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# VEGETABLE DRUGS CONTAINING CARDIAC GLYCOSIDE

## Content

### 1. MACROMORPHOLOGICAL TESTS

*Adonidis herba*  
*Convallariae herba*  
*Hellebori nigri rhizoma et radix*  
*Digitalis purpureae folium*  
*Digitalis lanatae folium*  
*Scilla bulbosus/siccata*  
*Strophanthi semen*

### 2. MICROSCOPICAL TESTS

Cross section:        *Digitalis purpureae folium*  
                              *Digitalis lanatae folium*  
                              *Strophanthi semen*

Powdered preparations:    *Digitalis purpureae folium*  
                                      *Digitalis lanatae folium*

### 3. PHYSICO-CHEMICAL AND CHEMICAL TESTS

#### 3.1. Test-tube reactions

3.1.1. Keller-Kiliani test

3.1.2. Kedde-test

3.1.3. Baljet-test

3.1.4. Legal-test

3.1.5. Xanthidrol-test

(*Digitalis lanatae folium*, *Adonidis herba*, *Convallariae herba*)

#### 3.2. Thin-layer chromatography of cardiac glycosides (*Convallariae herba*)

#### 3.3 Preparative scale isolation of crude cardiac glycosides from *Digitalis lanatae folium*

3.3.1. Thin-layer chromatography of *Digitalis lanatae* cardiac glycosides

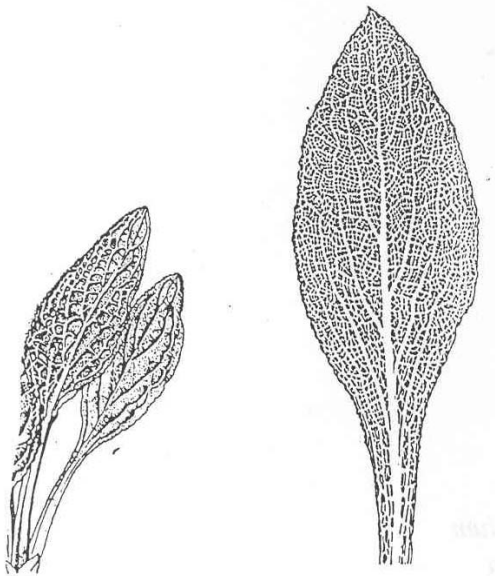
3.3.2. HPLC of *Digitalis lanatae* cardiac glycosides

## 1. MACROMORPHOLOGICAL TESTS

***Digitalis purpureae* folium**  
*Digitalis purpurea* L.  
(syn.: *Rhamnus frangula* L.)

Foxglove  
Scrophulariaceae

Ph.Eur., Ph.Hg.VIII.



Leaves are ovate, about 10-30 cm long and up to 10 cm wide, with a subacute apex and a crenate margin; petiolate with a decurrent base.

The veins are prominent on the undersurface and depressed on the upper surface, anastomosing near the margin.

Odour: faint

Taste: very bitter

***Digitalis lanatae* folium**  
*Digitalis lanata* Ehrh.

Thimble foxglove  
Scrophulariaceae

Used in pharmaceutical industry



The leaves are sessile and about up to 28 cm long and 6 cm wide, oblong-lanceolata. The margin is entire and in the basal half ciliate with long uniseriate trichomes, otherwise the leaf is glabrous. The main veins are few, they leave the midrib at a very acute angle, and travel for some distance towards the apex, while the smaller branches are inconspicuous thus giving an appearance simulating a parallel venation.

Odour: faint

Taste: very bitter

**Strophanthi semen**

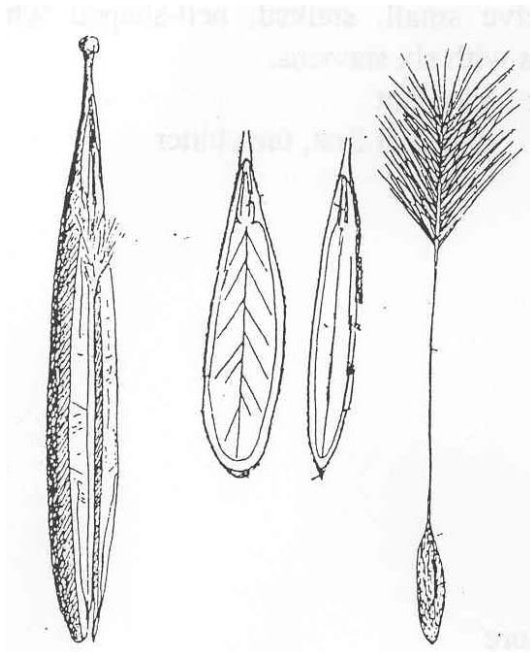
*Strophanthus kombe* Oliv.

*Strophanthus gratus* Franchet

*Strophanthus hispidus* D.C.

Strophanthus

Apocynaceae



The seeds are lanceolate or linear lanceolate and the testa is prolonged at the apex into a slender thread-like awn 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, at the apex is a broken point 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.

Odour: slight and unpleasant

Taste: bitter

**Adonidis vernalis herba**

*Adonis vernalis* L.

Hellebore, false. Yellow pheasant's eye

Ranunculaceae



Stem about 15-25 cm long, bearing feathery 2-3 pinnate leaves and a single large yellow flower, 40-80 mm across with ten or more petals and numerous stamens

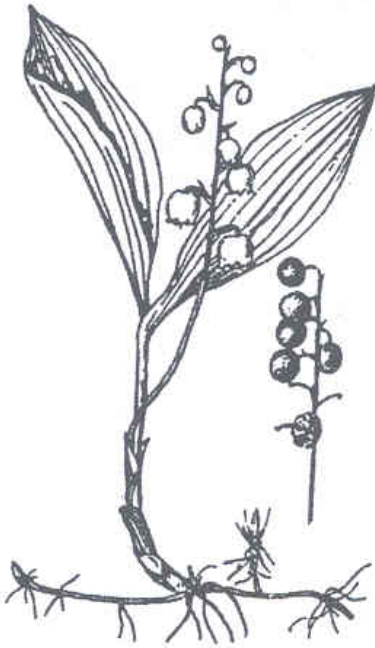
Odour: odourless

Taste: slight bitter

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**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.

Odour: pleasant

Taste: sweet at first, then bitter

**Hellebori nigri rhizome et radix**  
*Helleborus niger* L.

Hellebore  
Ranunculaceae



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

Odour: faint, fatty

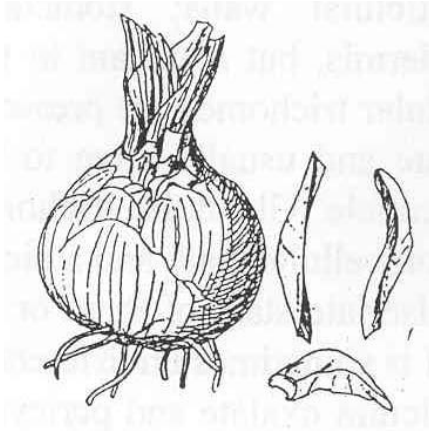
Taste: bitter and acrid

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**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 white, brittle when dry, flexible if allowed to absorb moisture from the atmosphere.

Odour: very slight

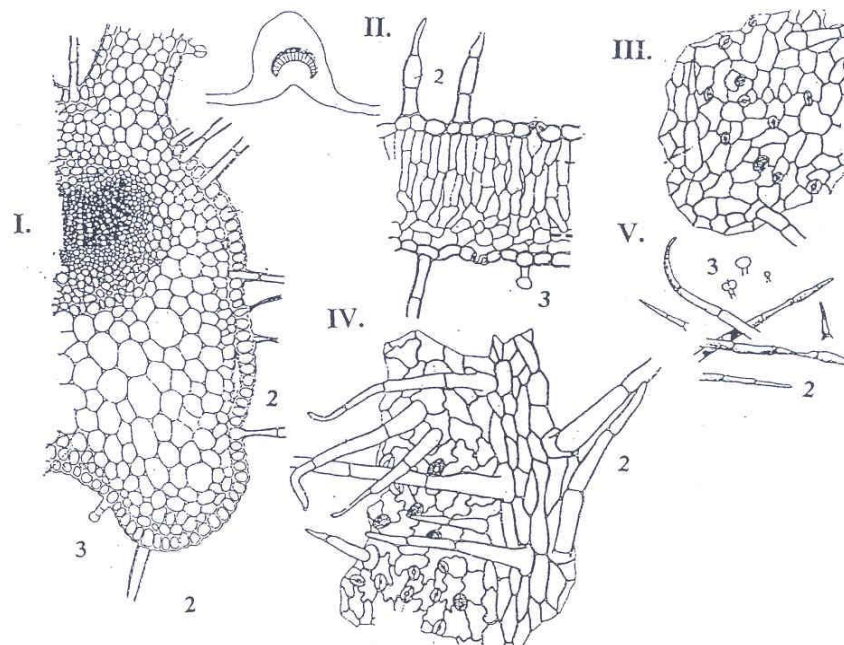
Taste: disagreeable, bitter, acrid

## 2. MICROSCOPICAL TESTS

### *Digitalis purpureae* folium

#### *Cross section and powdered preparation*

The midrib projects strongly on the lower surface and contains a meristele having a shallow gutter-shaped radiate xylem, beneath which is a narrow phloem and on the upper side a little parenchyma; below the phloem is a pericycle of small-celled collenchyma and the whole meristele is surrounded by an endodermis containing starch granules; the remainder of the tissue of the midrib is then-walled cellulosic parenchyma with about one layer of hypodermal collenchyma. The upper epidermal cells have straight or slightly wavy anticlinal walls and those of the lower epidermis have strongly wavy anticlinal walls; stomata of the ranunculaceous type are few in the upper epidermis, but abundant in the lower. Numerous covering trichomes and a few glandular trichomes are present on both surfaces. The covering trichomes are uniseriate and usually three to four cells long, with an acute apex and a finely warty cuticle. Glandular trichomes occur chiefly over the veins and usually have a short unicellular stalk and a bicellular, or more rarely unicellular, head, a few have a uniseriate stalk of three or four cells and a unicellular spherical head. The mesophyll is sometimes undifferentiated, but usually has a single layer of palisade cells. Calcium oxalate and pericyclic fibres are absent. On each marginal tooth there is usually one large water-pore, exceptionally two pores.



I-II. *Digitalis purpureae* folium cross section

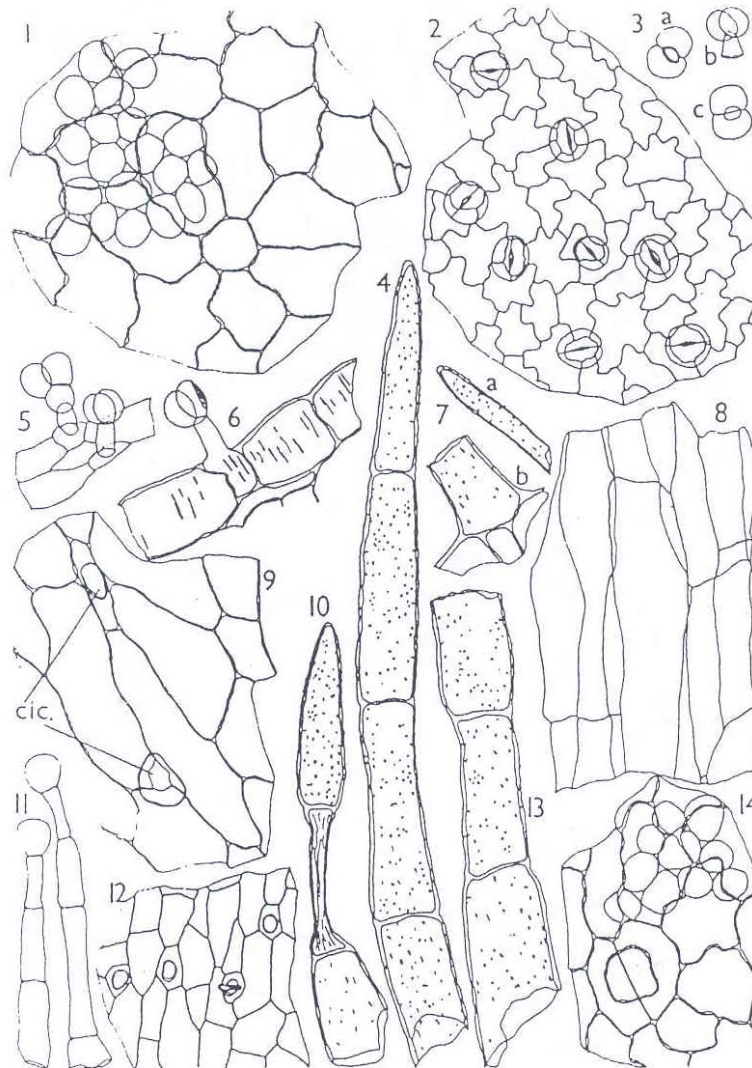
1. = vascular bundle;                      2. = covering trichomes;  
3. = glandular trichomes

III-IV. *Upper epidermis with stomatas*

V. *Covering and glandular trichomes*

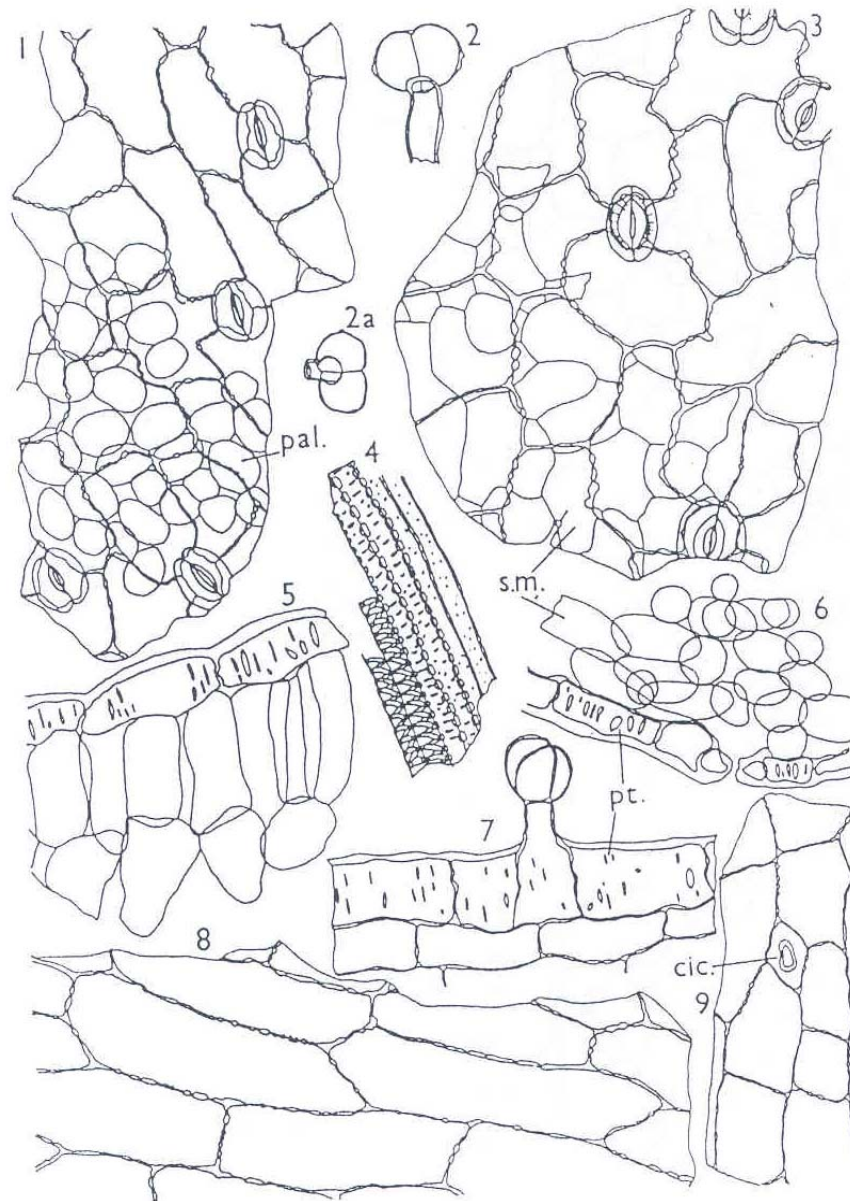


*Digitalis purpureae folium* – powdered preparation



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*



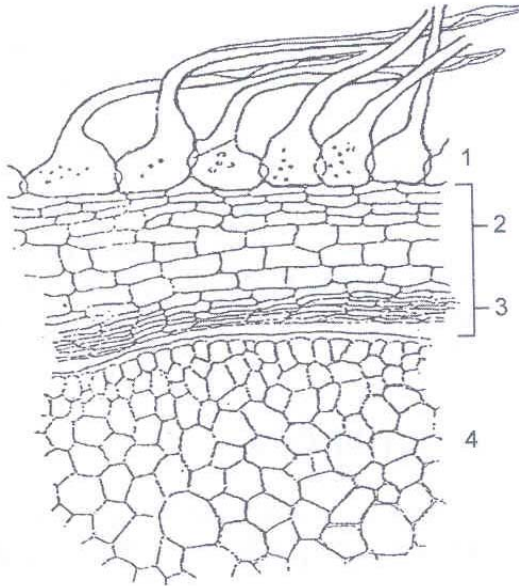
1. Upper epidermis in surface view showing anomocytic stomata and underlying palisade (pal.)
2. Glandular trichome in side view
- 2.a. Glandular trichoma from above
3. Lower epidermis in surface view with anomocytic stomata and underlying spongy mesophyll (s.m.)
4. Vascular tissue from a larger vein
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.)



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## Strophanthi semen

### *Cross section*



The epidermis is composed of elongated polygonal tabular cells, about 50 to 100  $\mu$  long and 20 to 30  $\mu$  wide and high the anticlinal walls being straight thickened and lignified. The upper surface of each epidermal cell is extended as a unicellular trichome bent over so as to be appressed to the seed. The free part of the trichome is about 500 to 800  $\mu$  long, and is strengthened by a single narrow strip of lignified thickening which extends along the entire length of the adaxial side of the trichome. This strip is connected with the ring-shaped thickening of the trichome base by struts of thickening which arise from it and converge to meet above in the point where the strip begins. The remainder of the testa consists of a narrow layer of more or less collapsed thin-walled parenchyma, in occasional cells of which calcium oxalate in clusters or broken crystals may be found, but such crystals are rare. The endosperm and embryo consist of moderately thin-walled parenchyma containing abundant fixed oil and aleurone grains.

1. = epidermis with unicellular trichomes
- 2., 3. = collapsed thin-walled parenchyma
4. = endosperm

### 3. PHYSICO-CHEMICAL AND CHEMICAL TESTS

#### 3.1 Test-tube reactions

(*Digitalis lanatae folium*, *Convallariae herba*, *Adonidis 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 chloroform (2 x 15 ml). Combine the chloroformic layers, dry over  $\text{Na}_2\text{SO}_4$  sicc. and prepare a stock solution of 25 ml (measuring cylinder).

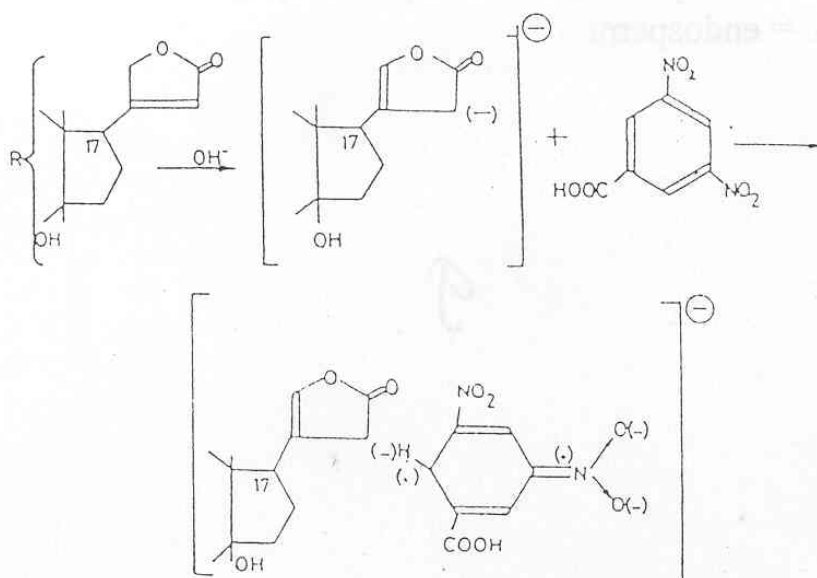
Carry out the specific reactions from 5 ml portions of these extracts.

##### 3.1.1. Keller-Kiliani 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.

##### 3.1.2. Kedde reaction

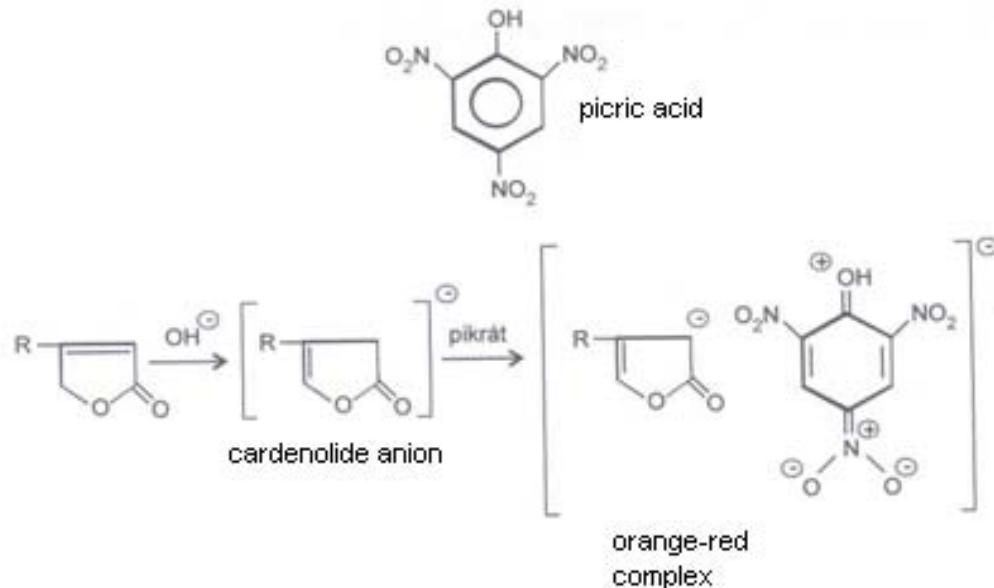
Dry 5 ml of the above chloroformic extract on a water bath, and dissolve the residue in 2 ml of alcoholic 3,5-dinitrobenzoic acid reagent and 1 ml of R-NaOH solution. The reaction mixture immediately turns purple-violet, which colour disappears after a few min.



##### 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.

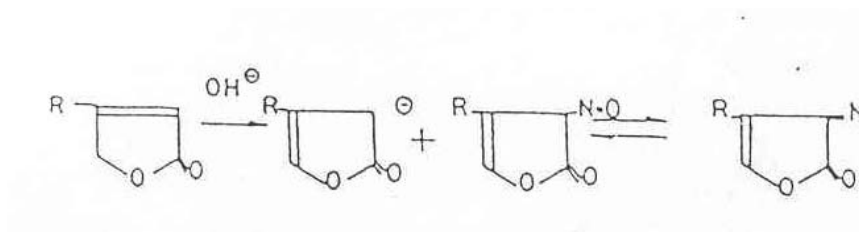
Blank: 3 ml of methanolic sodium picrate solution and 1ml of N-sodium hydroxide solution.



### 3.1.4. Legal reaction

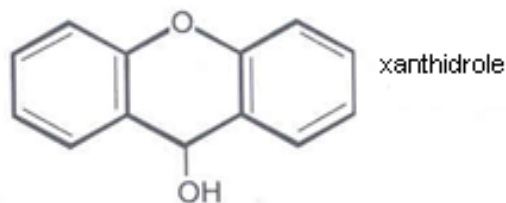
Dry 5 ml of the above extract on a water bath, and dissolve the residue in the mixture of 1 ml of water, a few drops of 10% sodium hydroxide and 1 ml of 0.3% nitroprussid sodium reagent. The mixture acquires at once a dark red colour.

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 xanthydrole reagent and heat it for 3 min on water bath.  
In the presence of deoxy sugars a reddish colour appears.



### Cardiac glycosides

| Crude drugs                     | Chemical reaction | Reaction of the steroid skeleton and the sugar moiety | Reaction of the unsaturated lactone ring at C-17 |                |                 | Reaction of the 2-deoxy sugars |
|---------------------------------|-------------------|---|--|----------------|-----------------|--------------------------------|
|                                 |                   | Keller reaction                                       | Kiliani reaction                                 | Kedde Reaction | Baljet Reaction | Legal reaction                 |
| <i>Digitalis lanatae folium</i> |                   |   |  |                |                 |                                |
| <i>Convallariae herba</i>       |                   |   |  |                |                 |                                |
| <i>Adonis herba</i>             |                   |   |  |                |                 |                                |

### 3.2. Thin-layer chromatography of cardiac glycosides (*Convallariae herba*)

Extract 5 g finely powdered crude drug with 30 ml of 50% ethanol and 10 ml of 10% lead acetate on a water bath at 60 °C for 15 min using reflux condenser. Filter the cooled extract and shake it with a 3:2 mixture of dichloromethane and isopropanol (3x30). Unify the lower layers, dry the liquid over anhydrous sodium sulphate, and evaporate to dryness. Redissolve the residue in 2 ml of chloroform-methanol (1:1).

#### TLC parameters

Sorbent: Silicagel G 60 F<sub>254</sub> 0.2 mm  
 Solvent system: Ethyl acetate 100  
 Methanol 13.5  
 Water 10

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Reagent: Vanillin - Sulphuric acid reagent (with heating at 100°C for a few min.)

Sample volume injected:

|                                  |          |
|----------------------------------|----------|
| Stock solution (1 mg/ml)         | 10-20 µl |
| Konvallatoxin standard (1 mg/ml) | 10 µl    |

### *Thin-layer chromatography of cardiac glycosides*

1. Sample (*Convallariae herba*, 20 µl)
2. Konvallatoxin (1 mg/ml; 10 µl)



## **3.2 Preparative scale isolation of crude cardiac glycosides from *Digitalis 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°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<sub>2</sub>SO<sub>4</sub> solution (3 g Na<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub> sicc. and evaporate the solvent under reduced pressure (max. 50°C).

### *Precipitation of the crude glycosides*

Dissolve the residue in 3 ml of chloroform and add at once 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. Use this solution for TLC studies.

### 3.2.1 TLC investigation of the crude cardiac glycosides

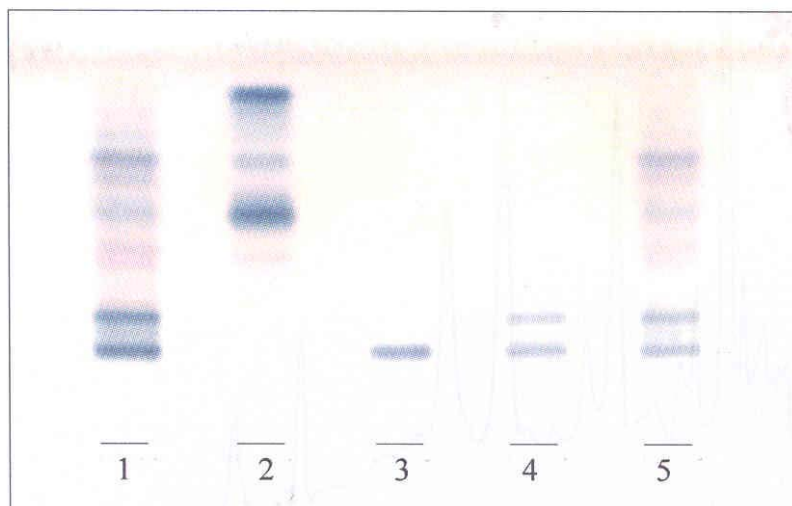
Sorbent: Silicagel G 60 F<sub>254</sub> 0.2 mm

Solvent system: Ethyl acetate 81  
Methanol 11  
Water 8

Reagent: Vanillin - Sulphuric acid reagent (with heating at 100°C for a few min.)

Standard solutions: a/ Lanatoside A-B-C-D mixture (1 mg/ml) 10 µl  
b/ Lanatozid C (1 mg/ml) 10 µl  
c/ Secunder glycosides (1 mg/ml) 10 µl

Calculate the R<sub>f</sub> values of the characteristic compounds in the isolated crude cardiac glycoside sample.



### TLC of crude cardiac glycosides from *Digitalis lanatae folium*

Sample:

1. *Digitalis lanatae* folium 10 µl
2. *Digitalis lanatae* folium 5 µl

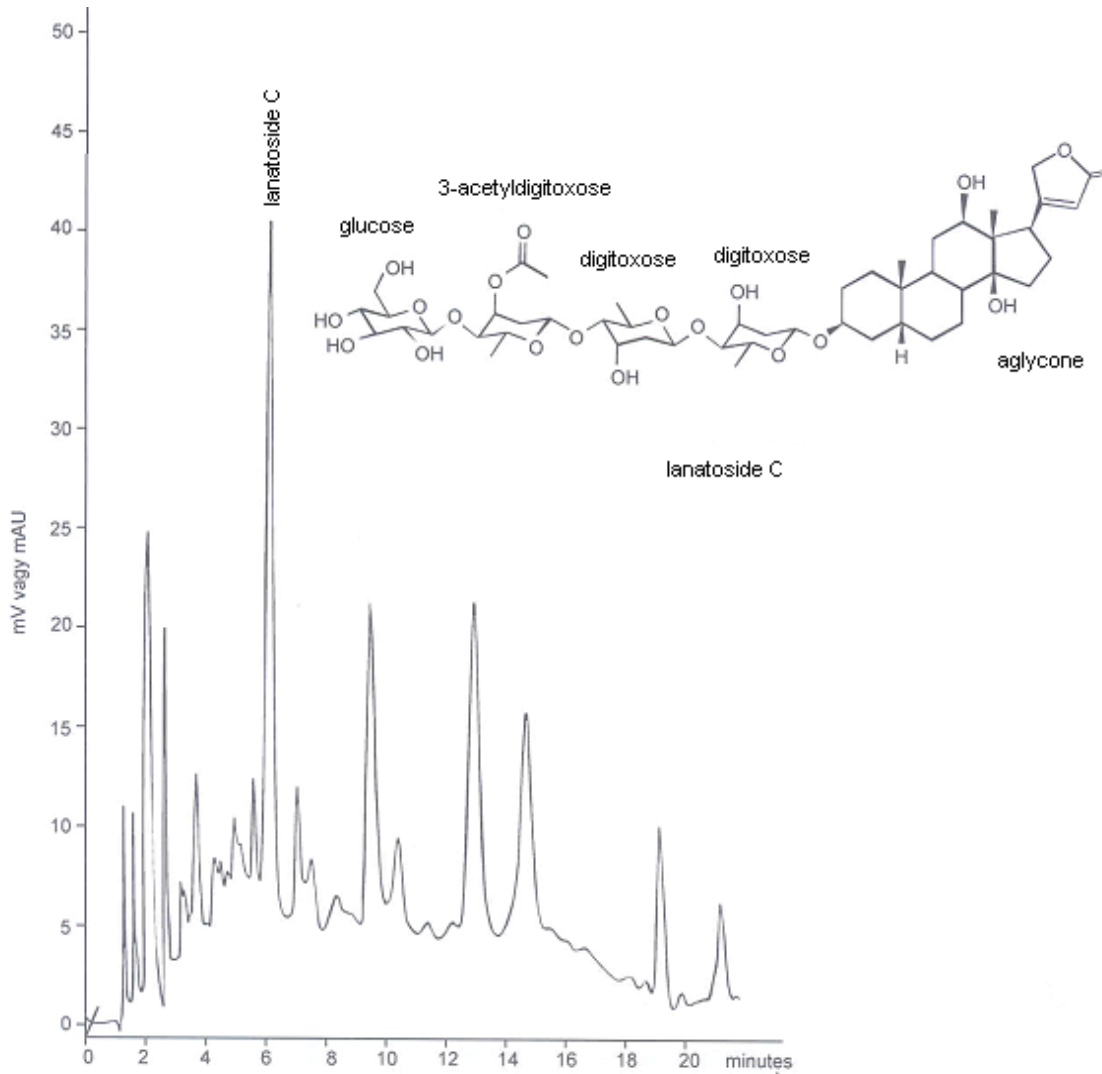
Standard solutions:

3. Secunder glycosides (1 mg/ml) 10 µl
4. Lanatoside C (1 mg/ml) 10 µl
5. Lanatoside A-B-C-D mixture (1 mg/ml) 10 µl

### 3.2.3 High performance liquid chromatography (HPLC) of *Digitalis lanata* cardiac glycosides

HPLC CONDITIONS:

**Instrument:** Spectra-Physics HPLC (P4000 quaternary gradient pump, Focus scanning UV-VIS detector)  
**Column:** Eurosphere 100-C8 (5 µm) Knauer (250x4 mm i.d.)  
**Mobil phase:** acetonitril-water (30:70, V/V)  
**Flow:** 1 ml/min



**HPLC chromatogram from extract of *Digitalis lanatae* folium**