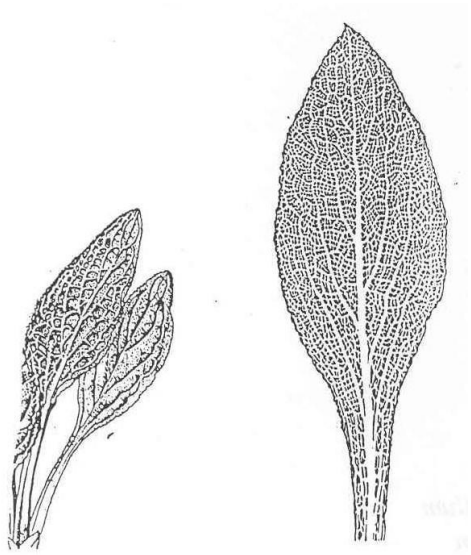
VEGETABLE DRUGS CONTAINING CARDIAC GLYCOSIDE

Content

1. MACROMORPHOLOGICAL TESTS

Digitalis purpureae folium — Digitalis leaf (Foxglove)
Digilalis purpurea L. — Scrophulariaceae
(syn.*: Rhamnus frangula* L.*)*

Ph.Eur.

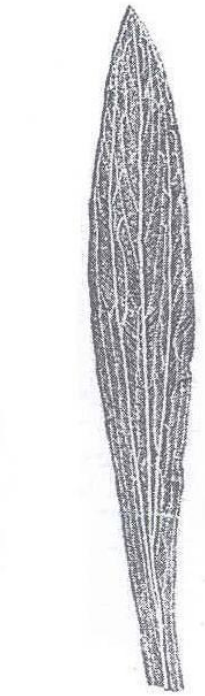


Digitalis leaf has a faint but characteristic odour. The whole leaf is about 10 cm to 40 cm long and 4 cm to 15 cm wide. The lamina is ovate lanceolate to broadly ovate. The winged petiole is from one quarter as long as to equal in length to the lamina

Digitalis lanatae folium
Digitalis lanata Ehrh.

Used in pharmaceutical industry

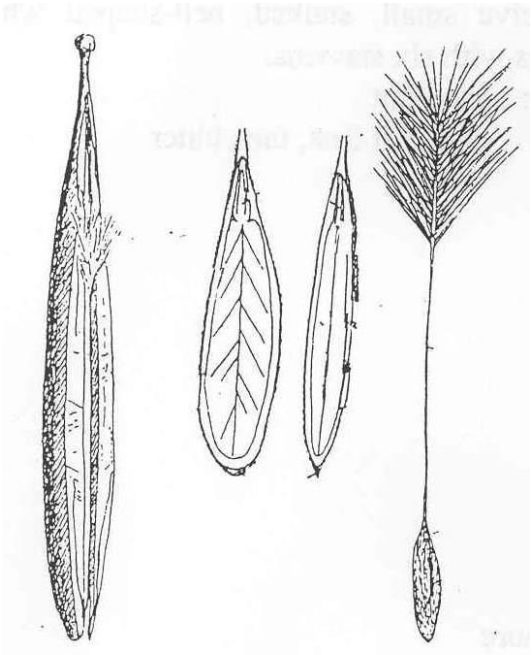
Thimble foxglove leaf
Scrophulariaceae



The leaves are sessile and about 28 cm long and 6 cm wide, oblong lanceolate. The margin is entire and in the basal half ciliate with long uniseriate trichomes, otherwise the leaf is glabrous. The main veins leave the midrib at a very acute angle, the smaller branches are inconspicuous giving an appearance simulating of a parallel venation.
Odour: faint

Strophanthi semen
Strophanthus kombe Oliv.
Strophanthus gratus Franchet
Strophanthus hispidus D.C.

Strophanthus seed
Apocynaceae



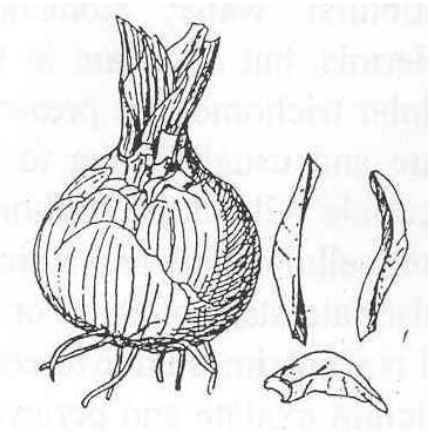
The seeds are lanceolate or linear lanceolate and the testa is prolonged at the apex into a slender thread-like own which terminates in a plume of silky hairs. The commercial seed is about 12 to 20 mm long, 3 to 5 mm broad and 2 mm thick. There is a broken point at the apex left by the removal of the awn and at the base a slight inconspicuous winged extension.

A ridge, which contains the raphe, runs from the apex along the central line of one of the broad faces of the seed for about two-thirds of its length. Near the apical end of this edge the hilum appears as a whitish point.

Trichomes give a silky sheen to the seeds.

Scilla siccata
Urginea maritima (L.) Baker
(syn.: *Scilla maritima* L.)

Squill



The drug consists of slices which are arcuate and concave-convex, being about 3 to 6 cm long and 3 to 8 mm wide and thick at the middle point.

They are somewhat translucent yellowish wite, brittle when dry, flexible if allowed to absorb moisture from the atmosphere.

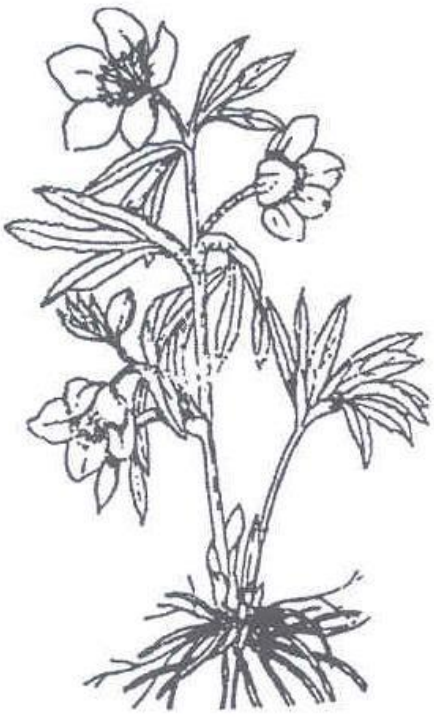
Convallariae herba
Convallaria majalis L.

Lily of the valley
Liliaceae

Leaves are broadly lanceolate, up to 15 cm long and about 5 cm wide, parallel-veined with entire margins. Flower stem carries eight to twelve small, stalked, bell-shaped white flowers with six stamens.

Hellebori nigri rhizome et radix
Helleborus niger L.

Hellebore
Ranunculaceae

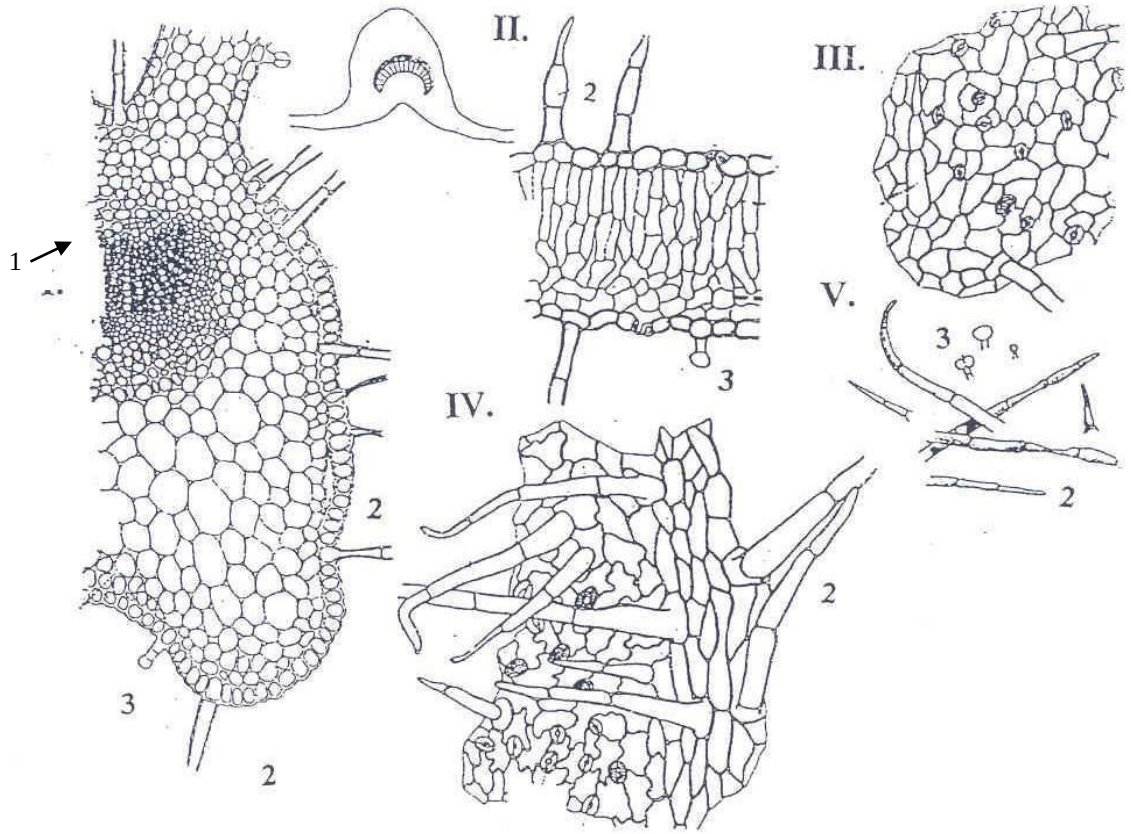


The rhizome is blackish, occuring as a tangled mass of short branches, bearing straight, slender, rather brittle black rootlets with a central cord.

2. MICROSCOPICAL TESTS

Digitalis purpureae folium

Cross section and powdered preparation

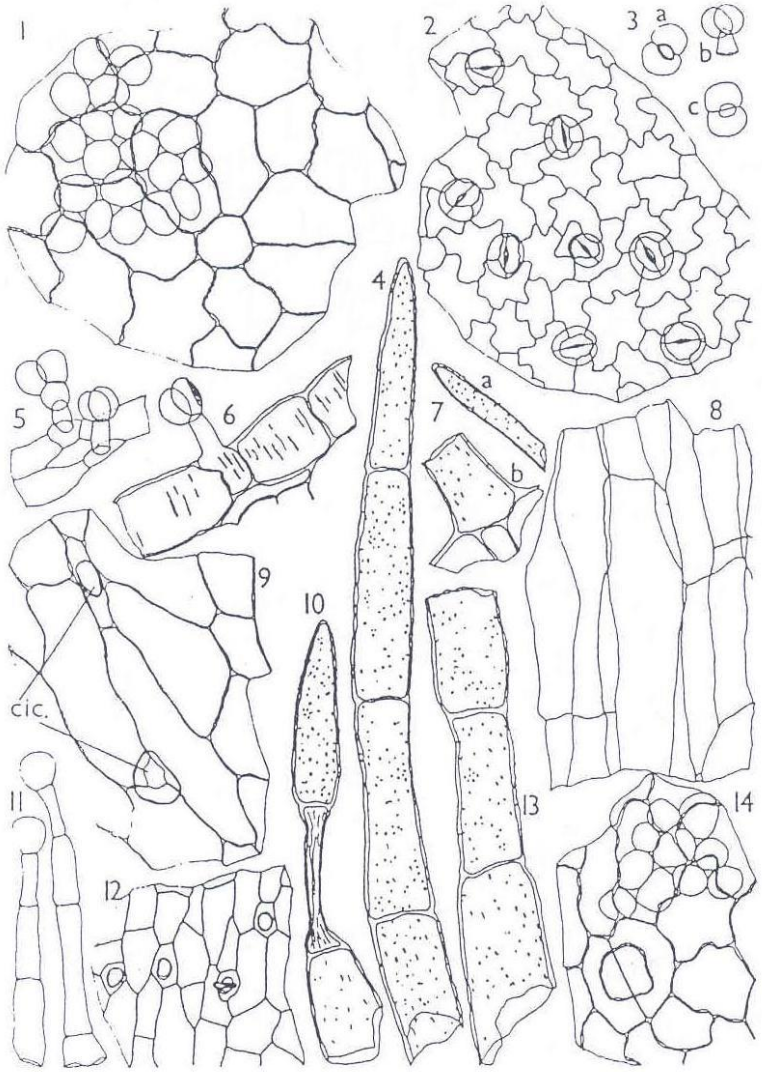


I-II. *Digitalis purpureae folium cross section*
1. = vascular bundle;
2. = covering trichomes;
3. = glandular trichomes

III-IV. *Upper epidermis with stomatas*

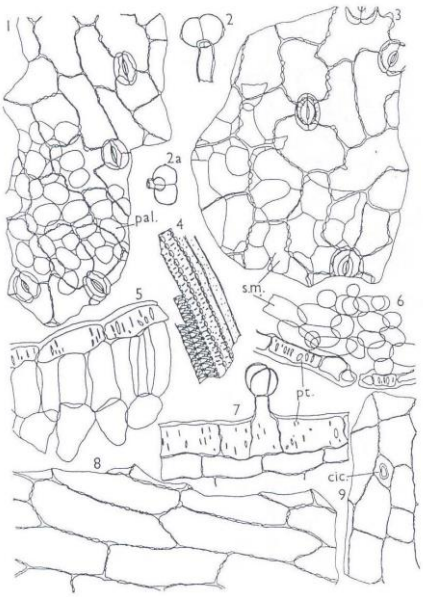
V. *Covering and glandular tirchomes*

Digitalis purpureae folium *– powdered preparation*



1. Upper epidermis in surface view with underlying palisade cells
2. Lower epidermis in surface view with anomocytic stomata
3. Glandular trichome with bicellular heads seen (a) from below (b) from the side and (c) from above.
4. Part of a covering trichome
5. Glandular trichomes attached to a fragment of the epidermis
6. Epidermis in sectional view showing pitting in the walls and a glandular trichome
7. Fragments of covering trichomes: (a) apical cell and (b) basal cell attached to a fragment of epidermis
8. Cortical parenchyma in longitudinal view
9. Epidermis in surface view showing cicatrices (cic.)
10. Part of a covering trichome showing a collapsed cell
11. Glandular trichomes with uniseriate stalks and unicellular heads
12. Epidermis from over a vein in surface view, showing cicatrices
13. Fragment of a large covering trichome
14. Upper epidermis in surface view showing a cicatrix and underlying palisade cells.

Digitalis lanatae folium *– powdered preparation*



1. Upper epidermis in surface view showing anomocytic stomata and underlying palisade (pal.)
2. Glandular trichome in side view

2.a. Glandular trachoma from above

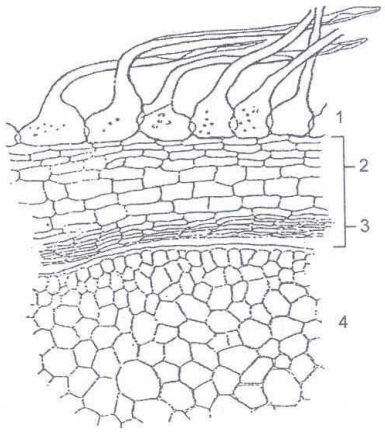
3. Lower epidermis in surface view with anomocytic stomata and underlying spongy mesophyll (s.m.)
4. Vascular tissue from a larger vein
5. Upper epidermis and palisade in sectional view
6. Lower epidermis with pits (pt.) and spongy mesophyll (s.m.) in sectional view
7. Epidermis over a vein in sectional view with pits (pt.) and a glandular trichome
8. Epidermis from over a vein in surface view
9. Epidermis in surface view showing cicatrices (cic.)

Strophanthi semen - *Cross section*

1. = epidermis with unicellular trichomes (elongated polygonal tabular cells, the upper surface of each epidermal cell is extended as a unicellular trichome bent over)

2., 3. = collapsed thin-walled parenchyma

4. = endosperm: thin-walled parenchyma containing abundant fixed oil and aleurone grains



PHYSICO-CHEMICAL AND CHEMICAL TESTS

3.1 Test-tube reactions

(Digitalis lanatae folium, Digitalis purpureae folium, Convallariae herba)

Extraction
Warm 2.0 g of each powdered crude drugs with 20 ml of 50% ethanol and 10 ml of 10% lead acetate on boiling water bath for 5 min. Centrifuge the cooled suspensions and extract the clear liquids with dichlormethan carefully (2 x 15 ml).
Combine the dichlormethane layers, dry over Na_2SO_4 sicc. and prepare a stock solution of 25 ml (measuring cylinder).

Carry out the specific reactions from 5 ml portions of these extracts.

3.1.1. Keller-Kilinai reaction

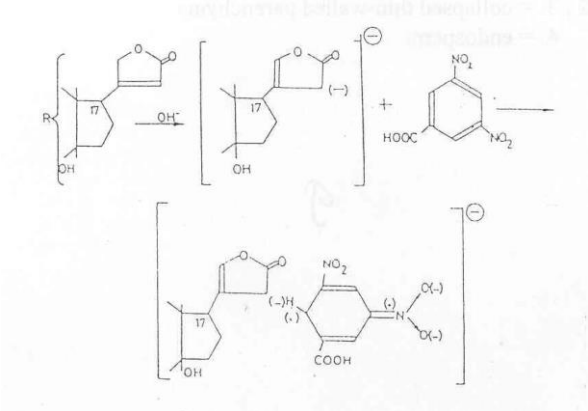
Dry 5 ml of the above extract on a water bath, and dissolve the residue in 3 ml of concentrated R-acetic acid. Add 1 drop of R-iron (III) chloride test solution to the liquid and carefully transfer it on concentrated R-sulphuric acid. A reddish brown ring forms at the interface, the upper acetic acid layer soon turns bluish green.

Explanation: digitoxose transform to furfurol in cc. acids, which give bluish green colour in cc. acetic acid. The brownish ring on the surface of the two phases is the reaction of the terpene skeleton (see Saponins).

3.1.2. Kedde reaction

Dry 5 ml of the above extract on a water bath, and dissolve the residue in 2 ml of alcoholic 3,5- dinitrobenzoic acid reagent and 1 ml of R -NaOH solution. The reaction mixture immediately turns purple-violet, which colour disappears after a few min.

Explanation: first, a cardenolide anion is formed, which is converred to a purple-violet anion by adding 3,5- dinitrobenzoic acid. Because of the hydrolysis of the lactone ring, the colour disappears in a few min. The reaction works with 5 member, α-, β- unsaturated γ-lactone ring, and it is based on the proton-dissociation catalised by alkalics.

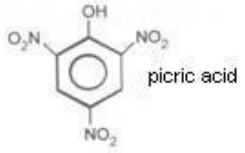


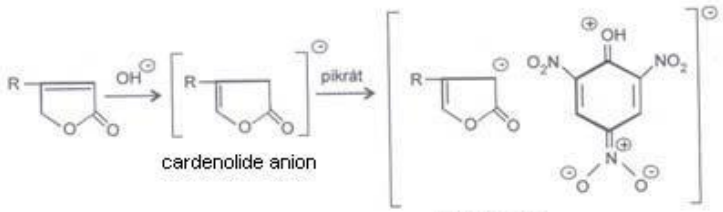
3.1.3. Baljet reaction

Dry 5 ml of the above extract on a water bath and dissolve the residue in 3 ml of methanolic sodium picrate solution. Add 1 ml of N-sodium hydroxide solution to the liquid. The mixture acquires at once a light wine-red colour.

Blank: 3 ml of methanolic sodium picrate solution and 1ml of N-sodium hydroxide solution.

Explanation: like in Kedde reaction, the colouric product is made by the connection of cardenolid-anion and a nitrogroup containing molecule.

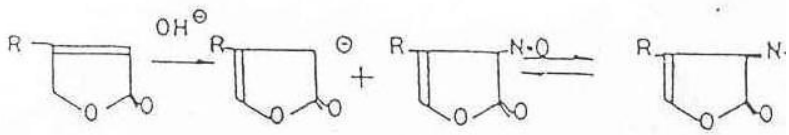




3.1.4. Legal reaction

Dry 5 ml of the above extract on a water bath, and dissolve the residue in the mixture of 1 ml of water, a few drops of 10% sodium hydroxide and 1 ml of 0.3% nitroprussid sodium reagent. The mixture acquires at once a dark red colour.

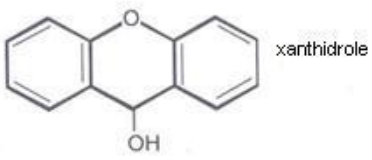
Blank: 1 ml water, some drops of 10% sodium hydroxide and 1 ml of 0.3% nitroprussid sodium reagent.



3.1.5. Xanthidrole reaction

Dry 5 ml of the above extract on a water bath, and dissolve the residue in 3 ml of xanthidrole reagent and heat it for 3 min on water bath. In the presence of deoxy sugars a reddish colour appears.

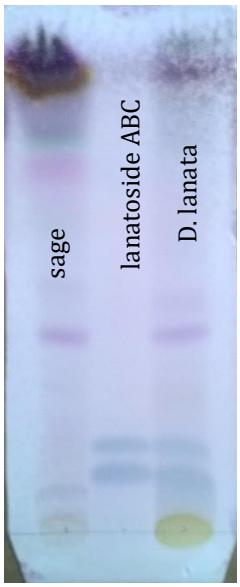
Explanation: the reaction serve for the detection of 2-desoxy sugars, like digitoxose, cymarose, oleandrose, diginose.



3.2. Thin-layer chromatography investigations

3.2.1 Thin-layer chromatography of Salviae folium contaminated with Digitalis.

Use 1 g sample to make extract with 10 ml ethyl-acetate, filter and evaporate to dryness. Dissolve in 0,5 ml methanol and use 10-20 µl for the TLC investigation. Use Lanatoside-mixture as standard.



TLC parameters

Sorbent: Silicagel G 60 F_{254} 0.2 mm

Solvent system:	Ethyl acetate	81
	Methanol	11
	Water	8

Reagent: Vanillin - Sulphuric acid reagent (with heating at 100^{o}C for a few min.)

3.2.2 TLC investigation of cardiac glycosides

Use 5 ml of the extract for test-tube reaction (3.1.) and investigate its 10-20 µl on TLC layer beside standards.

Sorbent: Silicagel G 60 F_{254} 0.2 mm

Solvent system:	Ethyl acetate	81
	Methanol	11
	Water	8

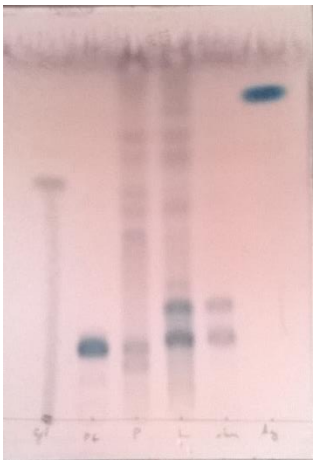
Reagent: Vanillin - Sulphuric acid reagent (with heating at 100^{o}C for a few min.)

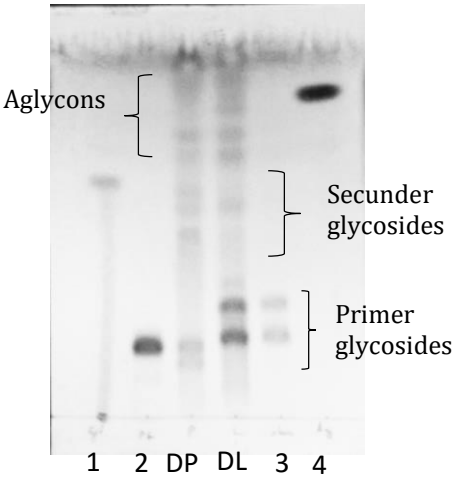
Standard solutions:

Standards	Type	Plant	Colour	Rf
1 Gitoxin	secunder	*D. purpurea*	light blue	0,57
2 Purpureaglycoside A	primer	*D. purpurea*	dark blue	0,20
3 Lanatoside ABC	primer	*D. lanata*	blue	0,22 0,26
4 Digitoxingenine	aglycon	*both*	light, later dark blue	0,81

Calculate the R_f values of the characteristic compounds in the plant extracts.

1. gotixin
2. purpureaglycoside A
   DP: Digitalis purpurea
   DL: Digitalis lanatae
3. Lanatoside ABC
4. Digitoxigenine





3.3 Preparative scale isolation of crude cardiac glycosides from *D. lanatae folium*

Extraction

Extract 10 g of dried, powdered Digitalis leaves with the solvent mixture of 100 ml ethyl acetate, 6 ml water and 2 ml R-ammonia solution by simple shaking or stirring in ultrasonic bath at room temperature for 30 min. After filtration reextract the crude drug for 30 min. with 100 ml of ethyl acetate.

Evaporate the solvent under reduced pressure (maximum temp. 40^{o}C).

Dissolve the residue in 20 ml of ethyl acetate, add 40 ml of water. Continue evaporation till the complete removal of ethyl acetate.

Purification

Add 10 g lead acetate crystals to the extract and shake it for 5 min. After filtration add Na_2SO_4 solution (3 g Na_2SO_4 in 5 ml of water) to the filtrate and centrifuge the reaction mixture (6 min; 3000 g). Remove the clean, yellowish extract to a separatory funnel and add 2 ml of 10% ammonia solution (pH=7.8). Extract it with chloroform (3 x 30 ml) and combine the chloroformic extracts, dry it over Na_2SO_4 sicc. and evaporate the solvent under reduced pressure (max. 50^{o}C).

Precipitation of the crude glycosides

Dissolve the residue in 3 ml of chloroform and add 30 ml of petroleum ether. Fine precipitation appears. After 10 min filter the mixture and collect the crude cardiac glycosides on a small filter paper. After evaporating the residues of petroleum ether dissolve the glycosides in 7 ml of 96% ethanol. Remove the extract into a small flask, evaporate the solvent and measure the isolate crude cardiac glycosides of *Digitalis lanata*. Dissolve the product in 0.5 ml of 96% ethanol.