10:90 v/v)

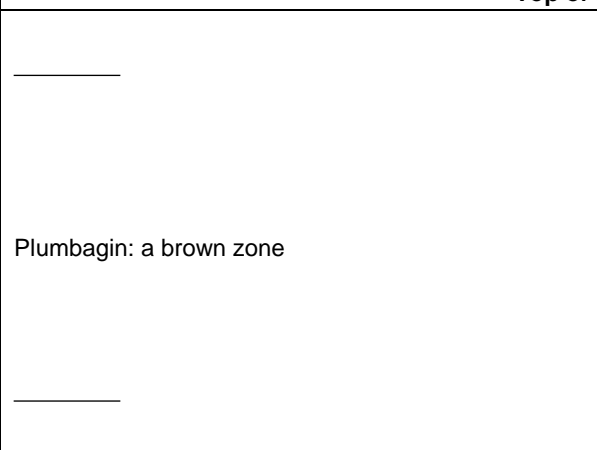
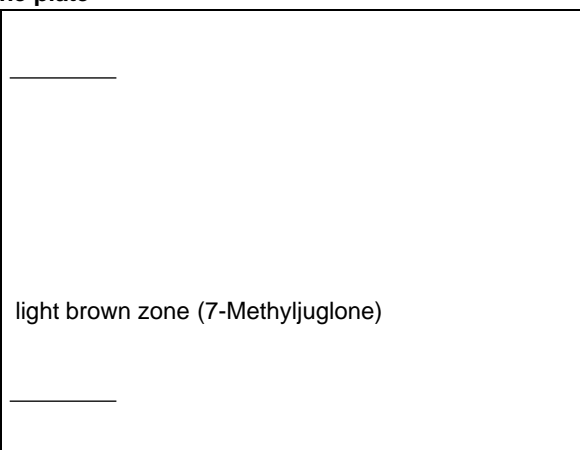
Application. 20 μL [or 6 μL] of the test solution and 3 μL [or 2 μL] of the reference solution as bands of 8 mm [4 mm].

Development. twice over 9 cm [or 5 cm] from the lower edge of the plate

Drying. in a current of air at room temperature for 5 min.

Detection. Spray the dried plate with a 100 g/L solution of potassium hydroxide in methanol. Allow the plate to dry in air for about 1 min and examine in daylight.

Results. see below the sequence of zones present in the chromatograms obtained with reference solution and the test solution. The chromatogram with the test solution does not show a brown zone corresponding to the zone of plumbagin in the chromatogram obtained with the reference solution. Furthermore, in the chromatogram obtained with the test solution, a brown zone is present below the zone due to plumbagin.

Top of the plate	
 <p>Plumbagin: a brown zone</p>	 <p>light brown zone (7-Methyljuglone)</p>
Reference solution	Test solution

ASSAY

Total flavonoid content

Stock solution. Into a 200 mL flask introduce 0.200 g of the powdered herbal drug (500) and 40 mL of ethanol (60 per cent v/v). Heat in a water-bath at 60 $^{\circ}\text{C}$ for 10 min, shaking frequently. Allow to cool and filter through a plug of

absorbent cotton into a 100 mL volumetric flask. Transfer the absorbent cotton with the residue back to the 200 mL flask, add 40 mL of ethanol (60 per cent v/v) and heat again in a water-bath at 60 °C for 10 min, shaking frequently. Allow to cool and filter into the same 100 mL volumetric flask. Rinse the 200 mL flask with a further quantity of ethanol (60 per cent v/v), filter and transfer to the same 100 mL volumetric flask. Dilute to 100.0 mL with ethanol (60 per cent v/v) and filter.

Test solution. Introduce 5.0 mL of the stock solution into a round-bottom flask and evaporate to dryness under reduced pressure. Take up the residue with 8 mL of a mixture of 10 volumes of methanol and 100 volumes of anhydrous acetic acid and transfer to a 25 mL volumetric flask. Rinse the round-bottom flask with 3 mL of a mixture of 10 volumes of methanol and 100 volumes of anhydrous acetic acid and transfer to the same 25 mL volumetric flask. Add 10.0 mL of a solution containing 25.0 g/L of boric acid and 20.0 g/L of oxalic acid in anhydrous formic acid and dilute to 25.0 mL with anhydrous acetic acid.

Compensation liquid. Introduce 5.0 mL of the stock solution into a round-bottom flask and evaporate to dryness under reduced pressure. Take up the residue with 8 mL of a mixture of 10 volumes of methanol and 100 volumes of anhydrous acetic acid and transfer to a 25 mL volumetric flask. Rinse the round-bottom flask with 3 mL of a mixture of 10 volumes of methanol and 100 volumes of anhydrous acetic acid and transfer to the same 25 mL volumetric flask. Add 10.0 mL of anhydrous formic acid and dilute to 25.0 mL with anhydrous acetic acid.

After 30 min, measure the absorbance of the solution at 410 nm, by comparison with the compensation liquid.

Calculate the percentage content of flavonoids, expressed as hyperoside, using the following expression, i.e. taking the specific absorbance of hyperoside to be 500:

$$\frac{A \times 1.235}{m}$$

A = absorbance at 410 nm;

m = mass of the herbal drug to be examined, in grams.

Total naphthoquinon content

Stock solution. Place 0.3000 g of the powdered herbal drug in the cartridge of a continuous-extraction apparatus (Soxhlet type). Moisten the powder with 1.0 mL of a tartaric acid solution (0.33 g tartaric acid in 1 mL water) and plug the cartridge with absorbent cotton. Add 70 mL of n-hexane to a 100 mL round bottom flask. Place the Soxhlet apparatus on the flask and heat under reflux for 1.5 h. Allow to cool. Transfer the liquid into a 100.0 mL volumetric flask. Rinse the round bottom flask twice with 10 mL n-hexane and transfer the rinsing liquid into the volumetric flask. Dilute the liquid to 100.0 mL with n-hexane.

Test solution. Evaporate 10.0 mL of the stock solution carefully at room temperature to dryness. Take up the residue with 10.0 mL magnesium acetate solution (5 g magnesium acetate in 1000 mL methanol). Measure the absorbance of the solution immediately, by comparison with the compensation liquid at 515 nm (A_1).

Compensation liquid. Evaporate 10.0 mL of the stock solution carefully at room temperature to dryness. Take up the residue with 10.0 mL of methanol.

Reference solution. Dissolve immediately before use 0.0150 g plumbagin in n-hexane and dilute to 100.0 mL with the same solvent. Dilute 10.0 mL of this solution to 100.0 mL with n-hexane. Carefully evaporate 10.0 mL of this solution without heating to dryness. Take up the residue with 10.0 mL magnesium acetate solution (5 g magnesium acetate in 1000 mL methanol). Measure the absorbance of the solution immediately, by comparison with the compensation liquid at 515 nm (A_2).

Calculate the percentage content of naphthoquinones, expressed as Plumbagin, using the following expression:

$$\frac{10 \cdot A_1 \cdot m_2}{A_2 \cdot m_1}$$

m_1 = mass of the sample to be examined, in grams.

m_2 = mass of plumbagin, in grams.

A_1 = absorbance obtained by the test solution.

A_2 = absorbance obtained by the reference solution.

Flavonoid glycosides

Liquid chromatography.

Test solution. To 0.3000 g of the powdered herbal drug (500) add 10 mL of ethanol. Sonicate for 15 min in a water-bath at 50 °C, then centrifuge (2000 RCF) for 5 min. Decant the clear supernatant into a 50.0 mL volumetric flask. Add another 10 mL of ethanol to the residue and repeat the extraction procedure two more times. Transfer the supernatant to the same 50.0 mL volumetric flask and dilute to 50.0 mL with ethanol. Filter through a membrane filter (nominal pore size 0.2 µm).

Reference solution (a). Dissolve 5.0 mg of hyperoside in methanol and dilute to 100.0 mL with the same solvent. Dilute 20.0 mL of this solution to 100.0 mL with methanol.

Reference solution (b). Dissolve 5.0 mg of isoquercitrine in reference (a) and dilute to 10.0 mL in a volumetric flask with the same solvent.

Column.

- size: l = 0.25 m, Ø = 4.6 mm;
- stationary phase: end-capped octadecylsilyl silica gel for chromatography (5 µm);
- temperature: 45 °C

Mobile phase.

- mobile phase A: mix of water and 0.1 % (v/v) acetic acid (glacial, analytical grade)
- mobile phase B: mix of acetonitrile and 0.1 % (v/v) acetic acid (glacial, analytical grade)

Time (min)	Mobile phase A (per cent (v/v))	Mobile phase B (per cent v/v)
0 -15	86 → 85	14 → 15
15 - 19	85	15
19 - 19.5	85 → 60	15 → 40
19.5 - 29	60 → 20	40 → 80
29 - 31	20	80
31 - 31.5	20 → 86	80 → 14
31.5 – 35	86	14

Flow rate. 1.2 mL/min

Detection. spectrophotometer at 351 nm.

Injection. 10 µL

Identification of peak. use the chromatogram obtained with reference solution (a) to identify the peak due to hyperoside; identify the peaks due to flavonoids with relative retentions between 1.0 and 1.14 with reference to hyperoside.

System suitability. reference solution (b):

- resolution: minimum 1.8 between the peaks due to isoquercitrine and hyperoside.

Calculate the percentage content of flavonoid glycosides calculated as hyperoside using the following expression:

$$\frac{A_1 \cdot m_2 \cdot p \cdot 0.2}{A_2 \cdot m_1 \cdot 0.6}$$

- A_1 = sum of the peak areas due to flavonoids which elute with relative retentions 1.0 and 1.14 with reference to hyperoside in the chromatogram obtained with the test solution
- A_2 = area of the peak due to hyperoside in the chromatogram obtained with reference solution (a);
- m_1 = mass of the herbal drug to be examined used to prepare the test solution, in grams;
- m_2 = mass of hyperoside used to prepare reference solution (a), in grams;
- p = percentage content of hyperoside in hyperoside.