CRUDE DRUGS CONTAINING ALKALOIDS
(tropane, ecgonin and piperidin structures)

THEME

1. MACROMORPHOLOGICAL EVALUATION
   - Belladonnae folium et radix
   - Hyoscyami folium
   - Stramonii folium
   - Cocae folium
   - Conii fructus

2. MICROSCOPICAL TESTS
   - Cross section: Belladonnae folium
                     Belladonnae radix
                     Hyoscyami folium
                     Stramonii folium
   - Clarified leaves: Belladonnae folium
                       Hyoscyami folium
                       Stramonii folium
   - Powdered preparations: Belladonnae folium
                            Belladonnae radix
                            Hyoscyami folium
                            Stramonii folium

3. PHYSICO-CHEMICAL AND CHEMICAL TEST

3.1. Test tube reactions
3.1.1. Universal reactions for detections of alkaloids:
   - Mayer-R (K₂HgI₄)
   - Wagner-R (KI I₂)
   - Hager-R (picrinic acid)
   - Dragendorff-R (KBI₄)

3.1.2. Specific reaction for the detection of alkaloids by Vitali reaction

3.2. TLC detection of tropane alkaloids in Solanaceae drugs
4. ASSAY OF ALKALOID CONTENT IN SOLANACEAE DRUGS

4.1. Main steps of determination of alkaloid content.
Extraction, purification, volumetric titrations.

4.2. Determination of alkaloid content by volumetric titration
A) in wateric (aquous) medium (Ph.Hg.VIII., Ph.Eur)
B) in nonaquous medium (Ph.Hg.VII.)

4.3. Apparatus for alkaloid extractions
A) Soxhlet apparatus
B) Lőrinc-Szász apparatus

Report
Evaluation of detection of alkaloid reactions
Detection of tropane alkaloids by TLC
Results of determination of alkaloid content in Solanaceae drugs
1. MACROMORPHOLOGICAL TESTS

**Belladonnae folium**
*Atropa belladonna* L.
Ph.Hg.VIII. Ph.Eur.

Belladonna leaves
Solanaceae

The leaves are dull or yellowish green, 0.5-4 cm long and a broadly ovate. The upper side is somewhat darker than the lower. It is thin, film-like, brittle and often badly attacked by insects.

The drug is odourless and bitter taste.

**Belladonna radix**
*Atropa belladonna* L.
Ph.Hg.VII.

Belladonna root
Solanaceae

The roots are 10-15 cm in length and 1 or 4 cm in diameter. The root breaks “mealy” and shows a whiteness or brownish interior. The outer surface is pale greyish-brown and wrinkled. The crude drug is odourless and bitter taste.

**Hyoscyami folium**
*Hyoscyamus niger* L.
Ph.Hg.VII., Ph.Eur.

Henbane
Solanaceae

The leaves are more or less broken but are characterised by their greyish-green colour, great hairiness, especially in the neighbourhood of the midrib and veins.

The leaves have a characteristic, heavy odour and a bitter, slightly acrid taste.
**Stramonii folium**

*Datura stramonium* L.  
Solanaceae

Ph.Hg.VIII., Ph.Eur.

Whole leaves are 8-25 cm long and 7-15 cm wide greyish-green in colour, thin, rittle, twisted and often broken.

The crude drug has slightly overpowering odour and disagreeable taste.

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**Cocae folium**

*Coca leaf*

*Erythroxylon coca* Lam.  
Erythroxylaceae

The leaf is 3-6 cm long, egg-shaped, the margin is entire, the venation is winged. Two sclerenchymaticus ribs are characteristic, parallel with the midrib, on the lower surface of the lamina.

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**Conii fructus**

*Hemlock fruit*

*Conium maculatum* L.  
Apiaceae (Umbelliferae)

The fruit is a broadly ovate, very similar to anise. It bears a small stylopod and the remains of the stigmas. Its ribs are twisty.
2. MICROSCOPICAL TESTS

**Belladonnae folium** (transverse section)

1. upper epidermis
2. trichome
3. Solanaceae trichome
4. palisade tissue
5. lower epidermis
6. Ca(COO)2 sandy crystal
7. collenchyma
8. phloem
9. xylem

**Belladonnae folium** (clarified leaf)

1. upper epidermis
2. stomata
3. palisade
4. cell containing Ca(COO)2 cryst.
5. main vein
6. lower epidermis
7. part of vein
8. glandular trichome

**Belladonnae folium** (powder preparation)

1. idioblast of sandy crystals of Ca-oxalate
2. glandular hair
3. palisade parenchyma
4. spongy parenchyma
5. venation
Belladonnae radix (transverse section)

1. periderm
2. cork tissue (dilatation zone)
3. parenchyma (secondary cortex)
4. cambium (of several layers)
5. xylem
6. trachea
7. idioblast cell (Ca-oxalate)

Belladonnae radix (powder preparation)

1. starch
2. parenchyma of the primer cortex
3. idioblast cell (Ca (COO)2 sandy crystal
4. trachea of pitted thicked wall
**Hyoscyami folium** (transverse section)
1. epidermis
2. stomata
3. covering hair
4. palisade parenchyma
5. spongy parenchyma
6. single Ca-oxalate crystal (crystal layer)
7. phloem
8. xylem

**Hyoscyami folium** (clarified leaf)
1. venation with cyclic trachea
2. palisade cells
3. Ca-oxalate single crystals
4. glandular trichome
5. covering hair
6. upper epidermal cells
7. stomata
8. base cell of covering hair

**Hyoscyami folium** (powder preparation)
1. Ca-oxalate single crystals
2. covering hair
3. trichome
4. venation
5. palisade
6. spongy
**Stramonii folium** (transverse section)

1. upper epidermis  
2. palisade parenchyma  
3. spongy parenchyma  
4. Ca-oxalate rosette (crystal layer)  
5. collenchymatous hypodermis  
6. phloem  
7. xylem

**Stramonii folium** (clarified leaf)

1. vein-isle  
2. venation with cyclic trachea  
3. palisade cells  
4. ca-oxalate rosette  
5. upper epidermis  
6. lower epidermis  
7. stomata

**Stramonii folium** (powder preparation)

1. Ca-oxalate rosette  
2. covering trichome  
3. Solanaceae trichome  
4. palisade parenchyma  
5. spongy parenchyma
DISTINCTION OF SOLANACEAE DRUGS ON THE BASIS OF THEIR CALCIUM OXALATE CRYSTAL FORMATIONS ACCUMULATING IN COLLECTOR CELL LAYER

1. epiderm
1a. covering trichome
1b. glandular trichome
1c. stoma
2. palisade parenchyma
3a. Ca oxalate sandy
3b. Ca oxalate rosette
3c. single crystal
4. spongy parenchyma
3. PHYSICO-CHEMICAL AND CHEMICAL TESTS

3.1. Test tube reactions.

3.1.1. Universal reactions for the detection of alkaloids

Stramonii folium.
Belladonnae radix

Extraction:
1 g of the crude drug powder is boiled with 20 ml solution of 0.2 M $\text{H}_2\text{SO}_4$
The extract is cooled and filtered through cotton. This sulphuric acid solution is the
"standard solution" which is used to the reactions:

A) 2 ml of standard solution + several drops of Mayer reagent ($\text{K}_2\text{HgJ}_4$)
B) 2 ml of standard solution + several drops of Wagner reagent ($\text{KJ J}_2$)
C) 2 ml of standard solution + several drops of Hager reagent (picrinic acid)
D) 2 ml of standard solution + several drops of Dragendorff reagent ($\text{KBiJ}_4$)

Remark: The listed reagents respectively complex heavy metal salts give precipitate or
coloured reaction-product with alkaloids.

3.1.2. Specific reaction for the detection of tropane alkaloids by Vitali reaction

Stramonii folium
Belladonnae radix
Belladonae folium

Extraction:
1 g of the crude drug powder is boiled with 20 ml solution of 0.2 M $\text{H}_2\text{SO}_4$ (1)
The extract is cooled and filtered through cotton and ~2 ml of 10 % $\text{NH}_4\text{OH}$ is
admixed (pH-9) and extracted with 20 ml of chloroform. (2) The chloroformic phase is
evaporated on waterbath.

Reaction:
The residue (the extract containing alkaloids) is solved in 5 drops of cc. Nitric acid and
evaporated again.
On the dry residue one KOH crystal is put and throw with 2 drops of ethanol. The
ethanolic solution and also the KOH-crystal show violet colouring (3).

\[ \text{I.} \] \[ \text{II.} \]
\[ \text{III. (violet product)} \]
Remarks:
(1.) The acid extracts contain the alkaloids (as form as salt) rather selectively, so the chlorophyll and other pigments remain in the drug back and don't disturb the colour-reaction.
(2.) Ammonia liberates the alkaloid-basics from their salts so they can be dissolved in organic solvent
(3). Essence of Vitali reaction:
During the reaction the tropic acid is nitrated forming 4-nitro-atropin-nitric acid ester (I).
Compound I. is decomposed and formed in parts 4-nitro-apoatropin(II) which is coloured violet or reddish violet by the effect of strong alkali (III).

3.2. TLC detection of tropane alkaloids

*Belladonae folium*

*Stramonii folium*

The extracts are prepared from 1 g of drugs. See 3.1.2. „Extraction: 1 g…

The chloroformic phase is evaporated on waterbath and the residue is dissolved in ethanol (1 ml) 10 and 20 µl of ethanolic extracts are investigated by TLC.

Reference solution:
- atropine 5 µl
- scopolamine 5 µl

TLC conditions: Kieselgel 60 F 254

Developing system: CHCl₃: CH₃OH: cc. NH₄OH - 100:18:2

Reagent: Dragendorff-R (KBiJ₄)

Valuation of the chromatogram:
- Rf of atropine: 0.4
- Rf of scopolamine: 0.8

4. ASSAY OF ALKALOID CONTENT IN SOLANACEAE DRUGS

4.1. Main steps of determination of alkaloid content

Extraction: The extraction is carried out with chloroform after liberation of the alkaloids from their salts by NH₄OH. (Consider that the alkaloids are mostly bound to organic acids in the plants.)

Purification:

The alkaloids (being present in chloroformic extract) are taken into aqueous solution by salt forming (of H₂SO₄). So the substances of impurity remain in organic solvent
back. From the acidic-aqueous solution the alkaloids dissolved in solution of organic solvent (after free up by basic).

Volumetric titration:

The alkaloid content of the above mentioned purified alkaloid extract is determined by volumetric titration in aqueous or non-aqueous medium.

4.2. Determination of alkaloid content by volumetric titration

Samples:

*Belladonnae folium*

*Stramonii folium*

3 g of crude drug powder - measured with mg accuracy - is smudged in a porcelain mortar with the mixture of 2 ml of 25 % NH₄OH and 8 ml of water.

1. The smudged crude drug is put in an Erlenmeyer flask having grinded neck and shaked with 20 ml of chloroform for 20 minutes, in ultrasonic bath. The solution is filtrated through cotton and the remained crude drug is extracted once more with 20 ml of chloroform for 15 minutes. Then it is also filtrated. The united chloroformic solution is shaked out with 2×10 ml of 2% H₂SO₄.

*The acidic solution is alkalined with cc NH₄OH to turn pH: 8-9 and shaked out with 3×20 ml of CHCl₃. The chloroformic solution is filtered through cotton wool covered by Na₂SO₄ sicc., (to remove the traces of water!), and evaporated on apparat-Rotedest (need to remove also the traces of NH₃!)*

A) Acid-basic volumetric titration in watric medium.

The residue (the purified alkaloid extract) is soluted in 10,00 ml of 0,02 n H₂SO₄ measuring solution then, in presence of 3-4 drops of methyl-red indicator, the excess of sulphuric acid is measured with 0,02 n NaOH back. Calculation of alkaloid content (%) in term of atropin

\[
\% = \frac{(10-a) \times 5,787}{b \times 10}
\]

a: number of ml of 0,02 n NaOH used up

5,787:1 ml of 0,02 n H₂SO₄ measures 5,787 mg of atropin

b: weight (g) of crude drug, in this case: 3
B) Volumetric titration in nonaqueous medium by perchloric acid.

The residue (the purified alkaloid extract) is dissolved in 5 ml of choloform and add 5 ml of ice acetic acid. The solution is titrated with perchloric acid (0,02 n HClO₄), in presence of 2 drops of gentiana-violet indicator.

Calculation of alkaloid content (%) in term of atropin

\[
\% = \frac{a \cdot f \cdot 5,787}{b \cdot 10}
\]

a: number of ml of 0,02 n HC1O₄ used up

f: factor of 0,02 n HC1O₄ solution

5,787: 1 ml of 2 n HC1O₄ measures 5,787 mg of atropin

b: weight (g) of crude drug, in this case: 3

II, Put the smudged crude drug in to the extractor socket of Soxhlet apparatus and carry out the extraction with CHCl₃ for 1 hour. Then evaporate the solution to 20 ml of volume and shake out with 2×10 ml of 2% H₂SO₄. Continue from*

III, Put the smudged crude drug into the extractor socket of Lőrincz-Szász apparatus. Carry out the extraction with benzene and 2×20 ml of 2% H₂SO₄.

Continue from.*
4.3. Apparatus for alkaloid extractions

A/ Soxhlet apparatus (Fig.1.)

a) a round flask solvent, rather to collection of solution
b) the Soxhlet appurtenance crude drug treated by alkali or the swollen crude drug
c) refluxing cooler

The Soxhlet apparatus is an universal solid-liquid extractor.
The portioning of the extraction is continuous. The exchange of fresh (clean) solvent (the liquid phase) is periodical through the suction pipe.
The temperature of the extraction is in agreement with the boiling point of the solvent (or it is a little higher than that).

Advantage of the apparatus

- It can be used - beside the alkaloid extraction - also for the extraction of different type of compounds.
- The higher temperature increases the effectiveness of the extraction.

Disadvantage of the apparatus

- It is not suitable for extraction of thermolabil compounds (at atmospherically pressure)
**B/ Lőrincz-Szász apparatus**

Its construction can be seen on the Fig.2.

The apparatus involves a solid-liquid: crude drug-organic solvent (e.g. benzene or toluene) and liquid-liquid (e.g. benzene-sulphuric acid) extractor.

It is suitable not only for the alkaloid extraction but the purification (the "exchange" of phase) too.

For the effect of the pressure-difference the organic solvent can circulate throughout the crude drug and the acidic-wateric phase. According to the construction, the organic phase and the wateric phase can intensive meet, so the alkaloids can be soluted into the acidic-wateric phase. The condition of the operating is the difference between the density of the organic and inorganic liquid phases. (in case of benzene-sulphuric acid apparatus of "light phase"; in case of chloroform-sulphuric acid apparatus of "heavy phase" has to be used.)

**Advantage of the apparatus**
- Extraction and purification can be carried out in the same apparatus
- It is suitable for extraction of thermolabil alkaloids too.

**Disadvantage of the apparatus**
- The operating of the apparatus is more complicated than the Soxhlet apparatus.

![Diagram of the apparatus](image-url)
Changing of alkaloid content during the extraction time

1. in the Soxhlet apparatus

2. in the Lőrincz-SzáSZ apparatus