CRUDE DRUGS CONTAINING INDOLE, PURINE AND MISCELLANEOUS (STEROIDAL, TROPOLONE) ALKALOIDS

1. MACROMORPHOLOGICAL EVALUATION

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<td>Coffea semen</td>
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<tr>
<td>Vincae minoris herba</td>
<td>Colea semen</td>
</tr>
<tr>
<td>Rauwolfiae radix</td>
<td>Thea folium</td>
</tr>
<tr>
<td>Passiflorae herba</td>
<td>Mate folium</td>
</tr>
<tr>
<td>Uncariae cortex</td>
<td></td>
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<tr>
<td>Secale cornutum</td>
<td>Colchici semen</td>
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<td>Strychni semen</td>
<td>Veratri rhizoma et radix</td>
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### 1. MACROMORPHOLOGICAL EVALUATION

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<thead>
<tr>
<th>Plant</th>
<th>Common Name</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thea sinensis L.</td>
<td>Tea</td>
<td>Camelliaceae</td>
</tr>
<tr>
<td>(Thea sinensis L.)</td>
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</tbody>
</table>

The leaves are obtained from the 6 m tall evergreen shrub. (Two variants are well known: var. sinensis has lance-shaped, 8-10 cm long leaves; var. assamica has larger longitudinally, pointing leaves). The leaves are lance- or elliptical shaped, coriaceous and naked, with neat serrated edges, the main rib protrudes to the lower surface. When quite young the leaves may be more or less hairy. The drug has a characteristic odour, it has an astringent bitter taste.

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<tr>
<td>Coffea arabica</td>
<td>Coffee</td>
<td>Rubiaceae</td>
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</table>

The beans are oval – concave on one side, flat on the other, with a central longitudinal groove, they are 8-10 mm long, 4-6 broad, grey-green when fresh and brown when roasted.

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<tr>
<td>Cola vera K.Schum</td>
<td>Kola nut</td>
<td>Sterculiaceae</td>
</tr>
</tbody>
</table>

It consists of the dried cotyledons of the seeds of Cola vera. The whole seeds are 2-5 cm in length and in the seeds usually imported there are two cotyledons. The dried cotyledons are usually of a dull, reddish-brown colour and more or less broken. They are usually graded as „halves” and „quarters”. Odourless, slightly astringent.
Secale cornutum
*Claviceps purpurea* Tulasne

Ergot

Hypocreaceae

Ergot is the dried sclerotium of a parasite ascus fungus the *Claviceps purpurea*, arising in the ovary of the rye.

The drug consists almost entirely of sclerotia, the amount of other organic matter being officially limited to not more than 1 per cent.

Each sclerotium is about 1.0 to 4 cm long and from 2 to 7 mm broad; fusiform in shape and usually slightly curved.

The outer surface, which is of a dark, violet-black colour, is often longitudinally furrowed and may bear small transverse cracks.

Ergot breaks with a short fracture and shows within white colouring. It has a characteristic odour and an unpleasant taste.

Strychni semen

*Nux vomica*

*Nux vomica*

Loganiaceae

Nux vomica consists of the dried, ripe seeds of *Strychnos nux-vomica*, a tree – 10-13 m high – found from India to North Australia.

The seeds are extremely hard, (as horn) light grey in color, silky, disc-shaped, 10-25 mm in diameter, 3-5 mm in thickness.

Most of the seeds are nearly flat and regular in shape but in the middle of the surface there is the hardly prominent hilum, from which a line – coming from the convergence of the hairs – run to the micropyle: at the margin of the seed.

It is odourless, taste very bitter.
Vincae minoris herba  
Vinca minor L.  
Common periwinkle  
Apocynaceae  
It is a perennial, evergreen, 20-30 cm tall plant. Rhizome and trailers develop, so it is very difficult to define the size of a plant, as its trailers stretch radially in every direction. Its vertical rhizome is short and brown-coloured. Its trailers have long internodes. The leaves are opposite, shortly petiolate, entire margin and egg-shaped, glabrous. The flowers stand single on the top of the axis. The characteristics of the flower of Apocynaceae are standard. The corolla is bluish-purple, with a conical tube and 5 asymmetrical lobes. The stamens number 5. The styles are united to form a column. The fruit consists of follicles. It is odourless, taste bitter.

Rauwolfiae radix  
Rauwolfia serpentina L.  
Rauwolfia root  
Apocynaceae  
The crude drug consists of the roots. They are 2-12 cm length and 4-20 mm in diameter. The roots are rarely branched or wound. The outer surface is greyish-yellow, light brown or brown with slight wrinkles (young pieces) or longitudinal ridges (older pieces). The recently dried drug has a slight odour which seems to decrease with age. Taste bitter.

Catharanthi rosei herba  
Catharanthus roseus (L.)  
(Vinca rosea L.)  
Madagascan periwinkle  
Apocynaceae  
It is a herbaceous subshrub, 40-80 cm high becoming woody at the base. The leaves are oppositely arranged oblong with a petiolate acute base, a rounded apex and an entire margin. The flowers are showy and reminiscent of those of the common periwinkle: constructed as a type 5, they are coloured violet, rose, white or white with a red eye. The fruit is a divergent follicle. It is odourless, taste bitter.
**Veratri rhizome et radix**  
*Veratrum album* L.  
White Veratrum rhizome  
Liliaceae

Ph.Hg.VIII.

The drug consists of the rhizome and the roots of the perennial plant *Veratrum album* L. growing sporadically in Hungary, too. The rhizome – yielding the drug – is 2.5-6 cm long, 2-4 cm wide truncated obconical, of carneous consistency, dark brown bark; white inside. The lower part gradually decays, the upper part ramifies. On the rhizome there are many rests of cicatrices and leaf vaginae, lending an annular surface to the rhizome. All around the rhizome, secondary roots grow, often 30 cm long, 3 mm wide outside light brown or straw-coloured, inside white. The odourless powder incites sneezing; taste permanently bitter, hot.

**Passiflorae herba**  
*Passiflora incarnate* L.  
Maypops (passionflower; mayhaw)  
Passifloraceae

Purple passionflower is a native, perennial vine. The slightly pubescent vines climb with tendrils that arise from the axils of the leaves. The vines can range from 2 to 6 m long. The alternate leaves (6 to 15 cm long and wide) are palmate with 3 lobes and finely serrated margins. The spectacular flowers are pale-lavender or, rarely, white, with five petals (3 to 4 cm long, 4 to 7 mm wide) and five sepals (2.5 to 3.5 cm long). The complex flower has a “crown” or corona of numerous fringelike segments that arise from above the petals. The corona is white or lavender with purple bands. The reproductive parts are interestingly arranged and add to the exotic beauty of the flower. The unique appearance of the flowers was purported, by early Spanish explorers, to represent the sufferings of Christ. Sweet-smelling, yellowish fruits develop in two to three months after flowering. The pulpy fruit, or “maypop”, is large and oval, about the size of a hen’s egg (4 to 10 cm long). The fruit contains many flattened, dark-colored seeds that are covered with an arillate pulp, which is the edible portion of the fruit.
Ilex paraguariensis
Aquifoliaceae
Mate tea

Active Ingredients:

Mate contains: Xanthene alkaloids (1-2% caffeine, 0.45-0.9% theobromine, 0.05% theophylline); tannin-like substances (4-16% caffeic and chlorogenic acids); the amines choline and trigonelline; amino acids; the flavonoids kaempferol, quercetin, and rutin; ursolic acid; vitamins B2, B6, C, niacin, and pantothenic acid, and volatile oil.

<table>
<thead>
<tr>
<th></th>
<th>( R_1 )</th>
<th>( R_2 )</th>
<th>( R_3 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coffee</td>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>Theobromin</td>
<td>( \text{H} )</td>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
</tr>
<tr>
<td>Theophyllin</td>
<td>( \text{CH}_3 )</td>
<td>( \text{CH}_3 )</td>
<td>( \text{H} )</td>
</tr>
<tr>
<td>Xanthin</td>
<td>( \text{H} )</td>
<td>( \text{H} )</td>
<td>( \text{H} )</td>
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</table>

Chlorogenic acid
Recently, Cat’s claw has become known as a healing plant with an ethnomedicinal background. There have been several reports on its constituents, in particular, oxindole alkaloids. It was found that 2 chemotypes of Uncaria tomentosa with different alkaloid patterns occur in nature. The roots of one type contain pentacyclic oxindoles and the other contains tetracyclic oxindoles. This difference should be considered when the plant is to be used for medicinal applications. Tetracyclic oxindole alkaloids act on the central nervous system, whereas pentacyclic oxindole alkaloids affect the cellular immune system. Recent studies have shown that the tetracyclic alkaloids exert antagonistic effects on the action of the pentacyclic alkaloids. Mixtures of these 2 types of drugs are therefore unsuitable for medicinal uses.

**Major chemical constituents**

Phillipson et al., 1978; Ugaz et al., 1994; Wagner et al., 1985
MICROSCOPICAL TESTS

Coffeae semen
I. Part of endospermium (cross section) with cells of thickened wall (pearllike)
II. Seed-coat with groups of stone cells of pitty thickened wall

Theae folium (cross section)
1. epidermis
2. stoma
3. unicellular trichomes
4. palisade parenchyma
5. spongy parenchyma
6. collateral vascular bundle
7. fibre group
8. bundle-cod
9. collenchyma
10. idioblast (lignified sclereid)
11. calcium oxalate cluster crystals
Transverse section of Veratri rhizoma

1. metaderma
2. starch
3. acicular crystals of calcium oxalate
4. endodermis of cells thickened in U-shaped
5. collateral, closed vascular bundle
6. amphivasal, closed vascular bundle
7. vascular bundle, horseshoe shaped
**Transverse section of Secale cornutum**

1. pigment layer containing sclerithrine
2. pseudoparenchymatous tissue (discontinous hypha tissue)

**Transverse section of Strychni semen**

1. Unicellular trichomes (clothing hairs) formed from the epidermal cells. The trichomes have an onion-shaped base and a wall thickened strongly by pits. They incline at angles 45° in the same direction adhese closely to seed.
2. compressed layer: brownish walled cells form several rows of seed-coat layer
3. endospermium, outer layer
4. endospermium, innerlayer
Uncaria tomentosa pulvis

Non shared characteristics of the bark.

A: microcrystals of Ca(COO)_2 from ray cells (longitudinal radial section)
B: phloem fibers
C: starch grains in cells of ray (transverse section)
3. PHYSICO-CHEMICAL AND CHEMICAL TESTS

3.1. Test-tube reactions

*Coffeae semen*

*Murexid test* (for purine alkaloids in *Coffeae semen*)

**Extraction**
Heat 0.5 g of drug powder with 5 ml of n H₂SO₄ till boiling then filter it through paper and add 5 ml of 6 n NH₄OH (pH=8-9) and shake it out with 5 ml of CHCl₃. The chloroformic phase is filtered through cotton wool and evaporated.

**Reaction**
To the dry residue 3 drops of cc H₂O₂ and 1-2 drops of cc HCl are added and evaporated again. A red colour appears which turn to violet from a few drops of NH₃ solution.

*Prepare microsublimate* from ~ 0.5 g of coffee powder, collect the obtained caffeine crystals and identify with Murexid test.

*Essence of Murexid reaction*
In acidic medium, for an oxidative effect the imidazole ring of the purin structure is decomposed and the two pirimidin rests are connected together in an alcalic medium forming ammonium salt of purpur acid $\rightarrow$ Murexid red.

*Secale cornutum*

Detection of trimethylamine and sclereritrine pigment in *Secale cornutum*

**trimethylamine test**
Moisten and rub the drug powder with R-NaOH. Vicious herring odour can be smelled.

**sclereritrine test**
1 g powder is shaken with 15 drops of 20 % sulphuric acid and 20 ml of chloroform for 5 min. The chloroformic solution is filtered. 15 drops of saturated NaHCO₃ wateric solution is added. The lower, aqueous phase shows first red then violet colouring.
Strichne semen
Detection of strychnine and brucine alkaloids in Strychni semen

strychnine test
Heat to boiling 2 g of drug powder in 20 ml of water and filter the liquid. Extract the filtrate with 10 ml of chloroform. Evaporate the chloroform layer to dryness. Moisten the residue with a few drops of concentrated R-sulphuric acid. Move a small potassium bichromate Crystal in the sulphuric acid. It will be followed by dirty violet-bluie stripes.

brucine test
Moisten the drug powder with a few drops of cc nitric acid, orangered colouring will be visible..

Veratri rhizome et radix
Identification of Veratrum alkaloids
Moisten the out surface of the root with 80 % sulphuric acid, first an orange yellow and then a red colouration occurs.

3.2. Caffein isolation from Coffeae semen, Theae folium, Mate folium, Colae semen

Extraction
5 g crude drug is cooked with 120 ml of dist. water for 15 minutes. After filtering and pressing of the drug the cooking is repeated with 80 ml of water (1).

Purification
To the united aqueous extracts 20 ml of 10 % alkalic Pb(Ac)₂ is added at 60-70 °C, the forming precipitate will be centrifuged (2). To the upper floating (the clean aqueous solution) 10 ml of Na₂SO₄ solution (4 g Na₂SO₄ cryst. + 10 ml water) is added, and the forming precipitate is centrifuged again (3).

Exchange of phase: the upper floating is alkalised to pH 8 and the solution is shaken out with 3 x 20 ml of CHCl₃. The untied chloroformic solution is dried over Na₂SO₄ sicc. and evaporated on Rotadest (4).
**Precipitation:** the greyish white residue is dissolved in 5 ml of dichlormethane. The solution is concentrated to 2 – 2.5 ml volume on waterbath (40-60 °C) and then 1-2 ml of petroleum ether is added. After a few minutes the forming precipitate (caffeine) is filtered and washed out with several ml of petroleum ether (5).

**Yield in per cent**
The mass \( (X_g) \) of the isolated caffeine is measured after drying of the crystals at 100 °C for 30 minutes.

\[
\text{Yield} \% = \frac{X \times 100}{10}
\]

**Remarks**
The centrifugation time is 5 minutes and the revolution per minute: 3000

1. The caffeine alkaloid is soluble in hot water.
2. The accompanying substances are removed in form of precipitate by alkaline Pb(Ac)\(_2\).
3. The excess of Pb\(^{2+}\) is removed in form of PbSO\(_4\) precipitate.
4. Caffeine is transferred into organic solvent.
5. The pure caffeine can be precipitated from the semipolaric solution by apolaric solvent.

**Universal alkaloid reactions**
from Veratri rhizoma

\(~ 0.5 \text{ g of Veratri rhizoma (powder) is warmed and boiled with 10 ml of 2 % H}_2\text{SO}_4 \text{ for 1-2 minutes. Then it is filtered and devided for 4 parts.}\)

- a) 2 ml of solution + few drops of Mayer-reagent
- b) 2 ml of solution + few drops of Wagner-reagent
- c) 2 ml of solution + few drops of Hager-reagent
- d) 2 ml of solution + few drops of Dragendorff-reagent

Veratri rhizoma et radix

<table>
<thead>
<tr>
<th>Mayer</th>
<th>Wagner</th>
<th>Hager</th>
<th>Dragendorff</th>
</tr>
</thead>
<tbody>
<tr>
<td>white precipitate</td>
<td>brown</td>
<td>yellow</td>
<td>dark-brown</td>
</tr>
</tbody>
</table>
3.3. **Caffeine detection by TLC**

Samples: Theae folium (green and black)
Coffeae semen

The caffeine isolated is dissolved in ~ 1 ml of CHCl₃ → 20 µl

Standard: caffeine solution 1 % - 10 µl

Developing system: ethylacetate-methanol-water (100 + 13.5 + 10)

Evaluation:

a) UV254
b) KJ-J2 and ethanolic HCl (96 % ethanol - 25 % HCl 1 + 1)

Rf of caffeine: 0.54

**Caffeine TLC**

\[
\begin{array}{cccc}
\text{caf. tee} & T1 & T2 & \text{mate cola} \\
\end{array}
\]

T1 = caffeine 10 µl  \quad \text{Rf} = 0.54
T2 = caffeine 15 µl

\[
\begin{array}{cccc}
\text{caf. tee} & T1 & T2 & \text{mate cola} \\
\end{array}
\]
4. ALKALOID DETECTION OF UNCARIA TOMENTOSA (CORTEX)

5 g of drug powder is extracted with ~ 40 ml of 2 % H₂SO₄ on supersoanic bath, then filtered and alkalined pH: 8-9, with cc NH₄OH and shake out with 2 x 20 ml of CHCl₃. The united chloroformic phase is dried with Na₂SO₄ sicc, filtered, evaporaled and the residue is dissolved in 1 ml of CHCl₃ → 40 µl TLC.

Developing system: ethylacetate - isopropanol – cc NH₄OH (100 + 2 + 1)

Standard: 1 % of pteropodine → 10 µl (Rᶠ: 0.8)

Evaulation:
  a) UV254
  b) Dragendorff reagent and NaNO₂ (10 %)

T = Pteropodine standard
U = Uncaria extract
5. ALKALOID DETECTION OF VINCAE MINORIS HERBA

5 g of drug powder is extracted with ~ 40 ml of 2 % H$_2$SO$_4$ on supersoanic bath, then filtered and alkalined pH: 8-9, with cc NH$_4$OH and shake out with 2 x 20 ml of CHCl$_3$. The united chloroformic phase is dried with Na$_2$SO$_4$ sicc, filtered, evaporaled and the residue is dissolved in 1 ml of CHCl$_3$ $\rightarrow$ 30 µl TLC.

5 µl of 1 % vincamin standard (R$_f$: 0.35)

Developing system: ethylacetate – methanol (9 + 1)

Evauation:

a) UV254
b) Dragendorff reagent and NaNO$_2$ (10 %)

T = vincamin
V = Vincae minoris herba extract