ВУЗ, III курс 6 семестр

Class 1.
Phytochemical analysis of raw materials containing alkaloids.

Aims: 1. To study the nomenclature, morphological and anatomical diagnostic signs of medicinal plant raw materials serving as a source of alkaloids and approved for use in medical practice, as well as basic information about the distribution and areal of medicinal plants and possible impurities to them.

2. To know the most important physico-chemical properties, pathways of alkaloid biosynthesis, methods of their extraction and purification from medicinal plant raw materials, as well as methods of their qualitative and quantitative determination in medicinal plant raw materials. 3.

3. To master the methods of isolation and purification of alkaloids from medicinal plant raw materials, to learn how to identify and quantify the alkaloids in medicinal plant raw materials.

Questions:

1.Chemical classification of alkaloids using basic structural formulas.

2.Physical and chemical properties of alkaloids.

3.Methods of isolation of alkaloids from plant raw materials.

4.Qualitative analysis of raw materials containing alkaloids.

5 Methods of quantitative analysis of raw materials containing alkaloids.

Laboratory work:

CAUTION MUST BE EXERCISED WHEN WORKING WITH ALKALOID-BEARING RAW MATERIALS! ALL RAW MATERIALS ARE POISONOUS!

Work 1. Methods for the extraction of alkaloids from medicinal plant material.

There is no single method for the extraction of alkaloids. Alkaloids are found in plants as salts of organic acids (oxalic, malic, tartaric, citric, etc.), less often as bases and sometimes as a special form - oxides.

As a rule, base alkaloids are soluble in organic solvents (chloroform, dichloroethane) and insoluble in water (except for caffeine, codeine and ephedrine, which are soluble in water). The alkaloid salts are insoluble in organic solvents, except alcohols, but mostly soluble in water.

To test raw materials for the presence of alkaloids, extraction is carried out. The raw material is uniformly ground, the remainder not ground is sieved.

1.The raw material is extracted with 1% hydrochloric acid solution by heating in a boiling water bath for 5-10 minutes. After cooling down, filtered and the filtrate is used for the detection of alkaloids.

2. 0.5 g of raw material powder is shaken up with 5 ml of diluted (15.5% - 16.5%) sulfuric acid and filtered. Qualitative reactions for alkaloids are carried out with the filtrate.

A disadvantage of the methods described above is that the extracts are crude and the common precipitation reagents may precipitate not only alkaloids, but also other compounds (choline, betaine, etc.). Therefore a definitive answer about the presence of alkaloids in medicinal plant material can be obtained when reactions are carried out with purified extracts.

Purified extracts of alkaloids are obtained according to the methods given in the private pharmacopoeia articles for the particular raw material.

Study one of the proposed pharmacopoeial articles on medicinal plant raw materials, sketch in a notebook the method of extraction and purification of the amount of alkaloids.

Work 2. Qualitative analysis of medicinal plant raw materials.

To prove the presence of alkaloids in the herbal raw material under study we use reactions that are characteristic of this whole group of compounds - precipitation reactions.

Precipitation reactions or common reactions are based on the fact that alkaloids form water-insoluble compounds when interacting with some substances. These are mainly complex iodides, complex inorganic acids, heavy metal salts and some acidic organic compounds.

The precipitation of some alkaloids dissolves in excess of the reagent, therefore reactions should be carried out by combining a drop of extraction with a drop of reagent on a watch glass, glass plates or porcelain cups.

Due to the fact that not all alkaloids precipitate to the same extent with common reagents, reactions are carried out simultaneously with several (5-7) different reagents.

In parallel, control experiments are conducted on the material, from which the alkaloids were previously washed with acidified alcohol: the raw material is placed for 5-7 days in a blotter with 5% tartaric acid solution in alcohol; after 2-3 days, the solvent is replaced with fresh one. Confirmation of the alkaloid nature of the precipitate gives a negative result with the same reagent in the control experiment.

Carry out qualitative reactions for alkaloids with different kinds of medicinal plant raw materials. Draw up the results in the form of a table:

Medicinal raw materials / Reagent	Results of a qualitative reaction					

The discovery of individual alkaloids in plant material is carried out by means of specific reactions for a given alkaloid.

Work 2. (UIRS) Quantification of alkaloids.

A variety of chemical structure of alkaloids, their physical and chemical properties, as well as individual characteristics of biochemical processes and the diversity of the chemical composition of plants does not allow to develop a single methodology for the quantitative determination of alkaloids in medicinal plant raw materials. For each type of raw material has developed its own methodology for determining the content of alkaloids.

All methods are divided into:

- - titrometric;
- - spectrophotometric;
- - gravimetric;
- - polarography.

Self-assessment questions:

1. State the main working steps in the quantification of alkaloids.
2. In what form are alkaloids found in medicinal plant material?
3. In what form are alkaloids extracted from medicinal plant material?
4. What is the basis of purification of the extraction obtained from raw materials in the quantitative extraction of alkaloids?
5. What techniques do you know for the quantitative determination of alkaloids in raw materials?

Guideline
developed 31.08.2022 Senior Instructor Nedilko O.V.

- **Application 1**

Reagents used for the detection of alkaloid salts

- **Bouchard reagent** (1.0 iodine + 2.0 potassium iodide + 50 ml water) - a brown precipitate is formed.
- **Wagner's reagent** (1.27 iodine + 2.0 potassium iodide + 100 ml water) - a brown precipitate is formed.
- **Mayer's reagent** (1.358 mercury bichloride (sulema) + 5.0 potassium iodide + 100 ml water). Most alkaloids form a white or yellowish precipitate. Caffeine, solacin, colchicine and others do not precipitate.
- **Reagent Marmet** (20.0 potassium iodide + 10.0 cadmium iodide + 100 ml hot water). A white or yellowish precipitate is formed, often dissolving in excess of the reagent. Caffeine does not react in this way; atropine, salonin, thiobromine etc. will precipitate only at a sufficiently high concentration.
- **Draugendorf's reagent** (solution 1: 0.85 mL bismuth nitrate + 40 mL water + 10 mL glacial acetic acid).(solution 2: 8.0 potassium iodide + 20 ml water). Mix equal amounts of solutions 1 and 2. To 10 ml mixture + 100 ml water + 20 ml ice-cold acetic acid. A brick red or orange-red colouring is produced. The Dragendorf reagent is considered to be the most sensitive among iodine containing reagents.
- **Sonenstein's reagent** (0.5 phosphomolybdenum acid + 50 ml water + 0.3 hydrochloric acid (0.1 n) to acidic reaction). An amorphous yellowish precipitate is formed. It is considered the most sensitive reagent for alkaloids.
- **Scheidler's reagent** (0.3 mL phospho-tungstic acid + 0.8 mL diluted hydrochloric acid + 10 mL water). With the salts of many alkaloids precipitates are formed, more often in the form of whitish plates.
- **Bertrand's reagent** (1% aqueous solution of silicomorphic acid). Precipitates many alkaloids from slightly acidic solutions. Precipitates whitish or yellowish plates.
- **5% solution of tannin.** Forms whitish or yellowish amorphous precipitation.
- **1% aqueous solution of picric acid.** Forms yellow precipitates, does not precipitate caffeine, colchicine, morphine, thiobromine and others. Atropine precipitates only from concentrated solution. In addition, picric acid may precipitate in strongly acidic media from mineral acids.