Pharmacognosy lab exercise 1



What is Pharmacognosy? What is a drug? How can we identify drugs? How to prepare microscopic preparations?



How to obtain course credits

<u>100% participation in practical lessons</u> – in case of one-time absence you will write a short paper on a given topic (2-3 standard pages)

<u>At least 60% of two tests (</u>12 points of 20 max.) Test 1 – 6th week of semester Test 2 – 10th week of semester

<u>Reports from lab exercises</u> – sketches of microscopic preparations, with designations of typical structures, possibly denote the magnification used for observation, sketches are made during the practical class and are handed in at the end of the that class



Pharmacognosy

Science about <u>drugs of natural origin</u> – plant, animal, microbial, which are used for treatment, prevention of diseases, and diagnostics and affection of physiological functions

Pharmacognosy relates to other pharmaceutical disciplines (pharmacology, medicinal chemistry, galenic pharmacy)

Starting material for isolation of natural medicinal products are <u>drugs</u>



Word origin: *droog waere* (flemish word for dry spices and herbs)

Modified or non-modified preserved **plants** or their parts; **animals** or their parts; or **products of metabolism** of plants, animals, or microbials (starch, mucilage, honey)

Drugs according to structure:

with organized body structure (cellular structure, *Malvae flos*) amorphous (products of metabolism, *Balsamum peruvianum*)

Drugs according to origin: plant-derived

animal-derived (whole animal – Cantharis, animal organs – glands, products – waxes, hormones, enzymes)

<u>Conservation</u> stops the decomposition of plant material after it has been removed from the mother plant

- freezing, freeze-drying
- preferred way of conservation drying



- direct sunlight is unsuitable
- in shade and well-ventilated rooms
- properly dried plant material contains most of its original constituents in the same amounts as before drying
- drying temperature, duration and method of drying depend on the constituents of the dried plant – plants containing volatile essential oils up to 40°C,
 - easily hydrolysed constituents (glycosides) fast drying with a higher temp.
 (enzyme inactivation) and then drying with usual drying temp. 60-70°C

Change of colour during the drying process:

- Plants with neutral cellular fluid almost no colour change
- Plants with acidic cellular fluid get dark
- Flowers with anthocyans content change colour from pinkish or red to purple or blue

We need to know:

Drying temperature Way of drying Length of drying Storage parameters (no direct sunlight, 5-15°C) Right labelling

Systematic classification of drugs

<u>Pharmaco-botanical</u> – according to the phylogenetic development of mother plants

<u>Pharmaco-chemical</u> – according to the chemical structure of content compounds

<u>Pharmacological</u> – according to the therapeutic effect of content compounds

<u>Biogenetic</u> – according to the biosynthetic origin of content compounds

Nomenclature of Drugs

Binomic – first part of name expresses mother plant (genus or species), second part expresses name of corresponding plant organ

Lini semen, Belladonnae radix, Conii fructus

One name - Lycopodium, Mel

Exceptions - *Liquiritiae radix, Cynosbati fructus*

It is necessary to state the mother plant and family each time

Folium (leaf) – plant leaves

Herba (herb) – whole aerial plant part

Flos (flower) - whole flowers or their parts (floral leaf petals), or whole inflorescence







Fructus (fruit) – whole fruit or infructescence, or fruit imitations

Pericarpium – pericarps of Rutacae family

Semen (seed) - seed or its part (germ)







Cortex (bark) – all kinds of plant tissues upon the cambium

Lignum (wood) – woody parts of plant only (xylem)





Stipes – drug contains peduncle only

Gemma - drug contains eye only

Sporae – drug contains spores only

Glandulae - drug contains glands only

Caulis - drug contains footstalk (stem) only

Strobilus - drug contains strobiles only







Drugs derived from underground plant organs

Radix – used for drug made of rhizomes, roots, bulbs and tubers



Nomenclature of plant products and most often used adjectives

Amylum - starch Balsamum - balm Resina – resin/oleoresin Gummi - mucilage Gummiresina – gum resin (bdellium) Cera - wax Oleum - oil, volatile oil

naturalis - natural *pulvis* - powdered *mundata* - peeled *maturus* - ripened *imaturus* - unripened amari - bitter dulcis - sweet

Review of traditional pharmacological effects of drugs

- astringent (adstringent)
- amarum
- anabolic
- analgesic
- anthelmintic
- antianemic
- antiarrhytmic
- antiasthmatic
- antidiabetic

- antidiarrhoic
- antidysmenoreic
- antiphlogistic
- antihemoroidal
- antihydrotic
- antirheumatic
- antisclerotic
- aromatic
- balneologic

Review of traditional pharmacological effects of drugs

- cardioactive
- cardiotonic
- carminative
- cholagogue
- cholekinetic
- choleretic
- corigens
- cytostatic
- derivans
- dermatic

- desinficiens
- diaphoretic
- diuretic
- emenagogue
- emetic
- expectorant
- gargarism
- gynecologic
- hypotensive

Review of traditional pharmacological effects of drugs

- lactagogue
- laxative
- metabolic
- nervine
- roborans
- sedative

- spasmolytic
- stimulans
- stomachic
- urologic
- venotonic

Drug analysis

- Using the demands of pharmacopaeia or other mandatory rules
- Identification
- Purity checking
- Content determination

Important – sample collection and preparation

Identification

- Sensoric (organoleptic) assays using our natural sensors
 - Smell
 - Taste
 - Vision
 - Touch
- Microscopic identification directed on characteristic signs (shape of skin cells, presence of typical structures/organelles containing essential oils, mucilages, starch grains, vascular bundles)
- Physico-chemical methods microsublimation, fluorescence, simple chemical reaction or thin layer chromatography

Purity checking

- Foreign matter, impurities
- Loss on drying
- Determination of total ash
- Determination of HCI-insoluble ash
- Optical rotatory power
- Index of refraction

Content determination

Direct / indirect determination of compounds

- Colorimetric methods
- Gravimetric methods
- Titration methods
- Biological methods
- HPLC, GC

Division of plant metabolites

- primary metabolites saccharides, amino acids, low-molecular carboxylic acids – mevalonate, pyruvic acid, shikimic acid
- secondary metabolites polysaccharides, alkaloids, terpenoids, phenolic glycosides, flavonoids, coumarines, anthocyanins, tannins, anthraglycosides...

Preparation of microscopic slides

- Temporary microscopic slides
- Permanent microscopic slides
- Surface specimens leaves, flowers
- Cuts barks, wood, root
 - transversal
 - tangential
 - radial

• Ways of cutting

- manual
- microtome cut thickness: 50 nm- 100 μm
 - Sledge-type microtome
 - Rotary microtome
 - Cryomicrotome
 - Laser microtome contact free slicing







Procedure of permanent slide preparation

- Softening and conservation
 - Alcohol-glycerol mixture, chlorine dioxide in acetic acid, formaldehyde
- Fixation
 - Immediate termination of biochemical reactions
 - Fixation mixtures FAA (formaldehyde/acetic acid/alcohol), chromic acid
- Clearing
 - Chloral hydrate, hydrogen peroxide, glycerol

- Fastening of object
 - Encapsulation into paraffin
 - it is necessary to remove water by treatment with ethanol (ethanol series)
 - Encapsulation into gelatin
 - Freezing
- Cutting and adhesion
 - Mixture of albumin and glycerol 1:1

Staining

 Before using stains paraffin must be removed by hydration with alcohol series

Staining according to the procedure

- progressive, regressive, succedaneous, simultaneous
- Staining according to the result
 - diffusive, differentiative
- phloroglucine, picric acid, iodine solution, saphranine, fuchsine, ferric chloride, congo red





Mounting

- Necessary to dehydrate
- Canada balsam, resins, liquid paraffin
- Enframement
 - lanoline-colophony glue, albumin, colorless
 nail polish

The most important information sources about natural products

- Text books for Pharmacognosy
 - Bruneton J.: Pharmacognosy, phytochemistry
 - Evans W.C.: Pharmacognosy
 - Dewick: Natural products
 - Wichtl M.: Herbal drugs and phytopharmaceuticals
 - European Pharmacopoeia

• internet

- Web of Science
- Science Direct
- Belstein Crossfire
- American Chemical Society
- SciFinder