CRUDE DRUGS CONTAINING SAPONINS

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Saponariae albae radix
Primulae radix
Liquiritiae radix
Hippocastani folium
Hippocastani semen
Ginseng radix
Sarsaparillae radix
Hederae folium

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1. MACROMORPHOLOGICAL EVALUATION

Saponariae albae radix
Gipsophila paniculata L.

Soap root
Caryophyllaceae

The entire drug consisting of the root and the short rhizome is 4 to 8 cm thick and 40 to 80 cm long, white-coloured outside, sometimes twisted, inside light yellow, radially cracked. The concentrically enclosed xylem is separated by brown cambium from the white bark. The pieces covered with cork are yellowish brown outside, with many transversally elongated protuberances united almost to a transversal ring.
The drug should be cut into oblique discs of 2 to 5 mm width or into small pieces.

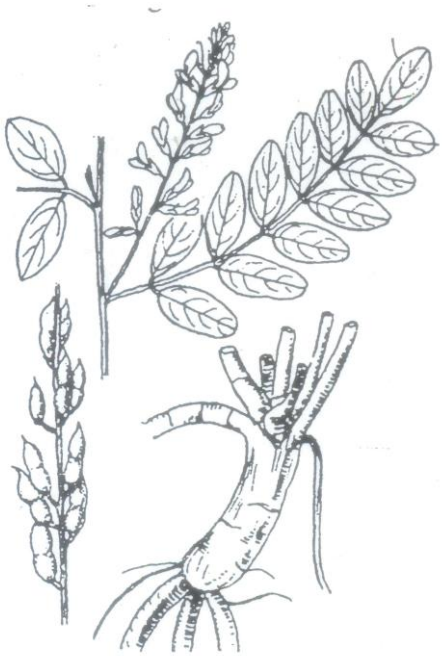
Primulae radix
Primula vulgaris Huds.
Primula veris L. ssp. *inflata* (Lehm.) Domin
Ph. Eur.

Primula root
Primulaceae

The coarsely torose, greyish-brown rhizome is straight or slightly curved, about 1 cm to 5 cm long and about 2 mm to 4 mm thick. The rhizome crown often bears the remains of stems and leaves. Attached to the rhizome are numerous brittle roots, about 1 mm thick and usually 6 cm to 8 cm long. The root of P. elatior is light brown to reddish-brown, that of P. veris light yellow to yellowish-white. The fracture is smooth.

Liquiritiae radix
Glycyrrhiza glabra L.
Glycyrrhiza glabra ssp. glandulifera
Glycyrrhiza uralensis
Ph.Eur.

Liquorice root
Fabaceae



The root has few branches. Its bark is brownish-grey to brown with longitudinal striations and bears traces of lateral roots. The cylindrical stolons are 1 cm to 2 cm in diameter; their external appearance is similar to that of the root but there are occasional small buds. The fracture of the root and the stolon is granular and fibrous. The cork layer is thin; the secondary phloem region is thick and light yellow with radial striations. The yellow xylem cylinder is compact, with a radiate structure. The stolon has a central pith, which is absent from the root. The external part of the bark is absent from the peeled root

Hippocastani folium
Aesculus hippocastanum L.

Horse-chestnut leaf
Hippocastanaceae

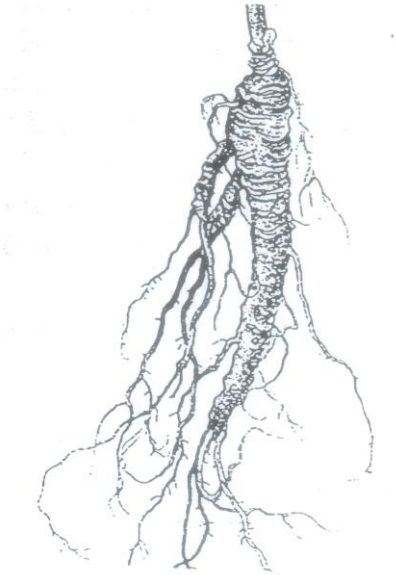
A large deciduous tree with a finely scaly, grey-brown bark. The leaves are opposite, palmically complex with 5-7 leaflets; these are obovate-lanceolate, up to 22 cm long, tapering to a wedge-shaped base, strongly ribbed; the petioles are long; the leaf-buds rather sticky.
The drug has no odour, taste is bitterish acrid.

Hippocastani semen
Aesculus hippocastanum L.

Horse-chestnut seed
Hippocastanaceae

The fruit is a leathery prickly capsule, it contains 1 or 2 seeds. The seeds are large, shining brown, with large whitish scar. They are odorless, their taste is sharp.

Ginseng radix
Panax ginseng C.A. Meyer
Ph. Eur.



Ginseng root
Araliaceae

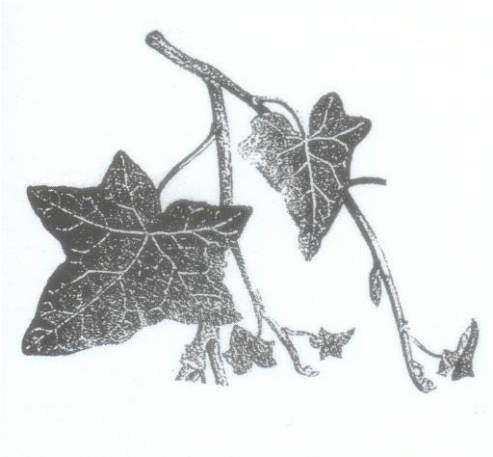
The principal root is fusiform or cylindrical, sometimes branched, up to about 20 cm long and 2.5 cm in diameter, and may be curved or markedly re-curved. The surface is pale yellow to cream and shows longitudinal ridges; stem scars may be seen at the crown. The fracture is short. The transversely-cut surface shows a wide outer zone with scattered orange-red resin canals and a finely radiate inner region. The rootlets, numerous in the lower part, are fine with a small diameter.

Sarsaparillae radix
Smilax species

Sarsaparilla root
Liliaceae

The crude drug consists of roots of the tropic plants of *Smilax* species. The roots are washed and dried by sun or fire and put in bundles.
The roots are 1-2 m in length and 3-5 mm in diameter, greyish brown coloured, longitudinally wrinkled and inside yellowish white.
The drug is odourless, its taste is mucilaginous.

Hederae folium
Hedera helix L.
Ph. Eur.



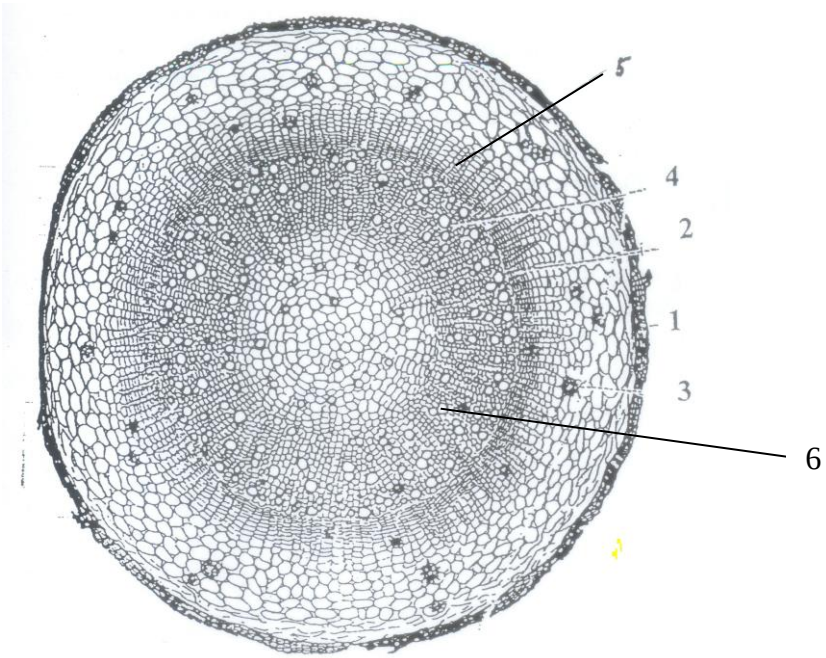
Ivy leaf
Araliaceae

Ivy is a woody climbing plant, often creeping and covering large areas on the ground, or climbing up to over 25 m. The leaves are simple, dark green and glossy of 2 kinds: those on flowering shoots are ovate to diamond-shaped; the others palmically 3-5 lobed, up to 10 cm long, the lobes are triangular.
The drug is odourless, its taste is bitterish, sharp.

2. MICROSCOPIC EVALUATION

Cross section of Saponariae albae radix

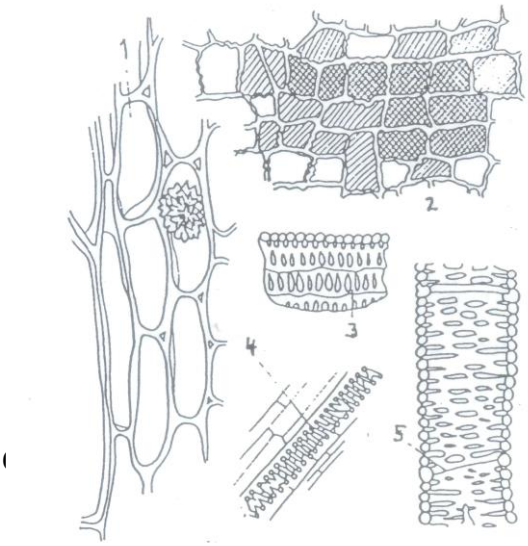
The soap root is secondary thickened, below the periderm, the cortex (dilatation zone and phloem) can be found, which is rich in $Ca(COO)_2$ rosettes.
The medullary rays of phloem and xylem also contain $Ca(COO)_2$ rosettes. In the phloem part we find only "soft" phloem constituents whitout any cell-wall thickening. The cambium consists of several cell-lines. The xylem contains trachea, tracheida and parenchymatous tissue. Xylem fibres can be found only in the elder roots.



1 = periderm
2 = phloem body
3 = $Ca(COO)_2$ rosettes
4 = wood body
5 = cambium
6 = trachea, tracheide

Powder preparation of Saponariae albae radix

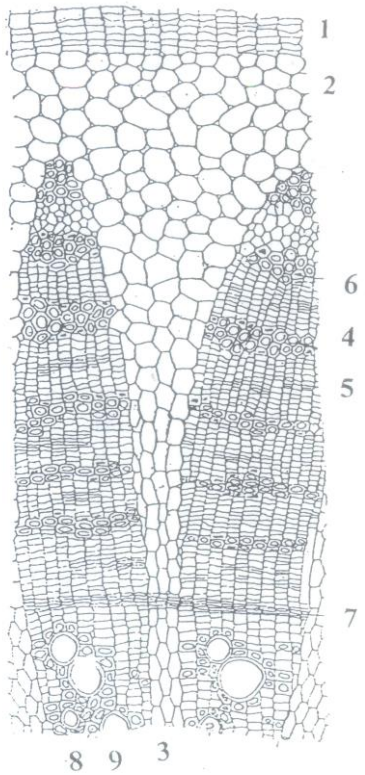
The powder can be characterized by the $Ca(COO)_2$ rosettes and tracheas with pitted or spirally thickened walls as well as oblique perforated transversal walls. In the unpeeled drug fragments of the cork also appear.



1 = Cortex-parenchym with cells containing Ca-oxalate rosettes
2 = cork in surface view
3 = trachea of pitted thickened wall
4 = trachea of spirally thickened wall
5= trachea of oblique perforated transversal walls

Cross section of Liquiritiae radix

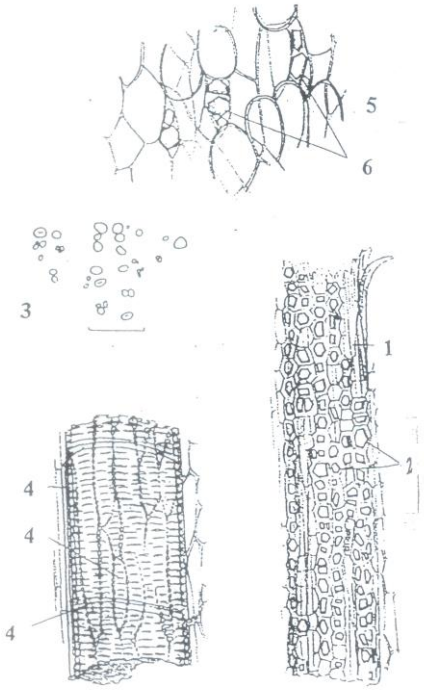
The external layer of the secondary thickened root is the periderm. Below the periderm is the primary cortex parenchym (dilatation zone). Then we find the phloem which is devided by phloem rays and pit medullary rays. The parenchymatic cells of the phloem contain starch. The phloem consists of hard and soft parts. The sieve tubes of the soft-phloem, close to the cambium are functionable (transportation), but further from cambium we find non-functional sieve tissue (keratenchym). The hard phloem consists of sclerenchymatous phloem fibre bundles and locular fibres containing single Ca-oxalate crystals.
The cambium is buit of several cell-lines. The wood part is devided by pit and xylem medullary rays. In the xylem medullary rays, tracheidas, tracheas, xylem fibres and locular fibres can be found.



1 = periderm
2 = outer cortex
3 = pit-medullary ray
4 = phloem fibre
5 = phloem parenchym
6 = locular fibre
7 = cambium
8 = xylem fibre
9 = trachea

Powder preparation of Liquiritiae radix

The powder is characterized by sclerenchymatous phloem fibre bundles and locular fibres containing Ca-oxalate single cubic crystals. The parenchym fragiles contain starch. In the unpeeled drug fragments of the cork also appear.

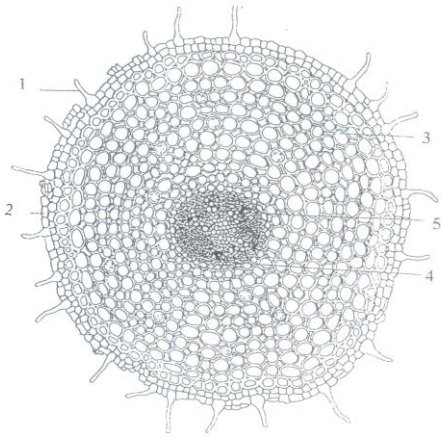


1 = phloem fibre
2 = locular fibres
3 = starch
4 = trachea of pittily thikened wall
5 = cortex parenchym
6 = $Ca(C00)_2$ single cubic crystals

Cross section of Primulae radix

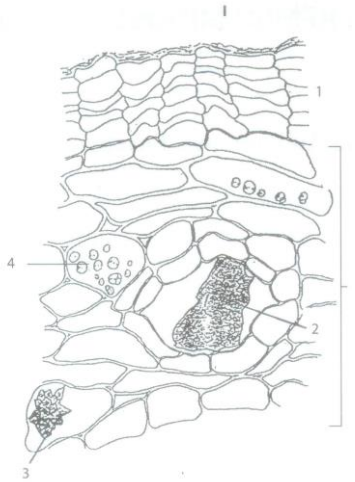
Primulae radix is a primarily thickened root. Some cells of the rhyzoderm transformed to root-hairs. Inside the rhizoderm we find the wide primary cortex. It consists of 15-20 starch containing parenchymatous cell-layers. Between the central cylinder and the cortex, a one cell-line thick endodermis can be seen. The endodermis consists of cells, wich radial walls has Caspary dotted thickenings. The central clinder contains xylem and phloem boundles which form rings in older root.

1 = root-hair
2 = rhyzoderm
3 = cortex parenchym
4 = endoderm
5 = xylem vessels



Cross section of Ginseng radix

Below of the periderm the cortex can be found, which consists of the external dilatation zone (cortex-parenchym) and the phloem, which is separated from the xylem by the cambium. The xylem is divided by xylem rays and pitty medullary rays.



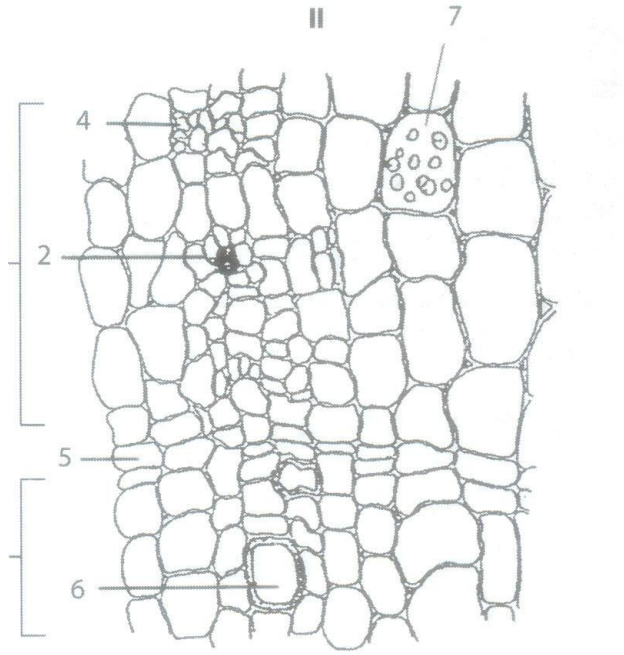
cortex

I. Periderm and dilatation zone
1 = periderm
2 = schizogene oil containing cell
3 = calcium oxalate cluster crystals
4 = starches in parenchima

II. Phloem- and xylem parts
2 = oil containing cells
4 = sive-tube group
5 = cambium
6 = trachea in xylem body
7 = starches in parenchyma

phloem

xylem



3. PHISICOCHEMICAL AND CHEMICAL QUALITATIVE INVESTIGATIONS

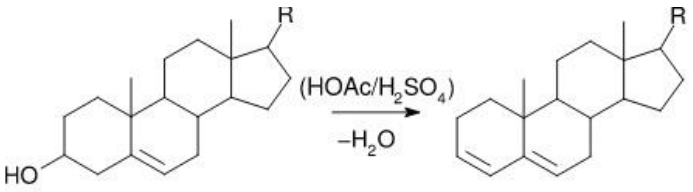
3.1. Detection of saponins (triterpenes, sterins) by Liebermann-Bourchard reaction.

Samples

- Saponariae albae radix
- Liquiritiae radix
- Hederae folium
- Hippocastani folium
- Hippocastani semen

Shake 1 g of powdered drug with 10 ml of chloroform in ultrasonic bath for 2 minutes, filter through a filter paper and evaporate the filtrate in a porcelain dish to dryness. Dissolve the residue in 1 ml of glacial acetic acid and transfer cautiously over 1 ml of cc. sulphuric acid in a test tube. The ring at the meeting surface of the liquid phases becomes brown or reddish brown; the colour of the glacial acetic acid layer is depending on the drug applied.

Reaction: Conjugated double bound system is forming by the loose of water molecule from the secondary alcohol group in the third position of the triterpene ring-system.



3.2 TLC detection of saponins

3.2.1. TLC examination of saponins in Hederae folium, Hippocastani semen, and Saponariae albae radix

Sample preparation:
Boil 1 g of crude drug powder with 20 ml of 70% ethanol on water bath for 20 minutes. Filter and evaporate the extract to 4.0 ml volume. Investigate 10 µl of the solution by TLC, beside saponin reference solution (0.1%, 10µl).

Chromatographic parameters:

Adsorbent:	Kieselgel G60 F_{254}
Developing system:	n-butanol-acetic acid-H_2O (3:1:1)
Test solution	aescin standard 2%, 5 µl
Dying reagent:	sulphuric acidic anis aldehyde
	spray and warm it at 100°C for 5 minutes
Evaluation:	visually

3.2.2. TLC examination of saponins in Ginseng radix (according to prescription of Ph. Eur.)

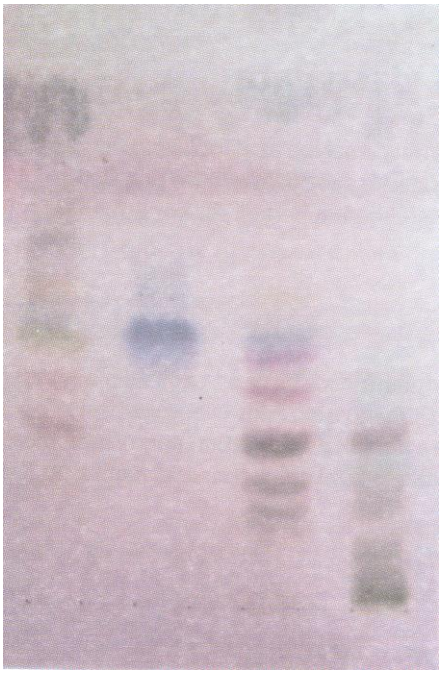
Boil 1 g of crude drug powder with 10 ml of 70% methanol on water bath for 15 minutes. Filter and fill up the extract to 10 ml. Study 10 µl of the solution by TLC.

Chromatographic parameters:

Adsorbent:	Kieselgel G60 F_{254}
Developing system:	ethyl acetate- H_2O -1-butanol (25:50:100 V/V)
	use the upper phase!
Dying reagent:	sulphuric acidic anis aldehyde
	spray and warm it at 100°C for 5 minutes
Test solution	aescin standard 2%, 5 µl and saponin 0,1% (Fluka), 10 µl
Evaluation:	visually (see below, layer **B**)

Evaluation ot the TLC layers:

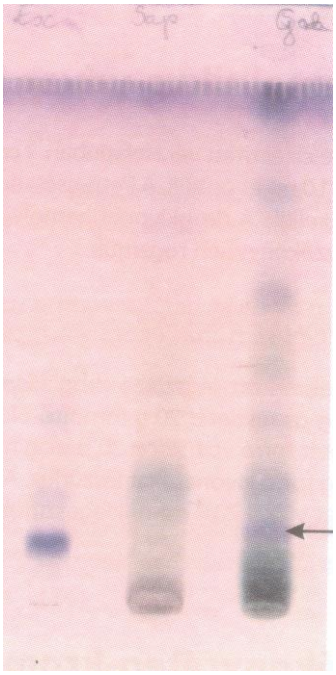
A) TLC examination of saponins



1. 2. 3. 4.

1. Hederae folium 10 µl
2. Aescin standard 2% 10 µl
3. Hippocastani semen 5 µl
4. Saponariae albae radix 10 µl

B) TLC examination of ginsenosids



Ginsenosid Rb1

1. 2. 3.

1. Aescin standard 2% 5 µl
2. Saponin 0,1% (Fluka) 10 µl
3. Ginseng radix extract 10 µl

4. QUANTITATIVE EVALUATIONS

4.1. Determination of foam number

Samples

Saponariae albae radix
Liquiritiae radix
Hederae folium

Definition of foam number

The biggest dilution of the extract (relative to 1 g of the crude drug) which gives 1 cm high foam if its 10 ml portion is shaken for 15 seconds in test tube of 16 mm and leaved to stand for 15 minutes.

Description of determination

Warm 0.25 g of crude drug with 100 ml of water for 30 minutes on water bath. Filter it on humidified cotton plug and complete its volume to 100 ml with water (stock solution).

Prepare the dilutions below of the stock solution complete to 10,0 ml with water:

	1. tube	**2. tube**	**3. tube**	**4. tube**	**5. tube**
stock solution	0.4 ml	1.0 ml	2.0 ml	4.0 ml	8.0 ml
water	9.6 ml	9.0 ml	8.0 ml	6.0 ml	2.0 ml

Shake the tubes for 15 sec and leave to stand for 15 min.
Search the dilution, where the thickness of the foam is 1.0 cm.

Calculation

$$F = \frac{10}{p}$$

F = foam number of crude drug
p = the amount (g) of crude drug in the test tube in wich the foam is 1 cm thick

4.2. Determination of haemolytic index (Ph.Hg.VII.)

Reagents

Buffer solution (pH = 7.4): 1.743 g of potassium hydrogen-phosphate (KH_2PO_4), 9.596 g of disodium hydrogen-phosphate (Na_2HPO_4 $2H_2O$) and 9.00 g of sodium chloride is solved with water to 1000 ml.

2% blood suspension. Freshly prepared solution of 10.00 ml of defibrinated bovine blood in buffer solution (500 ml).

Preparation of Extracts

Saponariae albae radix 0.5 g

Hederae folium 1.0 g

Weigh the powdered drug into a 250 ml flask and add 100 g of buffer solution. Heat the flask covered by a small glass funnel on hot water bath for 30 minutes, agitating the content of the flask four times by concentric swinging. Filter the hot extract into a 100 ml volumetric flask through a piece of cotton plug, placed between two layers of gauze. Leave the extract to cool down and then complete it with buffer solution to the sign.

Informative Test of Extract

Transfer 0.2, 0.4, 0.6, 0.8, 1.00 and 1.2 ml of extract into test tubes, complete the extracts to 5.0 ml with buffer solution, and add 5 ml of blood suspension to each test tube:

Test tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Drug extract (ml)	0.2	0.4	0.6	0.8	1.0	1.2	1.4
Buffer solution (ml)	4.8	4.6	4.4	4.2	4.0	3.8	3.6
Blood suspension (ml)	5.0	5.0	5.0	5.0	5.0	5.0	5.0

Homogenize the content of the test tubes by turning the mouth of the test tube upside down. Strong agitation should be avoided in order to prevent foaming. Observe the results of the informative test in 2 hours. The *limit* is indicated by the test tube the content of which is still transparent, but the next one is already opalescent, in the order of decreasing drug amount.

Calculate the hemolytic index (HI) of the drug with the help of the following formula:

$$HI = F \times \frac{10}{p}$$

F= blood factor

p = drug content of the extract filled into the test tube, which represents the limit.

Determination of the blood factor (F): Add increasing amounts of 0.02 % R-saponin solution (0.0200 g of saponin is solved to 100 ml with buffer solution) into 7 test tubes, starting from 1 ml, increasing by 0.5 ml in each test tube, and complete with buffer solution to 5.0 ml; finally add 5.0 ml of blood suspension into each test tube. Perform the informative test in the way described for the drug extract. Establish the apparent hemolytic index (hi_s) of R-saponin solution. The blood factor can be obtained by dividing the standard hemolytic index (25 000) with the apparent hemolytic index (hi_s)

$$F = 25\,000 / hi_s$$