PHARMACOGNOSY

Student _____

Group _____

Minsk BSMU 2024

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ РЕСПУБЛИКИ БЕЛАРУСЬ
БЕЛОРУССКИЙ ГОСУДАРСТВЕННЫЙ МЕДИЦИНСКИЙ УНИВЕРСИТЕТ
КАФЕДРА ОРГАНИЗАЦИИ ФАРМАЦИИ

ФАРМАКОГНОЗИЯ

PHARMACOGNOSY

Практикум
для студентов медицинского факультета иностранных учащихся

Минск БГМУ 2024

УДК 615.322(075.8)(076.5)
ББК 52.82я73
Ф24

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Представлена химическая структура действующих веществ лекарственных растений по всем группам биологически активных веществ, описаны методы их обнаружения и анализа. Приводятся схемы оформления протоколов по макроскопическому, микроскопическому и товароведческому анализам, методики количественного определения биологически активных веществ, рисунки микроскопического анализа.

Предназначен для студентов медицинского факультета иностранных учащихся, обучающихся по специальности 1-79 01 08 «Фармация» по учебной дисциплине «Фармакогнозия».

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TRAINING AND REGISTRATION CARD

Academic week	Theme of practical classes	Mark	Theacher's signature
1	Macroscopic analysis of medicinal plant raw material		
2	Microscopic analysis of medicinal plant raw material		
3	Quality control of MPRM. Determination of authenticity, crushing, content of impurities, moisture and ash. Morphological and chemical analysis of MPRM containing lipids		
4	**The final class**		
5	Polysaccharides. DRM and MPRM containing polysaccharides		
6	Vitamins. DRM and MPRM containing vitamins		
7	Essential oils. Analysis of MPRM containing essential oils. DRM and MPRM containing aromatic compounds		
8	Monoterpenes. DRM and MPRM containing acyclic, mono- and bicyclic monoterpenes		
9	Sesquiterpenes. DRM and MPRM containing sesquiterpenes		
10	Iridoids. Medicinal plant materials containing iridoids		
11	**The final class**		
12	Cardiac glycosides. Analysis of MPRM containing cardiac glycosides		
13	Saponins. DRM and MPRM containing saponins		
14	Saponins. DRM and MPRM containing saponins		
15	Phenolic glycosides and lignans. DRM and MPRM containing phenolic glycosides and lignans		
16	Anthracene derivatives. Analysis of MPRM containing anthracene derivatives		
17	**The final class**		
18	Coumarins and chromones. DRM and MPRM containing coumarins and chromones		
19	Flavonoids. Analysis of MPRM containing flavonoids		
20	Flavonoids. DRM and MPRM containing flavonoids		
21	Flavonoids. DRM and MPRM containing flavonoids		
22	Tannins. DRM and MPRM containing tannins		
23	Tannins. DRM and MPRM containing tannins		
24	**The final class**		
25	Analysis of MPRM containing alkaloids		
26	DRM and MPRM containing alkaloids with nitrogen in the lateral chain, derivatives of pyrrolizidine and tropane		
27	Chinolysidin and steroidal alkaloids (glycoalkaloids). DRM and MPRM containing these groups of compounds		
28	Isoquinoline and indole derivative alkaloids. DRM and MPRM containing these groups of compounds		
29	DRM and MPRM containing various groups of biologically active substances		
30	Animal origin medicinal products and natural products		
31	**The final class**		
32	Analysis of cut medicinal plant materials. Analysis of powdered and cut-pressed MPRM. Analysis of herbal collections		
33	Pass the practical skills		
34	Course work		

FOREWORD

The manual is an example of an innovative approach to the organization of laboratory studies on pharmacognosy, tk. optimizes the work under the supervision of the teacher and increases the productivity of studying the rich volume of material on pharmacological analysis of LRS.

The purpose of the laboratory journal is to facilitate and accelerate the assimilation by students of the diagnostic features of medicinal plant material, methods for determining authenticity and quality.

In the laboratory journal the safety in the laboratory is shown; the topics of laboratory studies, a brief description of the groups of biologically active substances, which are illustrated by the formulas of active substances are indicated.

In the journal there are: schemes for the design of protocols for laboratory studies on macroscopic, microscopic and commodity analysis, methods for quantifying various biologically active substances and for drawing microscopic signs of medicinal plant material. The student must find and sign diagnostic tests when analyzing raw materials. Using a laboratory journal, students will shorten the time for the registration of protocols, which will give an opportunity to pay more attention to the study of herbarium and medicinal plant material.

Independently studying the herbarium and medicinal plant raw materials, students fill out tables with Latin names: raw materials, plants and family; chemical composition; drugs and the use of medicinal plant raw materials in medicine.

At the end of the lesson, the teacher signs the protocol and conducts an out-of-control monitoring of the students' knowledge, on the basis of which the assessment for the lesson.

Class time card:

1. Discussion on the training subject (input control) is 45 minutes.
2. The practical part of the lesson is 90 minutes.
3. Registration of the laboratory journal is 20 minutes.
4. Individual interview with the teacher according to the protocol of the session (exit control) — 25 minutes

Requirements by the department of organization of pharmacy to students:

1. **Observe the safety rules in the lecture rooms of the department** (safety instruction is conducted), follow the internal regulations.
2. In laboratory classes to arrive **without delay, according to the schedule**. Late students for practical classes **are not allowed.**
3. In laboratory classes, students should have **lab coat, training workshops, hats, colored pencils**. Students without dressing gowns and training workshops for practical classes are not allowed.
4. Missed classes should be worked out within **2 weeks after the pass.**
5. Students who have not completed two weeks of missed practical classes, to subsequent studies, final lessons and credit without the permission of the dean of the faculty **are not allowed.**

I read the requirements of the department _____ 202_ _____ (signature)

SAFETY TECHNIQUE IN THE LABORATORY AT DEPARTMENT OF ORGANIZATION OF PHARMACY

The implementation of educational and scientific experimental work at the Department of Pharmacy Organization in educational and scientific laboratories is associated with the use of a variety of chemical substances (organic solvents, acids, alkalis), vegetable raw materials using a variety of chemical utensils, equipment and instruments. Therefore, in laboratory facilities there is always the possibility of affecting working students dangerous and harmful production factors that can lead to occupational injuries and occupational poisoning.

Under the influence of hazardous and harmful production factors, there may be:

1) mechanical injuries (abrasions, cuts, bruises, etc.) during the operation of equipment, with careless handling of glassware and appliances, if the rules for safe work with vacuum are not observed;

2) chemical burns when working with acids, alkalis and other caustic substances;

3) poisoning by dust of plant raw materials and vapors of harmful chemicals during work without exhaust ventilation and protection means;

4) thermal burns when working with electric and gas-fired heating appliances, as well as the ignition of harmful substances in the event of non-compliance with safe methods of working with them;

5) a fire can occur as a result of an explosion or when forming a mixture of organic solvent vapors, flammable liquids, combustible gases with air, reaching a certain concentration and having an open fire source.

In this regard, each student **is required to:**

1. To observe the established rules of the internal schedule, time of the beginning, the termination of work and a break on rest.
2. Observe the requirements established by the current labor protection regulations.
3. To carry out work in overalls and with the use of personal protective equipment.
4. Keep the workplace clean and tidy;
5. Do not take out of school or transfer it to others without the permission of the administration, and also use for your personal purposes any kind of chemicals, plant raw materials and preparations.
6. Perform only the work that is entrusted.
7. Timely inform the head (teacher) and the safety precautions department about the observed violations of the norms and rules of labor protection, industrial sanitation and fire safety rules.
8. It is prohibited to leave unattended devices and equipment unattended, in case of sudden interruptions in the supply of electricity, water, gas, appliances and equipment must be immediately switched off.
9. Repair, adjustment and testing of equipment and devices is allowed only by specially trained personnel.
10. It is prohibited to smoke outside designated areas.
11. It is forbidden to store outer clothing in laboratories.

At the end of the working time, the student must:

1. Check the disconnection of equipment, instruments, communications lines.
2. Tidy up the workplace, remove debris from the premises, put reagents, tools, etc. on the designated places.
3. Leaving the last (duty) is obliged to check the closing of windows and windows, disconnecting communications, power and lighting network, close the room and pass the key to the senior laboratory assistant.

A student who is guilty of violating norms, instructions and safety rules may be brought to disciplinary responsibility if the consequences of the violation or the danger that has arisen in case

of systematic violations do not require the application of another punishment to the offender in accordance with the current legislation.

In order to prevent a fire in the laboratory, it is forbidden:

1. Leave unattended heating devices switched on.
2. Use electric heaters with an open spiral.
3. Leave combustible wastes, oiled rags, used filter paper.
4. Carry out work when the ventilation is inoperative or malfunctioning.
5. Wash equipment, furniture, floors with organic solvents.
6. Transport flammable and highly flammable substances in an unsuitable container.
7. To block up approaches to workplaces and fire extinguishing means.
8. Dry any substances on heaters.

FIRE SAFETY REQUIREMENTS

Every student should know where the fire fighting equipment is and how to use them.

In the event of an explosion, fire or other emergencies, it is necessary to disconnect the electrical equipment and electrical equipment from the power grid and inform the head of the structural unit.

During a fire, you can not open windows and doors, as well as break glass. Leaving the room, you must close all the doors and windows behind you, since the influx of fresh air contributes to the rapid spread of fire.

In case of fire, call a volunteer fire brigade and take measures to extinguish the fire. If necessary, call the fire department at 101.

SAFETY REQUIREMENTS FOR WORK WITH CONCENTRATED ACIDS AND ALKALIES

1. All operations associated with the use of acids and caustic substances should be carried out in a fume hood or in conditions of installation above the workplace of local suction, with ventilation running using personal protective equipment.
2. Mixing or diluting chemicals, accompanied by heat release, to produce in heat-resistant and porcelain ware.
3. When heating chemical fluids in a test tube, it is necessary to direct it away from yourself and nearby persons.
4. When stirring the solution in flasks and test tubes, close them only with stoppers.
5. Do not leave the burners and other heating appliances on fire without supervision.
6. Do not store substances of unknown origin without labels.
7. Waste acid, alkali and other caustic substances poured into a special container.
8. Acids and alkalies must be pipetted only with a rubber pear, it is unacceptable to suck acids and caustic alkalies into the pipette by mouth, as this can lead to burns and poisoning.
9. Concentrated alkali, acid and other caustic substances should be stored in thick-walled glassware (with a capacity of no more than 2 liters), placed in metal or wooden boxes with lids, the walls and the bottom of which must be covered with non-combustible material.
10. Transfusion of acids and alkalis from bottles into smaller containers should also be carried out alone with a siphon and only under local exhaust ventilation.
11. For the preparation of acid solutions, they must be poured into water by a thin stream with continuous stirring, and not vice versa.
12. Large pieces of caustic alkali should be broken into small pieces in a specially designated place, having covered the broken pieces with a dense cloth or paper. Pieces of alkali take only with tongs.
13. Dispose of used acids and alkalis only in special containers, and upon completion of work, these acids and alkalis after neutralization should be drained into the sewage system.
14. At the end of work, wash hands with warm water and soap.

In emergency situations:

1. In case of spilling a concentrated acid solution, it must first be covered with sand so that it absorbs the acid. Sand collect in a container and remove from the premises to the collection point of waste. Spill contaminated spillage with water and wipe dry.
2. In case of spilling a concentrated solution of alkali and ammonia, they can be covered with sand or with sawdust. Clean the place after removing sand or sawdust wash with a weak solution of acetic acid.
3. In case of acid on the skin, in the eyes or mouth cavity, immediately rinse them with a flowing water stream for 10–15 minutes, then neutralize with 1–2 % sodium bicarbonate solution, eyes and mouth — 5 % with a sodium bicarbonate solution.
4. In case of contact with alkaline skin, eyes, or mouth, immediately rinse them for 10–15 minutes quickly with a flowing water jet, and then neutralize with 1–2 % boric acid solution.
5. In case of severe lesions with acid or alkali, after the end of the first aid, the victim must be sent to a medical institution.

With a view to daily prevention of exposure to harmful substances, students who have contact with them **are required to:**

1. At the end of work and workday, wash hands and face with soap.
2. Do not go to work clothes buffet, conference room, library.
3. Keep special clothing away from outer clothing.

ACCIDENT PREVENTION FOR WORKING WITH RAW MATERIAL CONTAINING STRONG ACTING SUBSTANCES

While working with medicinal plant raw materials containing potent substances, one should not touch the eyes, face and eat.

When preparing, processing, drying, sorting and packaging medicinal plant raw materials containing alkaloids and cardiac glycosides, protect the mouth and nose with a respirator or a moist gauze dressing, the eyes — with goggles.

After work, carefully shake out clothes, wash their face and hands with soap, wipe the respirator, glasses, gauze.

To work with medicinal plant raw materials containing potent substances, pregnant and lactating women are not allowed.

I read the requirements of the department _____ 202__. _____ (signature)

Practice № 1
MACROSCOPIC ANALYSIS OF MEDICINAL PLANT RAW MATERIAL

Control questions:

1. The concept of drug raw materials (DRM).
2. The classification of medicinal plant raw material (MPRM).
3. Pharmacognostic analysis and its purpose.
4. The concept of the identification, quality of the MPRM, the choice of a method for their determination.
5. The purpose, task and technique of macroscopic analysis.
6. Morphological groups of MPRM (leaves, herbs, flowers, fruits, seeds, buds, cortex, roots and rhizomes) and their diagnostic signs.
7. Various types of raw materials pharmacognostic determination.
8. Regulatory documentation for MPRM.
9. Structure of private pharmacopoeial monograph for MPRM.

INFORMATION

Macroscopic analysis of hingeless medicinal plant raw material

The term «pharmacognosy» derived from the Greek words «pharmakon», which means medicine and «gignosio» — the need for knowledge. It first appeared in Seydler's small scientific work called Analekta Pharmakognostica. At present, pharmacognosy as a science studies not only MPRM, but also products of both plant and animal origin.

Medicinal plant raw materials (MPRM) whole medicinal plants or parts of medicinal plants used for industrial production, pharmaceutical production of medicines, for which there are corresponding pharmacopoeial articles.

Leaves medicinal raw materials, which are dried or fresh leaves or individual leaves of a compound leaf.

Flowers are medicinal raw materials, which are dried individual flowers or inflorescences, as well as their parts.

Herbs are medicinal raw materials, which are dried or fresh above-ground parts of herbaceous plants (stems with leaves and flowers, partly with buds and unripe fruits).

Fruits are dried or fresh simple or complex fruits (fruits) and their parts.

Seeds whole seeds or individual cotyledons.

Bark is a medicinal raw material, which is the outer part of the trunks, branches and roots of trees and shrubs, located to the periphery of the cambium.

Roots, rhizomes, bulbs, tubers, corms dried or fresh underground organs of perennial plants, peeled or washed from the ground, freed from the remains of stems and leaves.

Primary processing products include essential and fatty oils, gums, resins.

Latin terms for types of medicinal plant raw material:

Calamus rhizome — Acori calami **rhizoma**
Birch leaves — Betulae **folium**
Birch buds — Betulae **gemma**
Hawthorn leaves and flowers — Crataegi **folium cum flore**
Senna leaves with fruits — Sennae **folium cum fructus**
Valerian rhizome with roots — Valerianae **rhizome cum radicibus**
Pumpkin seeds — Cucurbitae **semen**
Yarrow grass — Millefolii **herba**
Violet grass with flowers — Viola **herba cum flore**
Dill fruits — Anethi graveolentis **fructus**
Blueberry fruits fresh (dry) — Myrtilli **fructus recens (siccus)**
Licorice root — Glycyrrhizae **radix**

Chamomile flowers — Matricariae **flos**
Rhodiola rosea rhizomes and roots — Rodiolae roseae **rhizome et radix**
Fresh sea buckthorn fruits — Hippophaes ramnoides **fructus recens**
Corn columns with silks — Zeae maydis **styli cum stigmatis**
Willow bark — Salicis **cortex**
Icelandic moss thallus — **Lichen** islandicus

The name of the medicinal plant raw material consists of two parts: the medicinal plant Latin name (species or generic) in the genitive case is indicated in the first place, and the organ (or the product of primary processing) of the medicinal plant in the nominative case. For example, Frangulae cortex (producing plant — Frangula alnus Mill, family — Ramnaceae) or Ammi visnagae fructus (producing plant — Ammi visnaga Lam., family — Apiaceae).

The purpose of pharmacognitic analysis is to determine:

1. Identification.
2. Definition of MPRM quality.

The MPRM identification is established by: 1) macroscopic analysis; 2) microscopic analysis; 3) qualitative chemical analysis; 4) chromatographic analysis; 5) luminescence analysis.

The MPRM quality is established by: 1) merchandising analysis; 2) quantitative chemical analysis; 3) biological analysis.

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Carry out a macroscopic analysis of the leaves.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside	
9. Smell.	

Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

_____

TASK № 2. Carry out a macroscopic analysis of the flowers.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1.Type of an inflorescence or single flowers.	
2. The shape of the flower (actino- or zygomorphic).	
3. Inflorescence or flower size.	
4. Absence or presence of peduncle (its shape and size).	
5. Indumentum.	
6. Color.	
7. Smell.	

Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

_____

TASK № 3. Carry out a macroscopic analysis of the fruits.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Type of fruit (dry or fleshy).	
2. Shape.	
3. Size of the fruit (length, thickness, diameter)	
4. The features of the pericarp.	
5. The number of seeds, their shape and structure, the surface structure.	
6. Color.	
7. Smell.	

Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

_____

TASK № 4. Carry out a macroscopic analysis of the roots or rhizoma.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

_____

TASK № 5. Carry out a macroscopic analysis of the grass.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside and underside	
11. Smell	
12. Flower arrangement on the stem.	
13. **Flower.** Type of an inflorescence or single flowers.	

14. The shape of the flower (actino- or zygomorphic).	
15. Inflorescence or flower size	
16. Absence or presence of peduncle (its shape and size).	
17. Indumentum.	
18. Color.	
19. Smell	
20. **Fruits.** Type of fruit (dry or fleshy).	
21. Shape.	
22. Size of the fruit (length, thickness, diameter)	
23. The features of the pericarp.	
24. The number of seeds, their shape and structure, the surface structure.	
25. Color.	
26. Smell.	

Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion:_____

_____

TASK № 6. Carry out a macroscopic analysis of the bark.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size (thickness).	
3. The features of the outer surface.	
The features of the inner surface.	
4. Cork color and lenticle shape.	
5. The nature of the fracture.	
6. Smell.	

Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion:_____

_____

TASK № 7. Carry out a macroscopic analysis of the seed.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The features of the surface.	
4. Color.	
5. Smell.	

Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion:_____

Practice № 2
MICROSCOPIC ANALYSIS OF MEDICINAL PLANT RAW MATERIAL

Control questions:

1. Microscopic analysis purpose.
2. Rules and techniques for the preparation of microslades (fluxing, differentiation, using inclusive liquids).
3. Anatomical structure and microscopic diagnostic signs of leaves, herbs, flowers, fruits, seeds, roots, rhizomes, cortex, buds.
4. Microchemical reactions in microscopic analysis (on starch, mucus, fatty and essential oils, lignification).

INFORMATION

Microscopic analysis of medicinal plant raw material

Preparation of a surface preparation. The thin leaf surface preparations are prepared by boiling with 5 % sodium hydroxide solution. From thick and leathery leaves, if necessary, cross sections are prepared. Small leaves are used in one piece, separate units are taken from large leaves: the edge of the leaf, the leaf dent, the main vein portion, the leaf tip and the leaf base.

Examining a microscopic preparation of a surface leaf, pay attention to the main diagnostic features: the shape and the size of the epidermal cells, the type of stomata, the features of trichomes (hairs, glands), the presence and shape of crystalline inclusions, strengthening tissue, conceptacles, lactifers, secretory channels and etc.

Methods for stomatal index determining

5 × 5 mm part of the leaf blade is heated with 5 ml of chloral hydrate solution in a water bath for 15 minutes. Place the leaf on a slide and prepare the preparation from the surface. The underside epidermis is examined under a microscope with a × 40 lens and an eyepiece × 10. Count the number of epidermal cells (including trichomes) and the number of stomata.

Calculation of the stomatal index is carried out according to the formula:

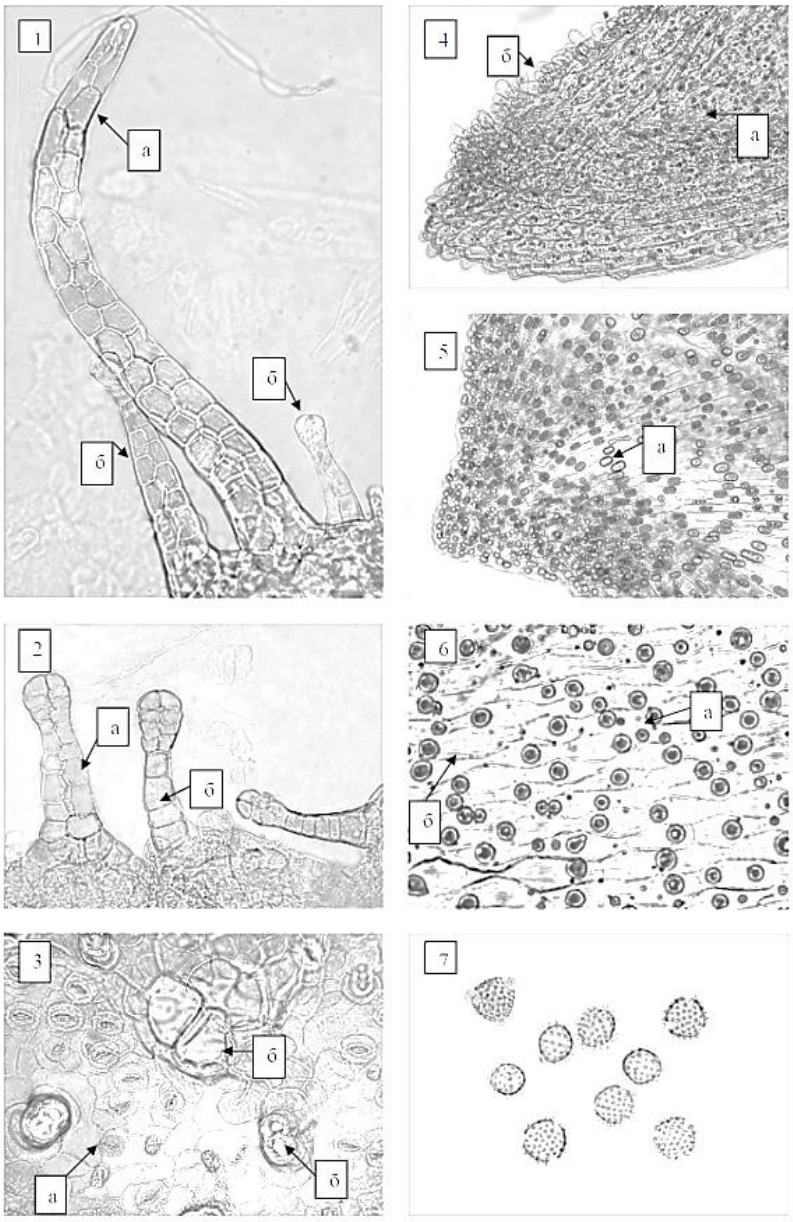
$$SI = \frac{S \cdot 100}{E + S},$$

where S — number of stomata; E — the number of epidermal cells, including trichomes, per unit area of the leaf (in the field of view of the microscope).

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Carry out a microscopic analysis and indicate the diagnostic signs of Calendulae flores.

The name of MPRM	
The name of the plant	
Family	



Calendulae flores microslide:
1 — a fragment of the tubular flower ovary epidermis (200×); 2 — fragment of the ligulate flower ovary epidermis (200×); 3 — a fragment of the envelope epidermis (200×); 4 — a fragment of the tubular flower corolla denticle (200×); 5 — a fragment of ligulate flower limb denticle (200×); 6 — a fragment of ligulate flower limb (400×);7 — pollen grains (200×)

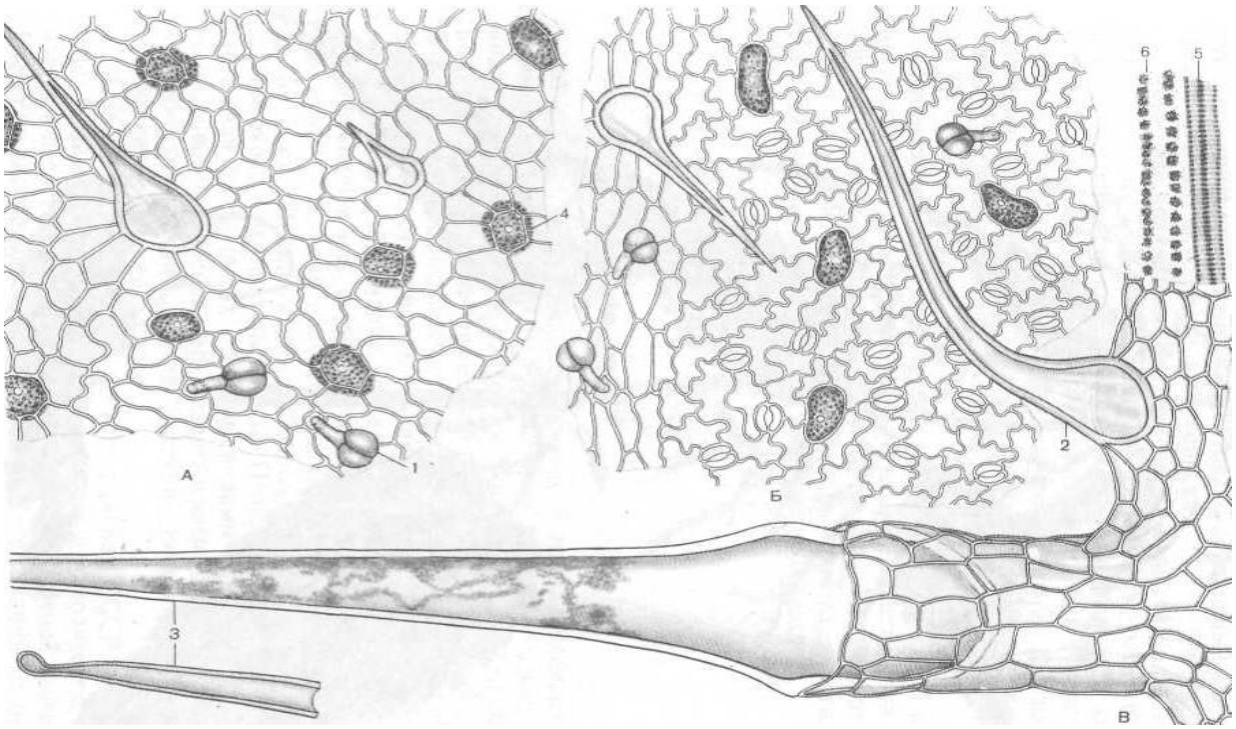
Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on microscopic feature.

Conclusion:_____

TASK № 2. Carry out a microscopic analysis and indicate the diagnostic signs of Urticae folia.

The name of MPRM	
The name of the plant	
Family	



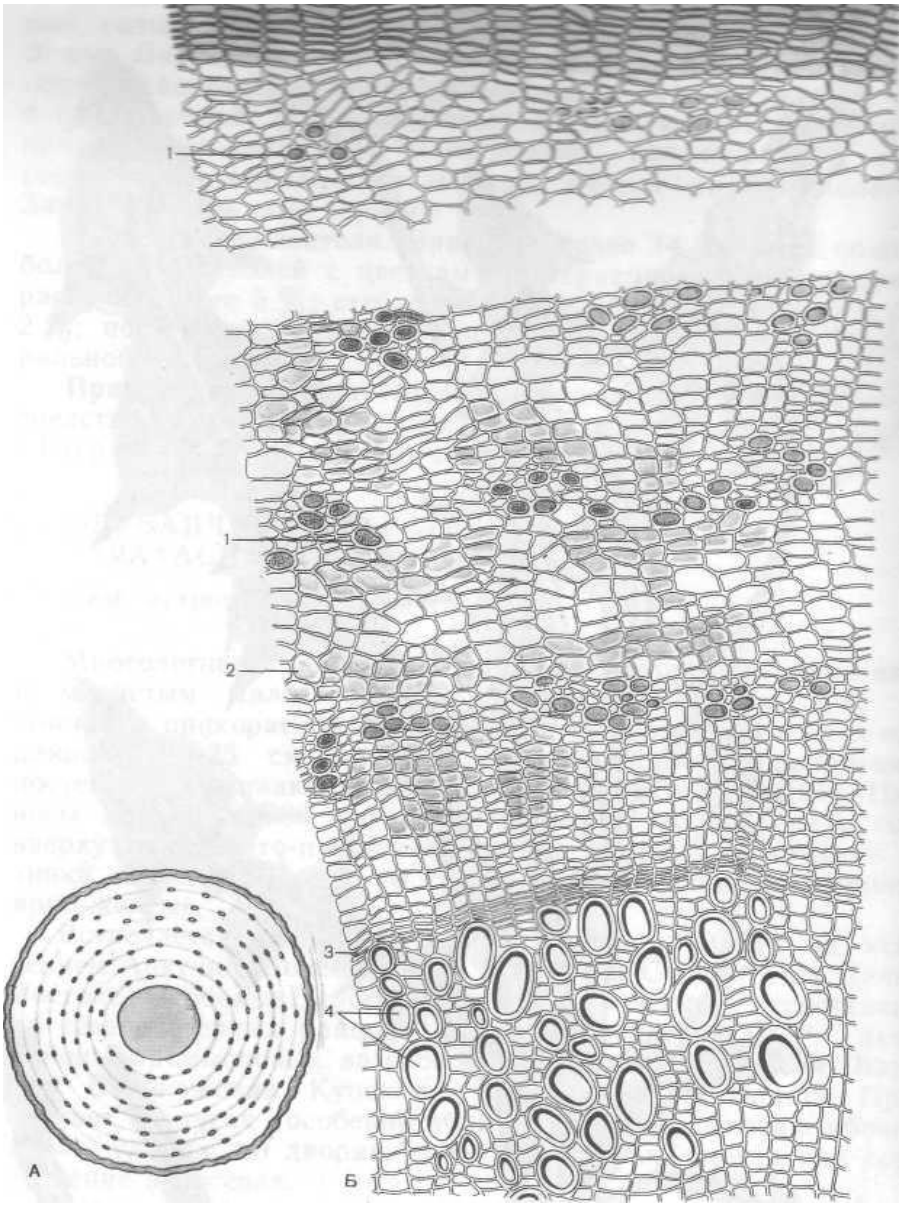
Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on microscopic feature.

Conclusion:_____

TASK № 3. Carry out a microscopic analysis and indicate the diagnostic signs of Taraxaci officinalis radices.

The name of MPRM	
The name of the plant	
Family	



Indicate the diagnostic signs of Taraxaci officinalis radices

A — cross-section of the root under the magnifying glass; B — transverse section ×280

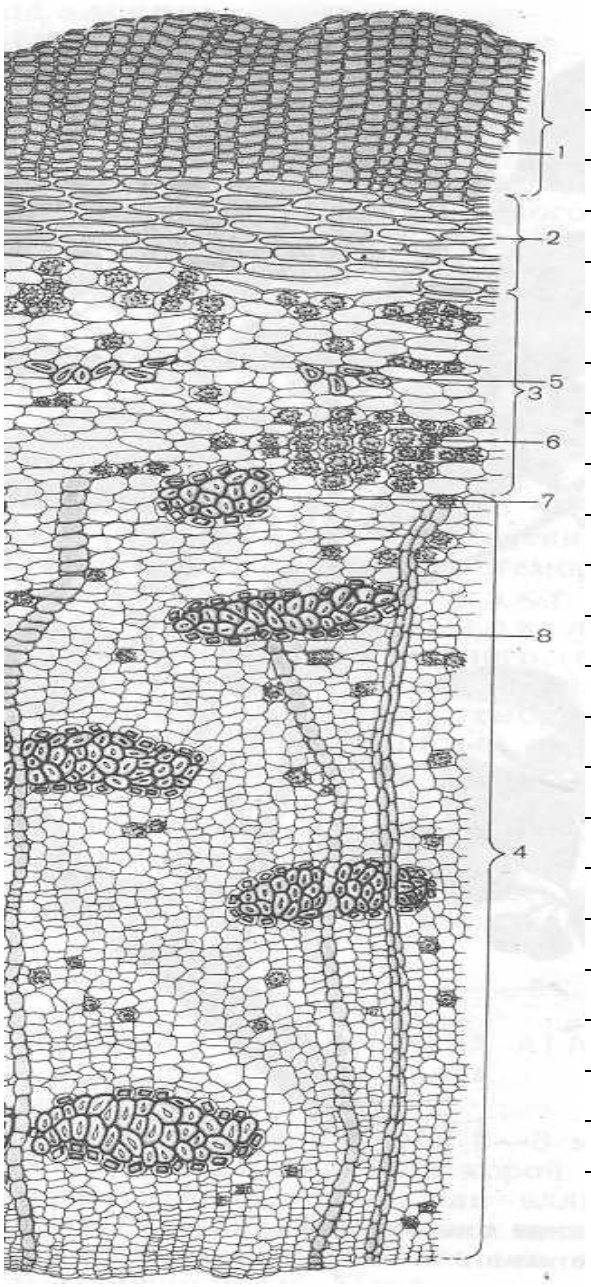
Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on microscopic feature.

Conclusion:

TASK № 4. Carry out a microscopic analysis and indicate the diagnostic signs of Frangulae cortex.

The name of MPRM	
The name of the plant	
Family	



Microscopic analysis of Frangulae cortex;
transverse section ×120

Compare your description with a description of the raw materials external features in the regulatory documentation (SF of the RB).

To give an opinion about the identification and quality of the MPRM on microscopic feature.

Conclusion:_____

Practice № 3
QUALITY CONTROL OF MPRM. DETERMINATION OF AUTHENTICITY, CRUSHING, CONTENT OF IMPURITIES, MOISTURE AND ASH. MORPHOLOGICAL AND CHEMICAL ANALYSIS OF MPRM CONTAINING LIPIDS

Control questions:

1. Purpose of commodity analysis.
2. MPRM commodities delivery.
3. The concept of a batch of raw materials.
4. Determination of sample sizes of MPRM.
5. Rules for sampling raw materials (point, combined, medium and analytical).
6. Purpose of analytical samples.
7. Determination of granulation.
8. Possible admixtures of MPRM and their classification.
9. Weight loss on drying. Determination. Analytical value of this indicator.
10. Humidity. Determination of humidity. The analytical value of this indicator.
11. The ash is general and ash insoluble in a hydrochloric acid. Its definition and analytical value.
12. Active and extractive substances, their definition and analytical value.

TASK № 1. Commodity analysis of medicinal plant raw materials.

_____

(Latin name of raw materials, plants and families)

Quantity of raw materials production units_____

The result of the package inspection (broken, not broken)

_____

The result of the batch homogeneity inspection (homogeneous, not homogeneous)

_____

The number of raw materials production units for dissection (sample size)

_____

Analytical samples mass for determination:

1) authenticity, granulation and admixtures content_____
2) humidity _____
3) ash and active substances content_____
4) microbial enumeration test_____
5) radionuclides_____
6) pesticides and toxic substances_____

Analysis results

Index numder	Found		ND, %
	g	%	
Organic admixture			
Mineral admixture			

* If the normative documents

Conclusion: raw materials (not) meet the requirements_____
ND

_____

_____
(by which indicators)

Signature of the analyst _____

TASK № 2. Determination of weight loss upon drying of medicinal plant raw materials.

The exact portion of the raw material specified in the pharmacopoeia is placed in a dry, pre-weighed box with a lid and dried in an oven at a temperature of 105 C until constant weight. The test is carried out in 2 parallels.

$$w = \frac{m_1 - m_2}{m_1} \times 100\ \%,$$

where m_1 — weight of raw materials before drying, g; m_2 — mass of raw materials after drying, g.

Practice № 4
THE FINAL CLASS

Methods of medicinal plant material pharmacognostic analysis:

1. The concept of DRM and MPRM.
2. The classification of MPRM.
3. Methods of pharmacognostic analysis.
4. The concept of the identification of the MPRM.
5. Methods for determining the identification of the MPRM.
6. Methods for determining the quality of the MPRM.
7. Morphological groups of MPRM and their diagnostic signs.
8. Various types of raw materials pharmacognostic determination.
9. MPRM product specification files.
10. Microscopic analysis of MPRM and its meaning.
11. Techniques for the preparation of microslades (fluxing, differentiation, using inclusive liquids).
12. Microchemical reactions in microscopic analysis (on starch, mucus, fatty and essential oils, lignification).
13. Anatomical structure and microscopic diagnostic signs of leaves, herbs, flowers, fruits, seeds, roots, rhizomes,cortex, buds.
14. Quality analysis and its meaning.
15. Rules for acceptance of MPRM in warehouses.
16. The concept of a raw materials batch. Documents accompanying a raw materials batch.
17. Determination of the sample size in the acceptance of the MPRM.
18. For which parameters the batch of raw materials is not subject to acceptance?
19. On what indicators should the batch be sorted, then submitted again for surrender?
20. Rules for sampling raw materials (point, combined, medium and analytical).
21. Purpose of analytical samples.
22. Determination of MPRM granulation. The value of this indicator.
23. Possible admixtures of MPRM and their classification.
24. Determination of the admixtures content.
25. Determination of mass loss during drying of MPRM. Analytical value of this indicator.
26. The ash is general and insoluble in a 10 % solution of hydrochloric acid. Its definition and analytical value.
27. Extractive substances, their definition and analytical value.
28. How do they handle with raw materials if, as a result of the tests, there is a discrepancy between the quality of raw materials and SF requirements?

Practice № 5
POLYSACCHARIDES. DRM AND MPRM CONTAINING POLYSACCHARIDES

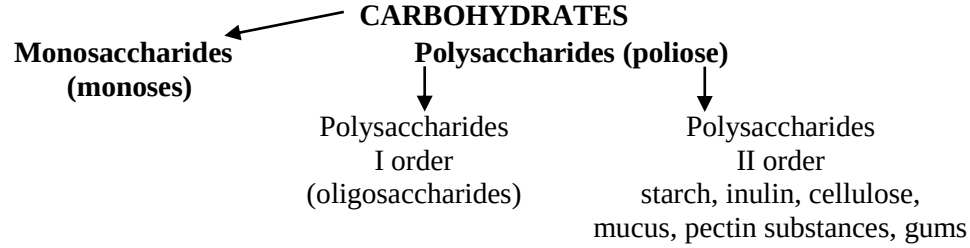
Control questions:

1. Definition and classification of carbohydrates.
2. Definition of the «polysaccharides», classification of polysaccharides.
3. Concept and classification of mucus.
4. Physico-chemical properties of mucus.
5. Methods for the extraction and purification of mucus.
6. Qualitative reactions to mucus.
7. Potency assay of mucus in MPRM.
8. Gums: definition, classification, physico-chemical properties, pharmacological action.
9. Pectin substances: definition, classification, physicochemical properties, pharmacological action.
10. Inulin: definition, classification, physicochemical properties, pharmacological action.
11. Latin and russian names of MPRM, producing plants and families of all plants of the training subject.
12. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
13. Rational methods of collecting raw materials, primary processing, drying and storage MPRM.
14. External and microscopic signs MPRM.
15. Chemical composition.
16. Medicines and useing.
17. MPRM containing polysaccharides: pharmacological activity, use.

INFORMATION

Carbohydrates are a broad class of organic compounds, which include high-molecular natural polymer compounds. Carbohydrates are the main nutritional and supporting material of plant cells and tissues. They make up up to 90 % of the total mass of the plant. Carbohydrates consisting exclusively of polyoxycarbonyl compounds are called holosides, their derivatives, whose molecules contain residues of other compounds, are called heterosides. Heterosides include all types of glycosides.

The classification scheme of carbohydrates can be represented as follows:



Oligosaccharides (polysaccharides of the first order) are constructed from a small number of monoses (usually 2–4) and are crystalline substances that are readily soluble in water.

Polysaccharides of the II order are biopolymers with a large molecular weight, which produce colloidal solutions or are generally insoluble in water and are built from monosaccharides and uronic acids connected with each other by a glycosidic bond. This are mostly amorphous substances, insoluble in non-polar solvents and alcohol. They are subjected to acid and enzymatic hydrolysis.

Polysaccharides can be classified by function (*reserve and structural*), by acidity (*neutral and acidic*), by the skeleton nature (*linear and branched*), by the degree of homogeneity of the blocks (***homopolysaccharides*** are constructed from identical monosaccharides, and ***heteropolysaccharides*** are constructed from different monosaccharides). The State Pharmacopoeia of the Republic of Belarus provides for the determination of the swelling index.

Swelling index is the volume in milliliters occupied by 1 g of MPRM, including adherent mucus, after swelling in aqueous solution for 4 hours.

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Identify the authenticity authenticity and quality of Plantaginis majoris folium.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

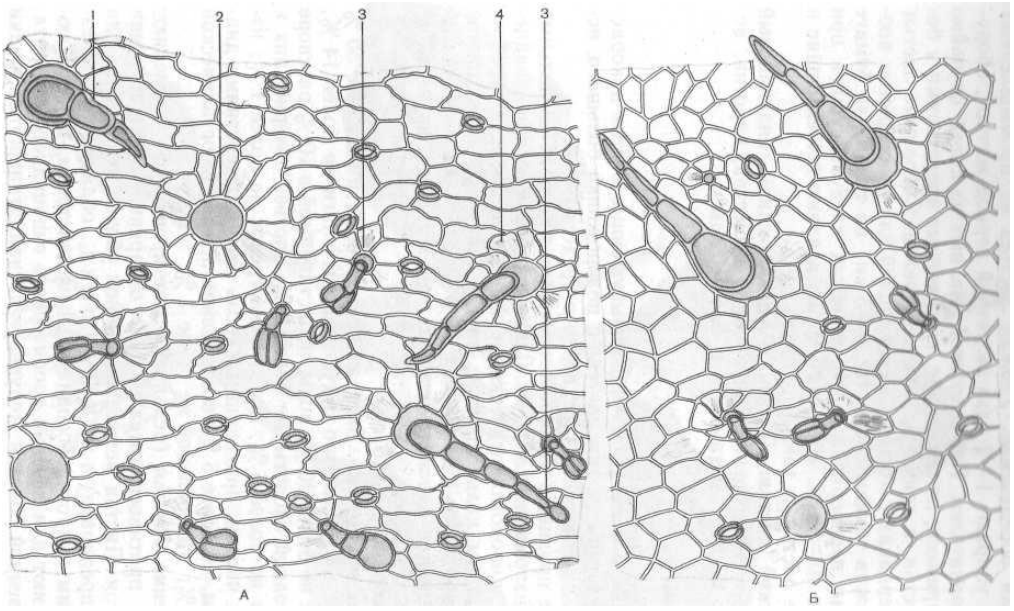
1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6.Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside	
9. Smell.	

Indicate possible admixtures:

1. _____

2. _____

b) Carry out a microscopic analysis and indicate the diagnostic signs of Plantaginis majoris folium.



To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion:_____

_____

TASK № 2. Identify the authenticity and quality of Farfarae folium.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6.Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside.	
9. Smell.	

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion:_____

_____

TASK № 3. Identify the authenticity and quality of Althaeae radices.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6.Smell.	
7.Shape.	

b) Carry out a microchemical reaction with the Althaeae radices powder.

1. With a solution methylene blue

Observation: _____

_____

2. Reaction of double staining: place the slice in ferric chloride solution for 20 minutes, blot with filter paper, add methylene blue solution and rinse with water.

Observation: _____

_____

3. With ink solution.

Observation: _____

_____

To give an opinion about the identification and quality of the MPRM on external feature.

Observation: _____

_____

TASK № 4. Determination of the swelling index of MPRM containing polysaccharides.

_____

(indicate the name of the raw material being analyzed)

1.0 g of hingeless or granulated raw material containing mucus is placed in a graduated cylinder with a capacity of 25 ml with a ground stopper and a graduation of 0.5 ml. Raw materials are moistened with 1 ml of ethyl alcohol 96 %, add water to the mark and thoroughly shake the mixture every 10 minutes for 1 hour to evenly wet the raw materials. After 1.5 hours, the cylinder is rotated about a vertical axis for settling the raw material floating particles. After 3 hours measure the volume of swollen raw material with surrounding mucus. Three tests are carried out in parallel. Calculate the average value of the swelling index.

Observation: _____

_____

Conclusion: _____

_____

DRM and MPRM containing mucus

Name of MPM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Althaea officinalis					
Althaea armeniaca					
Plantago major					
Plantago psyllium					
Tussilago farfara					

Name of MPM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Linum usitatissimum					
Laminaria saccharina					
Laminaria japonica					
Tilia cordata					
Tilia platyphyllos					
Fucus vesiculosus					
Cetraria islandica					

Practice № 6
VITAMINS. DRM AND MPRM CONTAINING VITAMINS

Control questions:

1. Definition of the «vitamins».
2. Classification of vitamins. Their physico-chemical properties.
3. Pharmacological action of vitamins.
4. Vitamins dispersal in the plant world.
5. Ascorbic acid qualitative and quantitative determination in Rosa fruits, according to SF of RB.
6. Thin layer chromatography of vitamins.
7. The role of vitamins in the life of the human body (Vit D, A, E, K, P, PP, B vitamins.
8. Carotenoids: concept, classification, physicochemical properties. Quality control of pharmaceutical products containing carotenoids.
9. Latin names of MPRM, producing plants and families of all plants of the training subject.
10. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
11. External signs of MPRM.
12. Microscopic diagnostic signs of powdered Rosa fruits.
13. Sustainable methods of collecting raw materials, MPM primary processing, drying and storage.
14. Structural formulas of ascorbic and dehydroascorbic acid.
15. Usage of MPRM and medicines containing vitamins.
16. Indicate the chemical composition, pharmacological activity and usage of MPRM containing vitamins.

INFORMATION

Vitamins (from Latin, vita — life) are organic compounds of various chemical nature that perform important biochemical and biological functions in living organisms. The body is required in very small quantities of them (from a few μg to several mg per day), but they have great importance for normal metabolism and vital activity. Vitamins can be considered as universal components of the living organisms cellular metabolism. They are involved in all biochemical processes not being a material for biosynthesis.

Today we know more than 20 vitamins. They are distinguished according to the alphabetic classification (A, B, C, etc.), chemical classifications, characterizing their chemical structure, and can be classified according to their pharmacological activity and physical properties. Vitamins are unitized into water-soluble and fat-soluble. The *water-soluble* vitamins are ascorbic acid, thiamine, riboflavin, pantothenic acid, pyridoxine, folic acid, cyanocobalamin, nicotinamide, biotin. The *fat-soluble* ones are retinol, calciferols, tocopherols, phylloquinones. Some flavonoids, lipoic, orotic, pangamic acids, choline, inositol belong to the vitamin-like compounds.

The vitamins qualitative determination in plants and their quantitative analysis are due to their chemical structure. Vitamins are applied for the prevention and treatment of hypo- and avitominosis and for vitaminization of food; they also use them in animal husbandry.

The vitamins localization is different: they are common in the plants green parts and in fruits. Vitamins are obtained by chemical and microbiological synthesis as well as from natural sources.

THE ALGORITHM OF LABORATORY WORK

TASK №1. Identify the authenticity and quality of Rosae fructus.

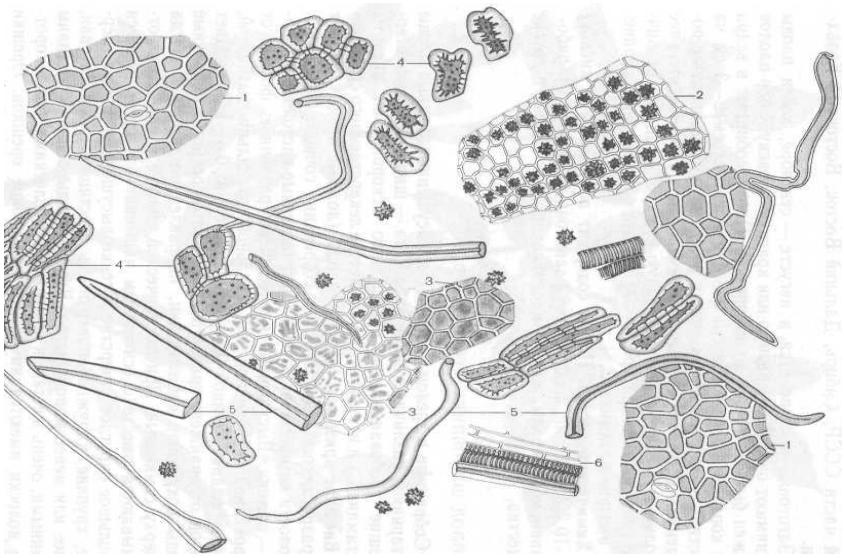
The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of fruit (dry or fleshy).	
2. Shape.	
3. Size of the fruit (length, thickness, diameter)	
4. The features of the pericarp.	
5. The number of seeds, their shape and structure, the surface structure.	
6. Color.	
7. Smell.	

Note the differences in the fruit of the section Cinnamomea from section Canina.

b) Carry out a microscopic analysis and indicate the diagnostic signs of powdered Rosae fructus.



To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

_____

TASK № 2. Carry out an ascorbic acid detection in Rosae fructus by thin layer chromatography.

1,0 g of the powdered raw material is placed in a test tube, pour 5 ml of alcohol 96 %, shake for 30 minutes, filter.

Apply the filtrate using a capillary to the F254 chromatographic plate next to the «witness» — ascorbic acid and place it in a chromatographic chamber with a solvent system: acetic acid–acetone–methanol–toluene (5:5:20:70).

Solvent run is at least 15 cm.

Dry the chromatogram in air under suction and view it under ultraviolet light at a wavelength of 254 nm.

Treat the chromatogram with 0.2 g/l sodium 2,6-dichlorophenolindophenolate solution. Note the nature of the color of the spots, calculate Rf and compare with the «witness».

Draw the results of chromatography.

	№ of spots	**Numeric value of Rf**	**Spots staining**

Conclusion: _____

_____

TASK № 3. To detect carotenoids in Sorbi fructus using thin layer chromatography.

Place 0.5 g of raw material in a flask, add 2.5 ml chloroform R, close with a stopper and stir for 1.5 hours. Filter through a paper filter.

Apply the filtrate using a capillary to a chromatographic plate next to the «witness» — β-carotene and place it in a chromatographic chamber with a solvent system: cyclohexane–ether (80:20).

Solvent run is at least 9 cm.

Dry the chromatogram in air under a draft, view in daylight.

Note the nature of the color of the spots, calculate Rf and compare with the «witness».

Draw the results of chromatography.

	№ of spots	Numeric value of Rf	Spots staining

Conclusion: _____

_____

TASK № 4. Carry out an ascorbic acid quantitative determination in Rosae fructus by titrimetric method (according to SF RB).

1) Preparation of MPRM: to powder whole fruits; 2.0 raw materials powder is placed in a porcelain mortar; rub with glass powder (0.5 g).

2) Extraction: gradually add 75 ml of water with stirring, infuse for 10 minutes and filter.

3) Quantitative determination — oxidation-reduction titration:, add 1 ml of the extract, 1 ml of 2 % hydrochloric acid solution and 13 ml of water in a conical flask with a capacity of 50–100 ml and mix;

titrate rapidly from the microburet by 0.001 mol / l 2,6-dichlorophenolindophenolate sodium solution, until the appearance of a pink color that does not disappear within 30–60 seconds. (Reduction of 2,6-dichlorophenolindophenolate sodium, which has a red color in an acidic media).

4) Calculation of results: calculate the percentage of ascorbic acid (X) according to the formula:

$$X = \frac{Y \times Y_1 \times T \times n \times 100 \times 100}{m \times Y_2 \times (100 - W)},$$

where Y — volume of titrated solution of sodium 2,6-dichlorophenolindophenolate, consumed for titration, ml; Y_1 — extraction volume corresponding to the entire sample, 30 ml; Y_2 — объем извлечения, взятый для титрования, 1 ml; m — sample weight, 2 g; W — weight loss when drying raw materials, %; T — 1 ml of a titrated solution of sodium 2,6-dichlorophenolindophenolate R corresponds to 0.0001 g of ascorbic acid; n — dilution ratio.

Note. If the extract is intensely colored or contains a high content of ascorbic acid (consumption of sodium 2,6-dichlorophenolindophenolate solution is more than 2 ml), detected by the first titration, dilute the original extract with water two or more times before re-titration. Take into account the dilution carried out in the formula when calculating.

On the basis of the analysis carried out, a conclusion is made about the raw materials quality.

Conclusion: _____

_____

DRM and MPRM containing vitamin C

Name of MPM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Rosa					
Ribes nigrum					

MPM and MPRM containing carotenoids

Name of MPM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Sorbus aucuparia					
Hippophae rhamnoides					
Calendula officinalis					

MPM and MPRM containing vitamins of the K group

Name of MPM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Urtica dioica					
Urtica urens					
Capsella bursa-pastoris					
Viburnum opulus					
Zea mays					

Practice № 7

ESSENTIAL OILS. ANALYSIS OF MPRM CONTAINING ESSENTIAL OILS. DRM AND MPRM CONTAINING AROMATIC COMPOUNDS

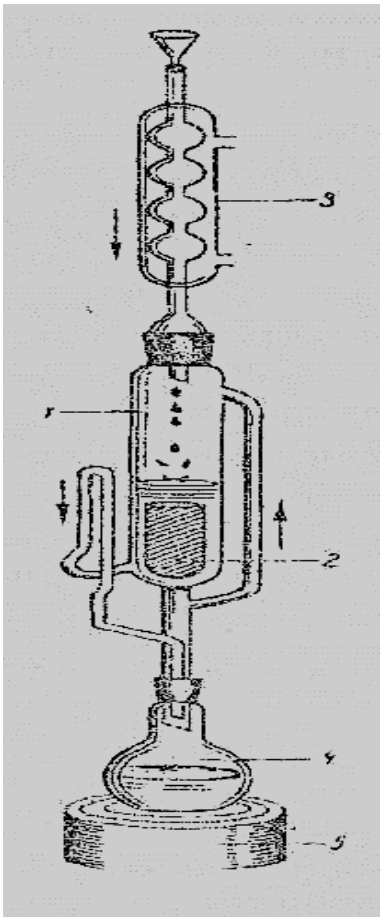
Control questions:

1. The concept of «terpenes» and their classification.
2. Definition of «essential oil». Classification of essential oils.
3. Physico-chemical properties and methods of isolation from plant matherial.
4. Qualitative and quantitative analysis of MPRM on the content of essential oils.
5. Analysis of essential oils.
6. Distribution of essential oils in the plant world.
7. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
8. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
9. Sustainable methods of collecting raw materials, DRM primary processing, drying and storage.
10. Chemical composition, formulas of thymol, carvacrol and anethole.
11. Medicines and their useing.
12. Identify the chemical composition, pharmacological activity and use of MPRM containing essential oils with a predominance of aromatic compounds.

INFORMATION

Essential oils are natural fragrances that have a strong volatility and cause a specific smell of plants. For this reason and also because of its «oily» consistency and the «greasy» spot on paper, which soon disappears, they received such an original name.

List the components of the Soxhlet apparatus



1. _____

_____

2. _____

_____

3. _____

_____

4. _____

_____

5. _____

_____

Classification of essential oils

Methods for quantitative determination of essential oil according to SF RB

Method	Device	Receiver for eo	P (EO)	Solvent in receiver	Features of the method
A					
B					
C					
D					
E					

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Identify the authenticity and quality of the Serpylli herba.

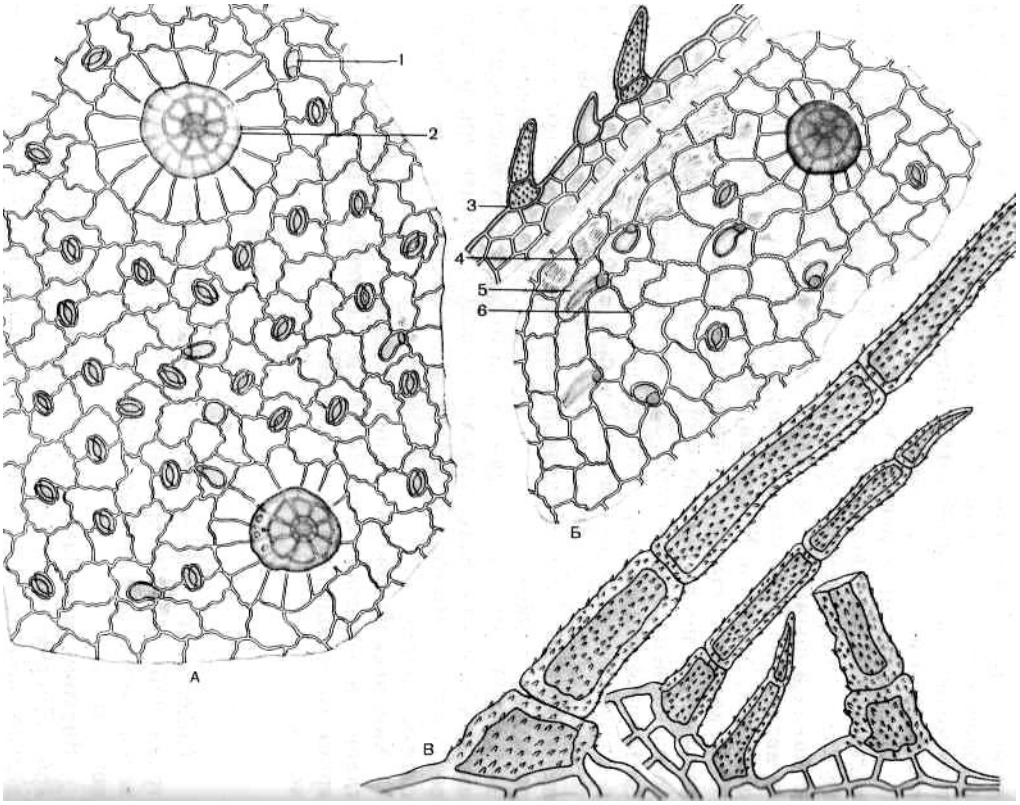
The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade.	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside.	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers.	
13. The shape of the flower (actino- or zygomorphic).	
14. Inflorescence or flower size.	
15. Absence or presence of peduncle (its shape and size).	

16. Indumentum.	
17. Color.	
18. Smell	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Serpylli herba.



To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

TASK № 2. Identify the authenticity and quality of the Origani herba.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

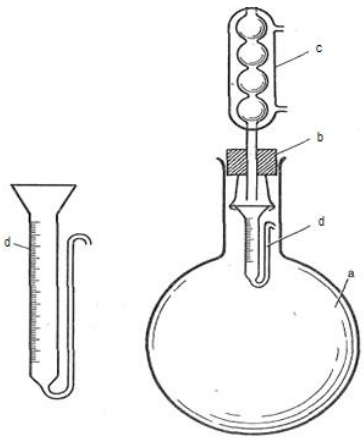
1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers.	
13. The shape of the flower (actino- or zygomorphic).	
14. Inflorescence or flower size.	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum.	
17. Color.	
18. Smell	

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

_____

TASK № 3. Carry out quantitative determination of essential oil in MPRM.



_____

(specify the name of the plant material being analyzed)

Quantitative determination of essential oil in raw materials is carried out by volume method by distillation of a sample of granulated raw material with water vapor, followed by measuring the volume of the obtained essential oil and its expression in volume-by-weight percentage. To do this, use the Ginsberg device (method B of GF RB) or a modified device Clevenger (methods C and D of GF RB). The distillation is carried out in a flask with a reflux condenser. According to the method B the receiver is strengthened inside the flask.

Sign all parts of the device to determine the content of essential oil in the MPRM

The device for determining the content of essential oil by the SP RB method B

a _____

_____

b _____

_____

c _____

_____

d _____

_____

Carry out a quantitative determination of essential oil in one of the types of MPRM: Chamomillae flores, Eucalypti folium, Pini silvestris gemma, Calami rhizoma.

1) To get acquainted with the private article of the SP of the Republic of Belarus on this type of raw materials.
2) Crush the raw material to the required particle size and take a sample on the hand scales.
3) Place in a 1000 ml flask.
4) Pour in the required amount of water in accordance with a private article of the SP RB.
5) Close the rubber stopper to which a graduated receiver is attached to the bottom, attach the return condenser.
6) Heat the flask with the contents on the electric stove to a boil and boil for the time indicated in the SF RB for this type of raw material.

Steam of water and essential oil condense in the return condenser and the liquid drains into the receiver. Essential oil is collected at the top of the receiver, since it is lighter than water, and water flows through the smaller elbow of the receiver back.

7) Measure the oil volume in the receiver after cooling it to room temperature.
8) Calculate the % content of essential oil in raw materials:

$$X = \frac{V \times 100 \times 100}{m \times (100 - W)},$$

where V — volume of essential oil (division price × number of divisions), ml; m — mass of raw materials, g; W — loss in mass when dried, %.

Calculate the content of essential oil in the raw material in ml/kg.

The results of the analysis are compared with the SF RB for this type of raw materials and give an conclusion about its quality.

Conclusion: _____

_____

DRM and MPRM containing essential oils with a predominance of aromatic compounds

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Pimpinella anisum					
Foeniculum vulgare					
Thymus serpyllum					
Thymus vulgaris					
Origanum vulgare					
Levisticum officinale					

Practice № 8
MONOTERPENES. DRM AND MPRM CONTAINING ACYCLIC, MONO- AND BICYCLIC MONOTERPENES

Control questions:

1. Definition of the «essential oils».
2. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
3. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
4. Rational methods of collecting raw materials, primary processing, drying and storage of pharmaceutical products.
5. Chemical composition and formulas of: geraniol, linalool, citral, menthol, cineole, carvone, camphor, borneol, α-pinene.
6. Medicines and their useing.
7. Identify the chemical composition, pharmacological activity and use of MPRM containing monotherphenes.

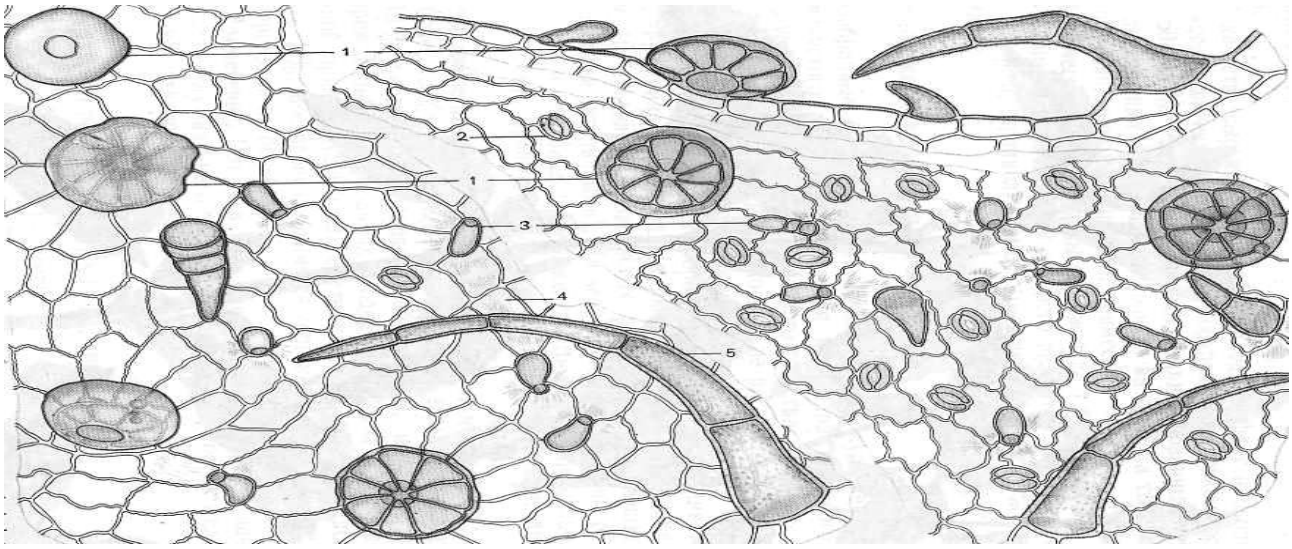
THE ALGORITHM OF LABORATORY WORK

TASK №1. Identify the authenticity and quality of the Mentha piperita leaves.

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside.	
9. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Menthae piperitae folium.



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To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

_____

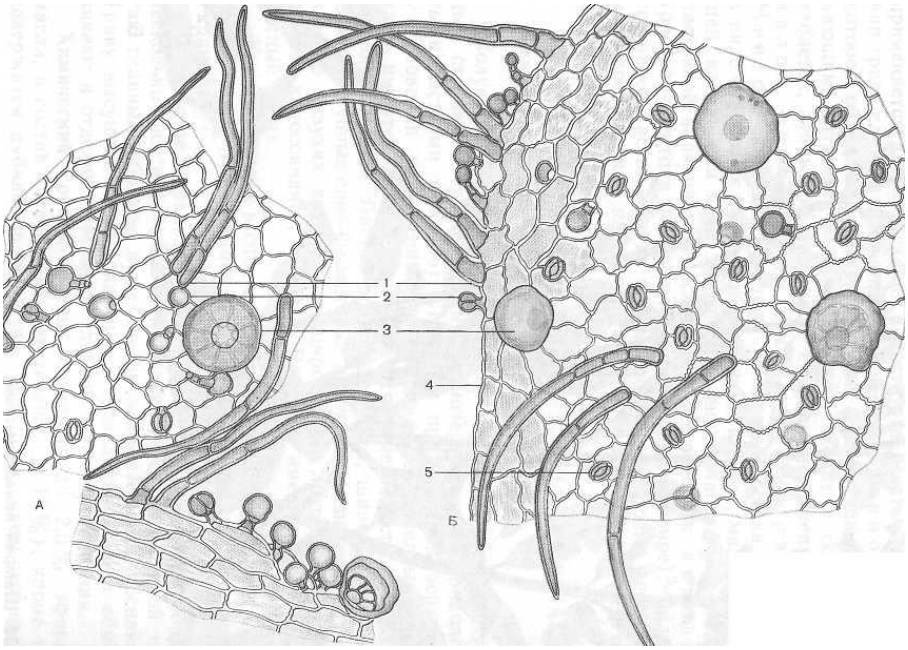
TASK № 2. Identify the authenticity and quality of the Salviae folium.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside and underside.	
9. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Salviae folium.



To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

TASK № 3. Identify the authenticity and quality of the Carvi fructus.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Type of fruit (dry or fleshy).	
2. Shape.	
3. Size of the fruit (length, thickness, diameter)	
4. The features of the pericarp.	
5. The number of seeds, their shape and structure, the surface structure.	
6. Color.	
7. Smell.	

To give an opinion about the identification and quality of the MPRM on external feature

Conclusion: _____

DRM and MPRM containing monotherphenes

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Coriandrum sativum					
Lavandula angustifolia					
Melissa officinalis					
Mentha piperita					
Salvia officinalis					
Carum carvi					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Eucalyptus viminalis					
Eucalyptus globulus					
Eucalyptus cinerea					
Anethum graveolens					
Juniperus communis					
Valeriana officinalis					
Pinus sylvestris					
Abies sibirica					

Practice № 9
SESQUITERPENES. DRM AND MPRM CONTAINING SESQUITERPENES

Control questions:

1. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
2. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
3. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
4. Chemical composition and formulas of bisabolene, chamazulene, matricin, ledole, alantolactone.
5. Medicines and their useing.
6. Identify the chemical composition, pharmacological activity and use of MPRM containing sesquiterpenes.

THE ALGORITHM OF LABORATORY WORK

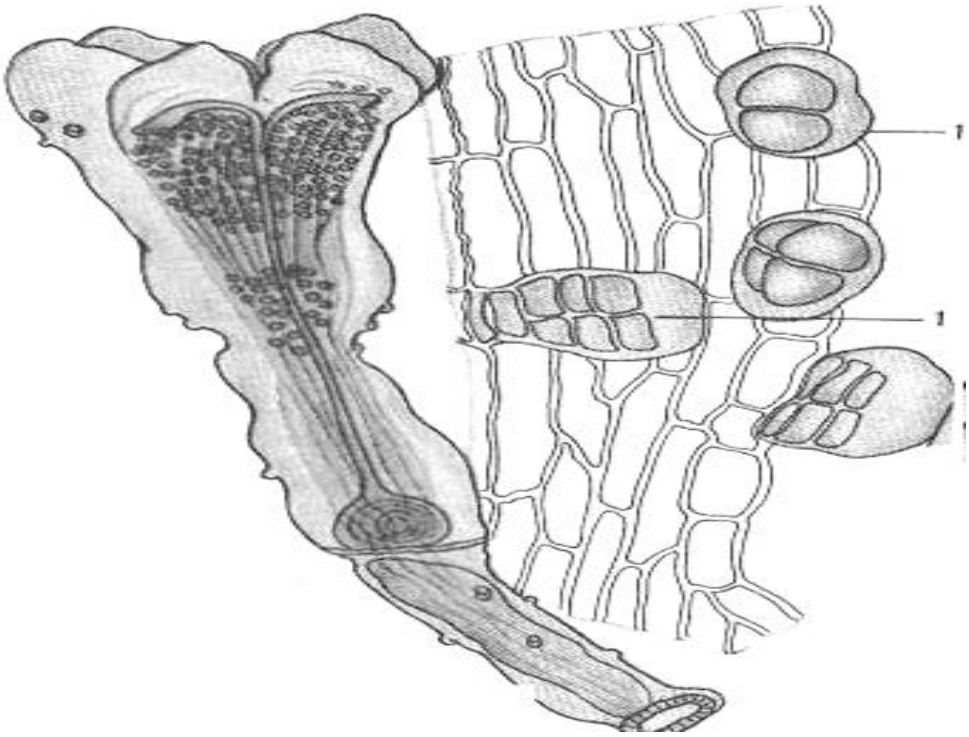
TASK № 1. Identify the authenticity and quality of the Chamomillae recutita flores.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of an inflorescence or single flowers.	
2. The shape of the flower (actino- or zygomorphic).	
3. Inflorescence or flower size.	
4. Absence or presence of peduncle (its shape and size).	
5. Indumentum.	
6. Color.	
7. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Chamomillae recutita flores.



c) Draw a longitudinal incision of the flower-bud, study possible admixtures: Matricaria discoidea, Tripleurospermum inodorum, Leucanthemum vulgare, Anthemis arvensis.

Conclusion:

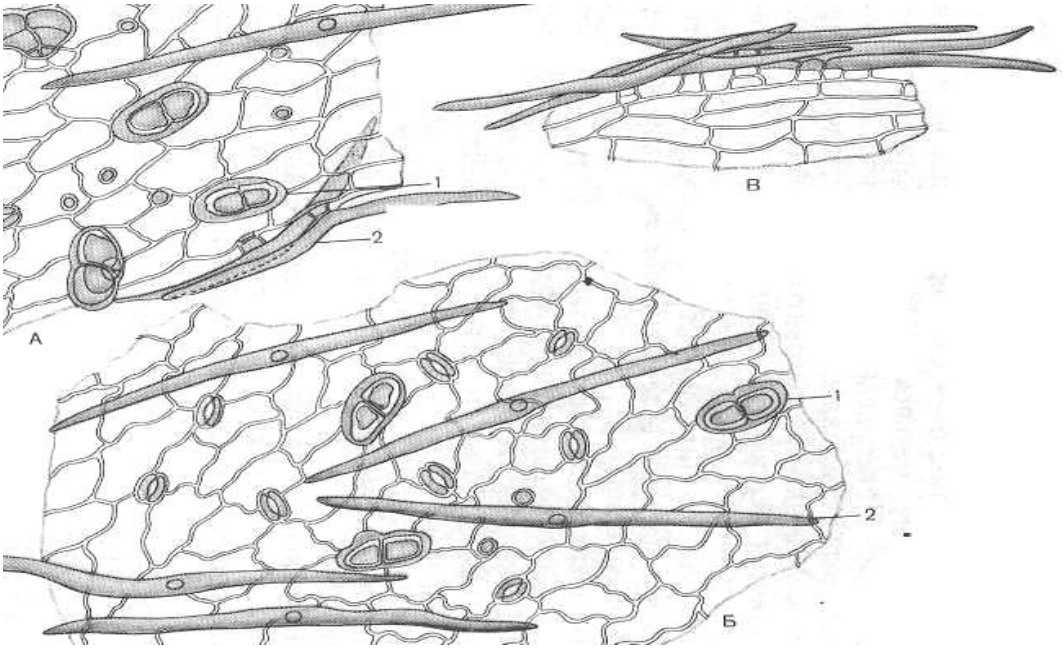
TASK № 2. Identify the authenticity and quality of the Artemisiae absinthii herba.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade.	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside.	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers.	
13. The shape of the flower (actino- or zygomorphic).	
14. Inflorescence or flower size.	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum.	
17. Color.	
18. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Artemisiae absinthii herba.



To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

TASK № 3. Identify the authenticity and quality of the Taraxaci officinalis radices.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

To give an opinion about the identification and quality of the MPRM on external feature.

Conclusion: _____

TASK № 4. To carry out tests for identity of the Calami rhizome.

a) Qualitation.

Place 1.0 g of crushed raw material in a 50 ml flask, add 10 ml of 96 % alcohol and bring to a boil in a water bath, cool and filteradd 0.2 ml of iron (III) chloride solution.

Observation: _____

_____

b) Qualitative analysis by thin layer chromatography.

Apply the solution using a capillary to the chromatographic plate next to the «witness» — alcohol solutions of thymol and anethole and place in a chromatography chamber with a solvent system: thyl acetate-hexane (1:9).

Chromatography is carried out for 20 minutes (solvent run 15 cm), then dry the chromatogram in air under draft.

Dry the chromatogram in air under suction.

Treat the chromatogram with an anisaldehyde solution and heat at a temperature from 105 to 110 °C for (8–10 minutes).

Draw the results of chromatography.

	№ of spots	**Numeric value of Rf**	**Spots staining**

Conclusion: _____

_____

DRM and MPRM containing sesquiterpenes

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Matricaria chamomilla					
Inula helenium					
Acorus calamus					
Arnica montana					
Arnica chamissonis					
Arnica foliosa					
Betula pendula					
Betula pubescens					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Achillea millefolium					
Humulus lupulus					
Artemisia absinthium					
Archangelica officinalis					
Taraxacum officinale					
Ledum palustre					
Zingiber officinale					

Practice № 10
IRIDOIDS. MEDICINAL PLANT MATERIALS CONTAINING IRIDOIDS

Control questions:

1. Definition of the «amarines» and iridoids. Their dispersal in the piant world.
2. Classification of iridoids, their physicochemical properties.
3. Methods of iridoids analysis.
4. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
5. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
6. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
7. External signs of MPRM.
8. Chemical composition and structural formulas of loganin, aucubin, valepotriates.
9. Medicines and their application.
10. Identify the chemical composition, pharmacological activity and use of MPRM containing iridoids.

THE ALGORITHM OF LABORATORY WORK

Bitternesses (Amara) — natural compounds of various chemical nature, which have a bitter taste and are used as agents that stimulate appetite and improve digestion.

Bitternesses are classified into pure bitternesses (Amara pura) and aromatic bitternesses (Amara aromatica).

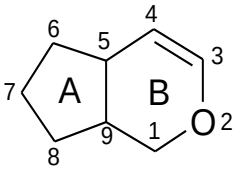
Bitter substances in plants can be found together with essential oils, and in this case they are called «aromatic bitternesses» (or sesquiterpene). Sesquiterpene bitternesses is represented mainly by lactones.

Pure bitternesses is mainly represented by iridoids, or monoterpene glycosides.

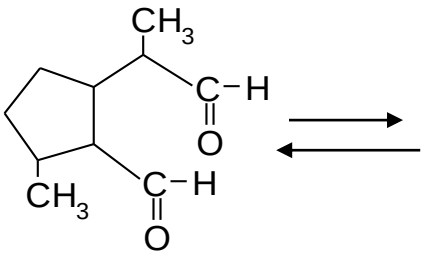
Iridoids are a group of plant-derived monoterpene compounds that have in their structure a partially hydrogenated cyclopentane-pyranic system.

Iridoids (or pseudoindicans) are a group of cyclopentanepyrane monoterpenes, whose name is associated with iridodial, which was obtained from the ancestral genus *Iridomyrmex*. Pseudoindicans are named for the ability to give a blue color in an acid medium.

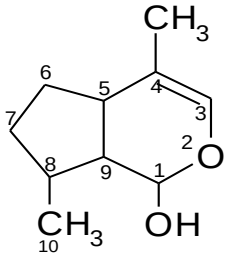
Iridoids are most often in the form of glycosides in plants, sometimes in free form (in the form of aglycons). The sugar part is represented by glucose, xylose, rhamnose, galactose.



Cyclopentanopyran



Iridodial
(dialdehyde form)



Iridodial
(Lactone form)

Method for determining the bitterness indicator

Bitterness index (BI) — the reciprocal of the highest dilution of the test substance, or fluid extract, which still felt the bitter taste. It is determined in relation to the bitterness of quinine hydrochloride, the value of which is assumed to be 200,000.

Determination of the correction factor. The tasting commission should consist of 6 people. Before tasting the mouth must be rinsed with water. In order to correct individual differences among the members of the tasting commission in determining the bitterness indicator, it is necessary to determine a correction factor for each member of the commission.

Basic solution. Dissolve 0.100 g quinine hydrochloride in 80 ml of distilled water in a 100 ml volumetric flask and adjust the volume of the solution with water to the mark (solution A). 1 ml of solution A is transferred to a 100 ml volumetric flask and the volume of the solution is adjusted to the mark with water (solution B).

Compensation liquid. A series of dilutions of solution B is prepared; 3.6 ml of the base solution are placed in the first tube, followed by 3.8 ml, then the volume is increased by 0.2 ml to 5.8 ml in the last tube. The volume of the solution in each tube is adjusted with water to 10 ml.

Determine the lowest concentration, which has a bitter taste. To do this, take 10 ml of the diluted solution in the mouth and move from side to side above the surface of the tongue during 30 s. If the solution does not feel bitter taste, it is spat out and waiting for 1 min. After that, rinse your mouth with water. After 10 minutes, repeat the test of the next solution in order of increasing concentration.

Calculate the correction factor for each member of the tasting commission according to the formula:

$$k = \frac{5{,}00}{n},$$

where n — the number of ml of the basic solution with the lowest concentration, in which the bitter taste is determined.

Persons who do not feel bitterness in the most concentrated solution are excluded from the commission for determining bitterness.

Sample preparation. If it necessary, the raw material sample is ground into a powder. To 1.0 g of the sample 100 ml of boiling water are added, heated in a water bath for 30 minutes, continuously stirring. Allow to cool and bring the volume of water to 100 ml. Mix well and filter, discarding the first 2 ml of the filtrate. This filtrate is designated C-1 and is considered as dilution factor (DF) = 100.

Test solutions. Prepare the next series of dilutions:

10,0 ml of *C-1* diluted to 100: C-2 (D_F = 1000);

10,0 ml of *C-2* to 100: *C-3* (D_F = 10 000);

20,0 ml of *C-3* to 100: *C-3A* (D_F = 50 000);

10,0 ml of *C-3* to 100: *C-4* (D_F = 100 000).

Each member of the commission starts testing with the most dilute solution of C-4 to find a solution that has a bitter taste. This solution is designated D and has a degree of dilution, which is denoted by Y.

Starting with solution D, solutions:

D, ml	1,2	1,5	2,0	3,0	6,0	8,0
Water, ml	8,8	8,5	8,0	7,0	4,0	2,0

Determine the amount of D (ml), which, when diluted to 10.0 ml with water, has a bitter taste (x).

$$BI = \frac{Y \cdot k}{x \cdot 0{,}1},$$

The average value of the bitterness indicator of all subjects.

THE ALGORITHM OF LABORATORY WORK

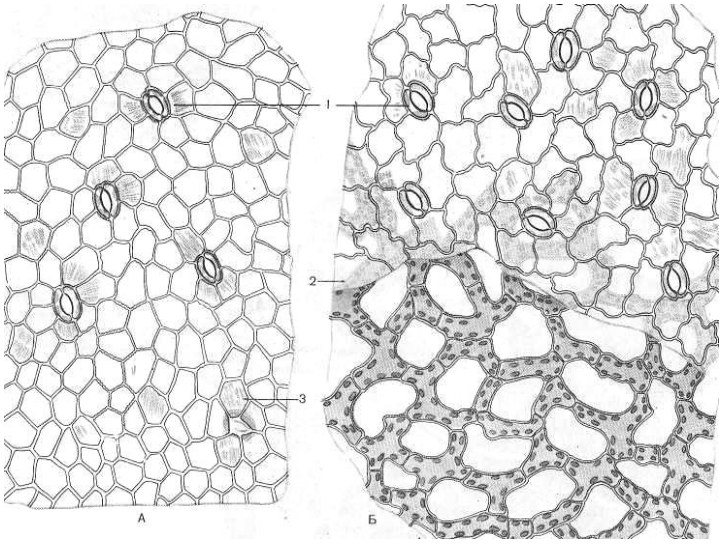
TASK № 1. Carry out a macroscopic analysis and indicate the diagnostic signs of Menyanthidis trifoliatae folia.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside	
9. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Menyanthidis trifoliatae folia.



To give an opinion about the identification and quality of the MPRM on microscopic and macroscopic diagnostic signs.

Conclusion: _____

_____

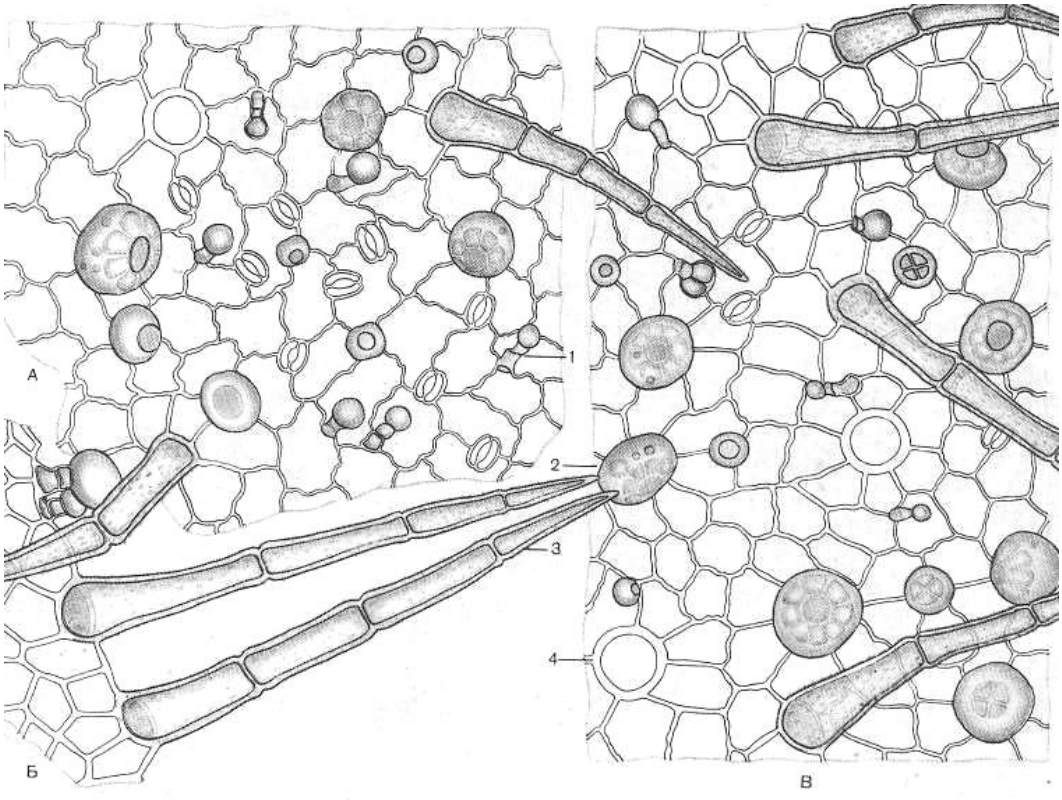
TASK № 2. Carry out a macroscopic analysis and indicate the diagnostic signs of Leonuri herba.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
11. And underside	
12. Flower arrangement on the stem.	
13. **Flower.** Type of an inflorescence or single flowers	
14. The shape of the flower (actino- or zygomorphic)	
15. Inflorescence or flower size	
16. Absence or presence of peduncle (its shape and size).	
17. Indumentum	
18. Color	
19. Smell	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Leonuri folia.



Conclusion: _____

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TASK № 3. Qualitative analysis of Leonurus leaves by thin layer chromatography Leonuri herba

Add 5 ml of 96 % ethyl alcohol to 0.5 g of crushed raw materials and heat in a water bath at 65 ° C for 5 minutes with shaking. Cool and filter.

Apply the filtrate to a chromatographic plate using a capillary and place it in a chromato-graphic chamber with a solvent system: chloroform–methanol–water (80:2:0.1).

Run the solvent for at least 10 cm, then dry the chromatogram in air under a draft.

Treat the chromatogram with a solution of dimethylaminobenzaldehyde (P2) and heat at a temperature of 100 to 105 C for 10 minutes.

Draw the results of chromatography.

	№ of spots	Numeric value of Rf	Spots staining

To give an opinion about the identification of the MPRM.

Conclusion: _____

_____

TASK № 4. Carry out a macroscopic analysis and indicate the diagnostic signs of Valerianae rhizomata cum radicibus.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	
7. Shape.	

To give an opinion about the identification and quality of the MPRM on microscopic and macroscopic diagnostic signs.

Conclusion: _____

_____

DRM and MPRM containing bitterness and iridoids

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Leonurus quinquelobatus					
Leonurus cardiaca					
Viburnum opulus					
Menyanthes trifoliata					
Valeriana officinalis					
Centaurium erythraea					
Gentiana lutea					
Paeonia anomala					

Practice № 11
THE FINAL CLASS

Polysaccharides

1. Chemical composition and physico-chemical properties of mucus.
2. Medical plant containing mucus: Laminaria, Linum, Tussilago farfara, Althaea, Plantago, Fucus vesiculosus, Tilia, Cetraria islandica. Use in medicine.

Vitamines

1. Classification. Features of drying and storage of vitamin-containing raw materials. Quantitative determination of ascorbic acid.
2. Medical plant containing vitamines: Ribes nigrum, Zea mays, Rosa, Hippophae rhamnoides, Calendula officinalis, Sorbus aucuparia, Urtica dioica, Capsella bursa pastoris, Viburnum opulus. MPRM and medicines using.

Terpenoids

1. The concept of terpenoids. Their classification.
2. Essential oils. Classification, chemical composition and physical properties, dispersal in the plant world. Localization of essential oil in MPRM. Methods of collection, drying and storage of essential oil raw materials. Methods of obtaining essential oils. Essential oil analysis and methods for determining essential oil in MPRM. Application.
3. DRM and MPRM containing acyclic monoterpenes: Coriandrum sativum, Lavandula angustifolia, Melissa officinalis.
4. DRM and MPRM containing monocyclic monoterpenes: Mentha piperita, Salvia officinalis, Eucalyptus, Carum carvi, Anethum graveolens.
5. DRM and MPRM containing bicyclic monoterpenes: Pinus silvestris, Juniperus communis, Abies sibirica, Valeriana officinalis.
6. DRM and MPRM containing sesquiterpenes: Matricaria chamomilla, Arnica, Betula, Inula helenium, Ledum palustre, Humulus lupulus, Achillea millefolium, Artemisia absinthium, Taraxacum officinale, Zingiber officinale, Acorus calamus.
7. DRM and MPRM containing aromatic compounds: Thymus vulgaris, Thymus serpyllum, Anisum vulgare, Foeniculum vulgare, Origanum vulgare, Levisticum officinale.

Iridoid

1. Classification, physicochemical properties, application in medicine.
2. DRM and MPRM containing iridoids: Centaurium, Menyanthes trifoliata, Valeriana officinalis, Viburnum opulus, Paeonia anomala, Leonurus, Gentiana lutea.

Fatty oils

1. Chemical composition and physico-chemical properties. Vegetable sources of fatty oils: Helianthus, Linum usitatissimum, Ricinus communis, Prunus dulcis, Prunus persica, Zea mays, Theobroma cacao. Reception and application of fats.
2. Know the formulas: ascorbic acid, dihydroascorbic acid, linalool, citral, geraniol, cineole, menthol, carvone, pinene, borneol, camphor, bornylisovalericanate, chamazulene, matricin, ice cream, carvacrol, anethole, thymol, loganin.

Practice № 12
CARDIAC GLYCOSIDES.
ANALYSIS OF MPRM CONTAINING CARDIAC GLYCOSIDES

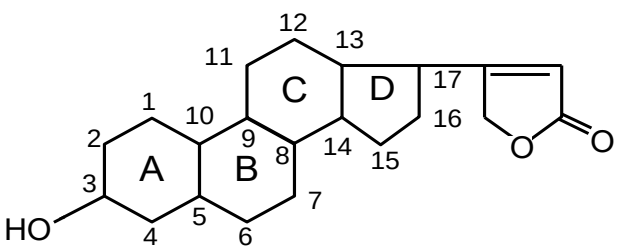
Control questions:

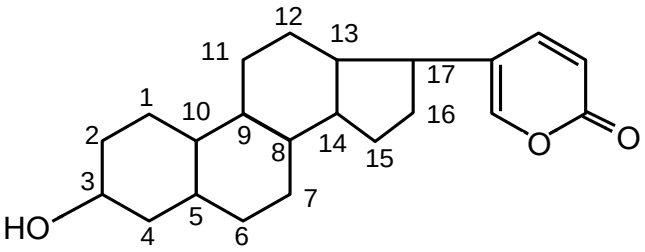
1. Definition of the «cardiac glycosides», classification of cardiac glycosides.
2. Features of the chemical structure of cardiac glycosides. Relationship between the chemical structure of cardiac glycosides and their biological activity.
3. Physicochemical properties of cardiac glycosides.
4. Cardiac glycosides dispersal in the plant world.
5. Methods for the cardiac glycosides extraction from MPRM and methods for their decontamination.
6. Qualitative reactions of cardiac glycosides detection and their specificity.
7. Methods for cardiac glycosides chromatographic analysis.
8. Biological methods for MPRM standardization. Definition of the «VALOR».
9. Physicochemical methods of cardiac glycosides quantitative determination in plant raw materials.
10. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
11. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
12. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
13. Chemical composition and formulas of purpureoglycosides A and B, lantozides A, B, C; digitoxin-genin, K-strophanthoside, K-strophanthin-β, cymarin, adonitoxin, konvalotoxin.
14. Application of MPRM containing cardiac glycosides.
15. Medicines and their application.
16. Indicate the chemical composition, pharmacological activity and application of MPRM containing cardiac glycosides.

INFORMATION

Ways and methods of using a raw material containing cardiac glycosides.

Cardioactive glycosides (cardiac glycosides) are heterozides which aglycones are steroids — cyclopentanepergid-rofenanthrene derivatives, which have an unsaturated lactone ring in C_{17}: five-membered butenolide (**cardedolides**) or six-membered cumulane (**bufadienolides**).





All cardiotonic glycosides aglycons have methyl at C_{13} and hydroxyl groups at C_{14}. There may be a β-oriented methyl, aldehyde or carboxyl group at C_{10}. The carbohydrate part of the cardioglycoside molecule contains from 1 to 5 monosaccharides, always joining through the oxygen atom at C_3.

According to the nature of the substituting group at C_{10} the medicinal plant raw material containing the cardenolides is divided into two groups:

Digitalis group — cardioglycosides, which are characterized by the methyl group (-CH3) presence at C10 and possessing a cumulative effect.

Strophanthus group — cardioglycosides, which are characterized by the aldehyde (-CHO) or alcohol (-CH2OH) groups presence and with no possessing the cumulative effect.

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Carry out ***quantitative determination*** of cardiac glycosides by photoelectric colorimetry.

1. **Preparative of MPRM**: powder raw materials, sift through a sieve with 1 mm hole diameter and take an exact sample (about 1.5 g), place in a flat bottomed flask with a 100 ml capacity, add 30 ml of 96 % ethanol, mix, stopper and leave for 18-20 hours in a dark place.

2. **Filtration and decontamination.** The resulting alcohol extract is filtered through a paper filter into a graduated cylinder with ground stopper, discarding the first 20 drops of the filtrate. Measure the volume obtained (filtrate A). Add an equal volume of distilled water to the filtrate A, add 7.5 g of Al_2O_3, close with a stopper, shake for 2-3 minutes and filter through a dry paper filter (discarding the first 20 drops of the filtrate) into a dry flask (filtrate B).

3. **Preparation of a cardiac glycosides colored complex.** Test solution: transfer 8 ml of filtrate B into a dry test tube, add 1 ml of 1 % alcohol solution of picric acid, 1 ml of 5 % NaOH solution and mix thoroughly.

At the same time, prepare a compensation solution consisting of 8 ml of 50 % ethanol, 1 ml 1 % alcohol solution of picric acid and 1 ml of 5 % NaOH solution. Keep both solutions (test and compensation) in a dark place for 10 minutes.

Determination of the test solution optical density on a background of a control solution on a photoelectric calorimeter in a 1 cm thick cuvette (green light filter). Using the obtained value of the solution optical density, find the content of the cardiac glycosides sum in 1 ml of the solution (C) using the calibration graph.

The percentage of the cardiac glycosides sum X is calculated by the formula:

$$\text{X, \%} = \frac{\text{A} \times \text{V} \times (V_1 + V_3) \times V_4 \times 100}{\text{m} \times V_2 \times V_3 \times (100 - \text{W}) \times A_{1\text{см}}^{1\%}} = \frac{A \times 83}{m \times A_{1\text{см}}^{1\%}},$$

where A — optical density of the solution, value from experiment; V — volume of extractant for extraction of the sum of cardiac glycosides, 30 ml; V_1 — volume of water taken to dilute filtrate A, value from experiment, ml; V_2 — volume of filtrate B taken for colorimetry 8 ml; V_3 — volume of filtrate A taken for determination, value from experiment, ml; V_4 — the total volume of the final (colorimetric) solution is 10 ml; m — sample of raw materials, 1.5000 g; W — weight loss upon drying, 10 % (conditional); $A_{1\text{см}}^{1\%}$ — specific absorption rate of the sum of cardenolides equal to 140.

TASK № 2. Carry out ***qualitative reactions*** of cardiac glycosides detection in MPRM.

The filtrate B, remaining after quantitative determination, should be transferred to the evaporation cup, evaporated in a water bath to dryness. Dissolve the dry residue in 3.5 ml of 70 % ethanol and carry out qualitative reactions.

1) reactions to a five-membered lactone ring:

Legals test. To 0.5 ml of extraction, add an equal volume of 10 % NaOH solution and mix. Carefully add on the tube wall 3 drops of 5 % sodium nitroprusside.

Observation: _____

Balye test. To 0.5 ml of extraction, add 0.5 ml of 10 % NaOH solution and 5 drops of 2.5 % picric acid alcoholic solution.

Observation: _____

Kedde test. To 0.5 ml of extraction, add 8 drops of 10 % NaOH solution and the same 2 % 3,5-dinitrobenzoic acid alcohol solution.

Observation: _____

2) reaction to deoxysugars:

Keller-Kiliani test (to carry out with «Adoniside» or with Erysimum herb extract). To 0.5 ml of «Adoniside» add an equal volume of glacial acetic acid with iron trace amounts. Carefully place 0.5 ml of concentric sulfuric acid with iron trace amounts.

Observation: _____

3) reaction to the steroid structure:

Lieberman-Burkhard test. Evaporate 0.5 ml of extraction in a test tube to dryness. To the residue add 0.5 ml of acetic anhydride and **carefully!** couch together 0.5 ml of conc. sulfuric acid on the test tube wall.

Observation: _____

Based on the analyzes carried out, a conclusion is drawn on the content of cardiac glycosides in the raw materials.

Conclusion: _____

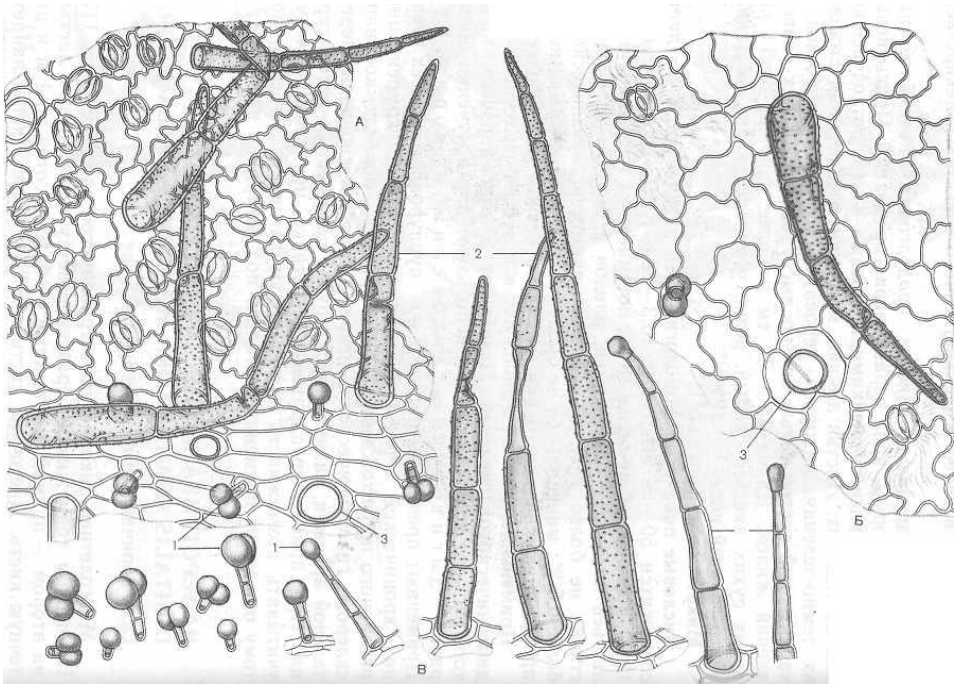
TASK № 3. Carry out a macroscopic analysis and indicate the diagnostic signs of Digitalis folium.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside	
9. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Digitalis folium.



To give an opinion about the identification and quality of the MPRM on microscopic and macroscopic diagnostic signs.

Conclusion: _____

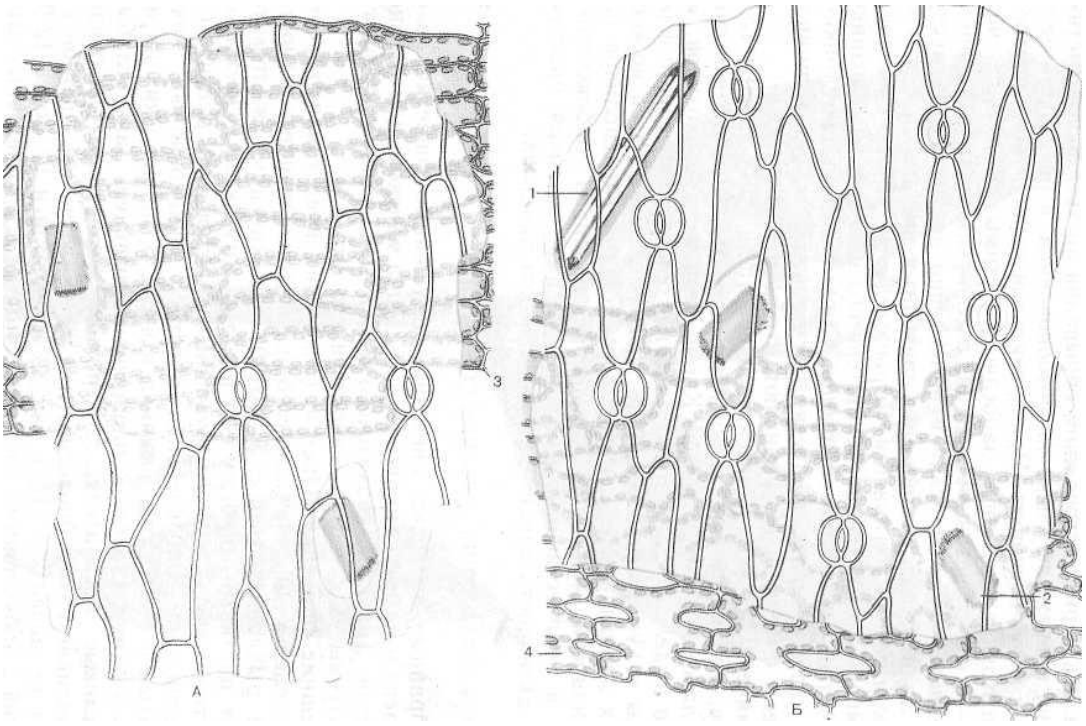
TASK № 4. Carry out tests for identication of Convallariae folium.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside.	
9. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Convallariae folium.



c) Carry out a qualitative analysis using thin layer chromatography of Convallariae folium.

To 1.0 g of powdered raw material, add 20 ml of 50 % alcohol, 10 ml of 100 g / l lead acetate solution and heat with reflux in a water bath for 2 minutes. Cool and centrifuge. The supernatant is shaken twice with chloroform (15 ml portions), then shaken twice with a mixture of chloroform and alcohol (1: 1) in 15 ml portions. Chloroform layers are combined, filtered through a paper filter with 2 g of sodium sulfate anhydrous and evaporated to dryness. Dissolve the residue in 1 ml of a mixture of chloroform and alcohol (1: 1).

Apply the filtrate on the chromatographic plate by the capillary next to the «witness» — a solution of konvaltoxin in a mixture of chloroform and alcohol (1: 1) and place in a chromatographic chamber with a solvent system: CHLOROFORM — ETHANOL — WATER (40: 9: 1)

Chromatography is led for 20 minutes (the range of the solvent is not less than 12 cm), then the chromatogram is dried under air in the draft.

Process the chromatogram with a solution of vanillin and heat at a temperature of 100 to 105 °C for 5 minutes.

Draw the results of chromatography.

	№ of spots	Numeric value of Rf	Spots staining

To give an opinion about the identification of the MPRM.

Conclusion: _____

_____

To give an opinion about the identification and quality of the MPRM on microscopic and macroscopic diagnostic signs and on the chromatography results.

Conclusion: _____

_____

DRM and MPRM containing cardiac glycosides

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Digitalis purpurea Digitalis grandiflora Digitalis lanata					
Strophanthus kombe					
Adonis vernalis					
Convallaria majalis					
Erysimum diffusum					

Practice № 13
SAPONINS. DRM AND MPRM CONTAINING SAPONINS

Control questions

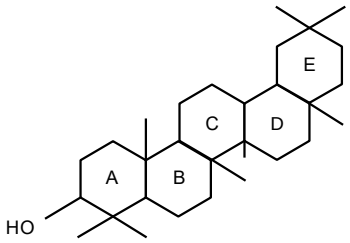
1. Definition of the «saponins», classification of saponins.
2. Dispersal of saponins in the plant world, their localization in organs and tissues.
3. Chemical structure of saponins and their classification.
4. Physico-chemical and biological properties of saponins.
5. Methods for saponins extraction from MPRM and methods for their decontamination.
6. Methods for detection and quantification of saponins in MPRM.
7. Chemical composition and structural formulas of α- and β-amyrin, oleanolic and ursolic acids, glycyrrhizic acid, diosgenin, dammaran.

INFORMATION

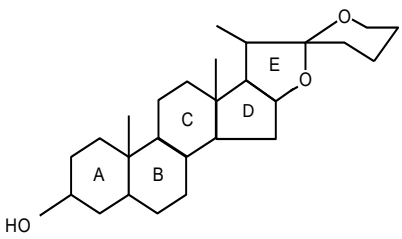
Saponins (Sapo — soap) are a group of natural glycosides that are hydrolyzed into a carbohydrate complex and aglikon-sapogenin.

Aqueous solutions of saponins give abundant ***persistent foam*** with shaking, have acidic or neutral reactions, *are toxic to cold-blooded*, have ***hemolytic and surface activity***.

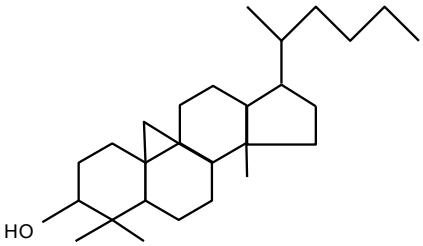
According to their structural and chemical characteristics saponins are divided into triterpene and steroid glycosides.



Triterpene saponin
β-amyryn type



Steroid saponin
spirostanol type



Tetracyclic triterpene
saponin cycloartan type

THE ALGORITHM OF LABORATORY WORK

TASK № 1 Carry out qualitative reactions and chromatographic determination of saponins in MPRM.

_____

(specify the name of the plant material being analyzed)

Qualitative reactions to MPRM for the detection of saponins

Prepare extraction: take 3 flat bottom flasks for 30 ml, place in each of them 0.5 g of powdered raw material. Add 10 ml of isotonic sodium chloride solution (extract No. 1) to one flask, 10 ml of distilled water in another flask (extraction No. 2). Both flasks are heated in a boiling water bath for 10 minutes. After cooling, filter through a piece of cotton wool.

1. *Test based on the biological properties of saponins*.

Erythrocytes hemolysis. To 1 ml of extraction N 1 add 1 ml of 2 % suspension of erythrocytes in isotonic solution.

Analytical effect: _____

2. *Test based on physical properties of saponins.*

Foam test. In 2 tubes of the same diameter and height, add 0.5 ml of extraction No. 2. Add 1 ml of 0.1N HCl to one tube, then 1 ml of 0.1N NaOH in the other, then shake the tubes intensely. If a foam is formed equal in volume and persistence in both test tubes — triterpene saponins; in alkaline medium, the foam is several times larger in volume and firmness — steroid saponins.

Analytical effect: _____

3. Test based on the chemical properties of saponins.

Saponins settling. To 0.5 ml of extract No. 2 add 3 drops of a saturated solution of the normal lead acetate. A precipitate forms (triterpene saponins).

To 0.5 ml of extraction No. 2, add 3 drops of a saturated solution of hydroxide lead acetate. A precipitate forms (steroid saponins).

Analytical effect: _____

_____

	Glycyrrhiza roots	**Tribuli terrestris herbae**
Triterpene saponins		
Steroidal saponins		

Conclusion: _____

_____

Detection of saponins in MPRM by chromatography in a sorbent thin layer

Place 1.0 g of crushed raw material into a test tube, add 10 ml 70 % ethanol, heat to boil for 10 minutes filter.

Apply alcohol extract to the starting line of the chromatographic plate with a capillary and a «witness» next to it. (alcohol solution of pure saponin). Solvent system: glacial acetic acid-water-butanol (10:40:50).

When the system separates, remove the top layer of the mixture.

Chromatography for 20 minutes (solvent run 12 cm), then dry the chromatogram in air under draft. Observe color under UV light 254 nm.

Treat the chromatogram with an anisealdehyde solution and develop in a drying cabinet at a temperature of 100–105 °C for 5-10 minutes.

Mark the color of the spots, calculate the Rf values and compare them with the witness.

Draw the results of chromatography

	№ of spots	Numeric value of Rf	Spots staining

Conclusion:

_____

_____

TASK № 2 Carry out a saponins potency assay in Glycyrrhizae radicum in accordance with the SP RB.

Preparation of MPRM: 2.0 g of the powdered raw material is placed in a flask with the capacity of 150 ml, add 20 ml of the nitric acid and acetone mixture (1:20) and shake for 1 h.

Filtration and decontamination: The extract is filtered into a 100 ml volumetric flask. Residue in a flask rinse with 10 ml of acetone and filter through the same filter. Extraction is filtered through the same filter in a 100 ml volumetric flask. The rests of the raw material should be washed with acetone to the volume of the liquid in a 100 ml volumetric flask. The contents of the volumetric flask are quantitatively transferred to a 200 ml beaker, rinsing with 40 ml of 96 % alcohol.

Preparation of precipitate: With vigorous mixing add an ammonia concentrated solution dropwise until the light yellow curdy precipitate appearance (pH 8.3-8.6). The mother liquor along with the precipitate is filtered through a paper filter. A beaker and a filter with precipitate are rinsed with portions of acetone. The filter with precipitate is transferred to a beaker in which precipitation was carried out, add 50 ml of water and mix. The resulting solution is quantitatively transferred to a volumetric flask with a capacity of 250 ml. Filter is rinsed with small portions of water several times, add them to the solution in a volumetric flask. Bring water to a volume of 250 ml. Dissolve 30 ml of the resulting solution with water to a volume of 500 ml.

Measurement of optical density: Measure the optical density of the resulting solution at 258 nm using water as a compensation solution.

Calculation of results: The percentage of glycyrrhizic acid in percent is calculated by the formula:

$$\frac{A \times 823 \times 417}{m \times 11000},$$

where: A — optical density of solution; m — sample weight, g.

On the basis of the analysis carried out, a conclusion is made on the goodness of raw materials.

Conclusion: _____

_____

Practice № 14
SAPONINS. DRM AND MPRM CONTAINING SAPONINS

Control questions:

1. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
2. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
3. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
4. Application of DRM and MPRM containing saponins.
5. Indicate the chemical composition, pharmacological activity and application of MPRM containing saponins.

THE ALGORITHM OF LABORATORY WORK

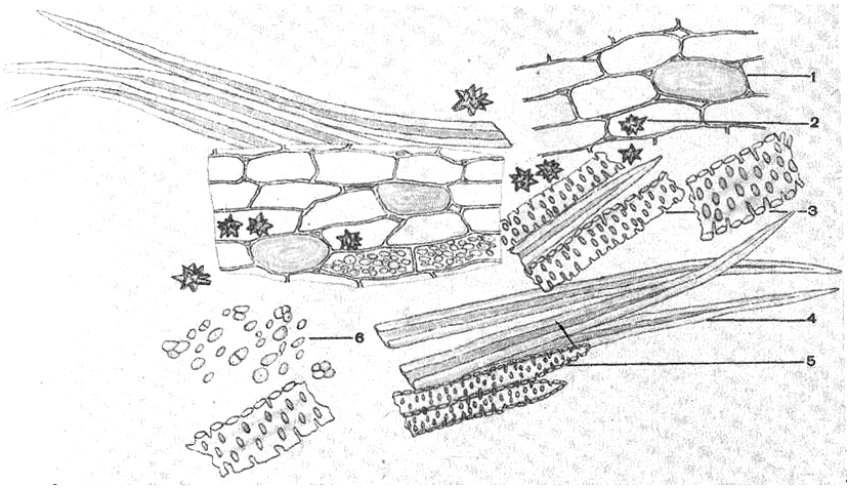
TASK № 1. Determine authenticity and quality of Glycyrrhizae radicum.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Glycyrrhizae radicum powder.



To give an opinion about the identification and quality of the MPRM on microscopic and external diagnostic signs.

Conclusion: _____

_____

TASK № 2. Carry out a macroscopic analysis and indicate the diagnostic signs of Polemonii rhizoma cum radicibus.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

To give an opinion about the identification and quality of the MPRM macroscopic diagnostic signs.

Conclusion: _____

_____

DRM and MPRM containing saponins

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Oplopanax elatus					
Aesculus hippocastanum					
Glycyrrhiza glabra					
Glycyrrhiza uralensis					
Polemonium caeruleum					
Aralia mandshurica					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Panax ginseng					
Tribulus terrestris					
Primula veris					
Hedera helix					
Rhaponticum carthamoídes					

Practice № 15
PHENOLIC GLYCOSIDES AND LIGNANS.
DRM AND MPRM CONTAINING PHENOLIC GLYCOSIDES AND LIGNANS

Control questions:

1. Definition of the «phenol glycosides» and «lignanes».
2. Chemical structure and physico-chemical properties.
3. Substances dispersal in the plant world.
4. Appearance and biology of the studied plants.
5. Their areals, habitats and areas of cultivation.
6. Rational methods of procurement, primary processing, drying and storage of medicinal products.
7. MPRM external signs.
8. The raw materials anatomical structure diagnostic signs having importance in the study: Vaccinium vitis-idaea leaves, Arctostaphylos leaves.
9. Chemical composition and formulas of hydroquinone, arbutin, n-tyrosol, salidroside (rodiolozide).
10. Application of MPRM and medicines.
11. Indicate the chemical composition, pharmacological activity and application of MPRM containing phenol glycosides.
12. Indicate the chemical composition, pharmacological activity and application of MPRM containing lignanes.

INFORMATION

To phenolic nature substances it is customary to refer aromatic compounds (C6), which contain a benzene nucleus with one or several hydroxyl groups in their molecule.

The chemical classification of phenolic compounds was based on the biogenetic principle. All phenols can be divided several basic groups, arranged in the order of the molecular structure complexity:

C_6	Simple phenols, phenolic glycosides
C_6-C_1	Benzoic acids
C_6-C_2	Phenolic alcohols, phenol-acetic acids
C_6-C_3	The derivatives of the phenylpropane series
C_6-C_2-C_6	Oxytylbenes
C_6-C_3-C_6	Flavonoids
C_6-C_3-C_3-C_6	Lignans
Compounds consisting of 2 or 3 condensed rings	Naphthoquinone, anthraquinone
Polymeric phenolic compounds	Tannins

Phenolic glycosides are the form of phenolic compounds in which the hydroxyl group is reacted to sugar molecules. The simplest form of this combination is phenyl-O-glycosides. Also benzoic acid and phenolic alcohols are derivatives to this group. The first phenolic glycoside obtained from plants is salicin — salicylic alcohol β-glucoside. It was singled out by the French scientist Leroux (1828) from Salix cortex.

Lignans are the natural phenolic substances, derivatives of the phenylpropane series $(C_6$-$C_3)_2$ dimers, connected together by C-C bonds between the average carbon atoms of the side chains. The variety of lignans is due to the phenyl nuclei location, their saturation degree, the degree of the side chains saturation, and the degree of γ-carbon atoms oxidation.

Xanthones are the natural phenolic compounds with C_6–C_1–C_6 general formula.

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Carry out tests for identication and quality of Vitis idaeae folium.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside.	
9. Smell.	

Give an opinion on the authenticity and quality of pharmaceutical products based on external signs.

Conclusion: _____

_____

TASK № 2. Carry out tests for identication and quality of Uvae ursi folium.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside.	
9. Smell.	

Give an opinion on the authenticity and quality of pharmaceutical products based on external signs.

Conclusion: _____

_____

b) compare macroscopic differences between folium Vitis idaeae and Uvae ursi folium.

Leaf drawing	Form	Size	Glands	Veins	Leaf edge
Vaccinium vitis-idaea leave					
Arctostaphylos leave					

c) thin-layer chromatography.

To 0.5 g of powdered raw material add 5 ml of a mixture of equal volumes of ethanol and water and heat under reflux for 10 minutes. Hot extract is filtrated. The filter is rinsed with a mixture of equal volumes of ethanol and water and brought to a volume of 5 ml with the same solvent.

Apply the filtrate on the chromatographic plate by the capillary next to the «witness» — the solution of arbutin and gallic acid in ethanol and place in a chromatographic chamber with a solvent system: METHANOIC ANHYDROUS ACID — WATER — ETHYL ACETATE (3 : 3: 44).

Chromatography is carried out for 20 minutes (the range of the solvent is not less than 15 cm), then the chromatogram is dried at a temperature of 105 to 110 °C until the solvents smell disappears.

Process the chromatogram with 10 g/l of dichloroquinonchloroimide solution (in ethanol), and then with 20 g/l sodium carbonate anhydrous solution.

Mark the color of the spots, calculate the Rf values and compare them with the witness.

Draw the results of chromatography.

	№ of spots	Numeric value of Rf	Spots staining

Conclusion: _____

_____

TASK № 3. Carry out a macroscopic analysis and indicate the diagnostic signs of Rhodiolae roseae rhizomata cum radicibus.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

Give an opinion on the authenticity and quality of pharmaceutical products based on external signs.

Conclusion: _____

_____

DRM and MPRM containing phenolic glycosides

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Arctostaphylos uva-ursi					
Vaccinium vitis-idaea					
Rhodiola rosea					
Salix alba					
Salix viminalis					

DRM and MPRM containing phenolic lignans

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Schisandra chinensis					
Eleutherococcus senticosus					
Silybum marianum					
Podophyllum peltatum					

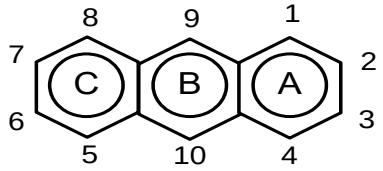
Practice № 16
ANTHRACENE DERIVATIVES.
ANALYSIS OF MPRM CONTAINING ANTHRACENE DERIVATIVES

Control questions:

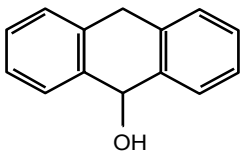
1. Definition of the AD, classification of AD.
2. Chemical structure of AD. Relationship between the chemical structure of AD and their biological activity.
3. Physical and chemical properties of AD.
4. Methods of extraction and decontamination of AD.
5. Methods of AD qualitative analysis.
6. Methods for quantitative analysis of MPRM containing AD.
7. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
8. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
9. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
10. MPRM external signs.
11. The raw materials anatomical structure diagnostic signs having importance in the study: Cassia acutifolia leaves, Frángula álnus leaves.
12. Chemical composition of raw materials and formulas of anthracene, anthraquinone, anthranol, anthrone, danthron, chrysacin, frangulaemodina, aloe-emodin, chrysophanol, alizarin, rubritic acid, sennoside A.
13. Application of MPRM and medicines.
14. Indicate the chemical composition, pharmacological activity and application of MPRM containing AD.

INFORMATION

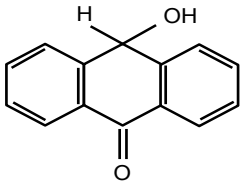
Anthracene derivatives are group of natural compounds based on anthracene. The degree of oxidation of the middle ring (ring B) can be different — to anthranol, anthrone, oxyanthron or anthraquinone.



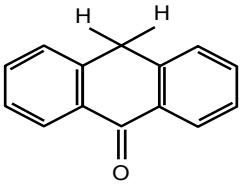
anthracene



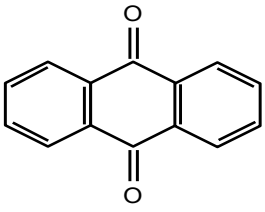
anthranol



oxyanthoron
anthraquinone



anthrone



danthron

Most anthracene derivatives is refered to the **anthraquinone type**, since anthrone and anthranol are labile and are readily oxidized by air oxygen to anthraquinones.

Anthraquinone derivatives are usually in the form of glycosides or aglycons — derivatives of 1,8-dioxoanthraquinone, or chrysacin (emodin) and 1,2-dioxoanthraquinone, or alizarin.

MPRM containing derivatives of emodin or chrysacin are used as laxatives. Alizarin derivatives have a litholytic effect.

THE ALGORITHM OF LABORATORY WORK

Chemical analysis of plant raw materials containing AD

TASK № 1. Carry out qualitative reactions for the detection of AD on samples of raw materials: Frangula alnus cortex, Rubiae rhizoma et radix, Sennae folium, Rumex confertus radix, Rhamni catharticae fructus, Rhei palmatum radix.

Reaction with alkali. Boil 0.2 g of crushed raw material (1–3 mm) for 2 minutes in a test tube with 1 ml of 10 % alcohol solution of sodium or potassium hydroxide. Cool, add 5 ml of water and filter into a separatory funnel. Add 10 % hydrochloric acid solution to a slightly acidic reaction and 10 ml of chloroform. After mixing and separating the liquids, separate the yellow-colored chloroform layer. Shake 5 ml of chloroform extract with 3 ml of 10 % ammonium hydroxide solution. Note the color of the ammonia layer: cherry red — 1,8-dioxyanthraquinones; purple — 1,4-dioxyanthraquinones; violet — 1,2-dioxyanthraquinones.

Observation: _____

_____

MPRM				
Analytical effect				
AD Group				

Sublimation. Place 0.2 g of crushed raw material (1–3 mm) at the bottom of a dry test tube and carefully heat it on an alcohol lamp, holding the test tube almost horizontally. After the test tube has cooled, apply 1–2 drops of 5 % alcohol solution of sodium hydroxide onto the sublimate. Note the color of the alkaline solution.

Analytical effect: _____

_____

Thin layer chromatography

0.3 g of the powdered raw material is placed in a test tube, add 3 ml of 70 % ethanol and heat to boiling. After cooling, filter and apply filtrate to the chromatographic plate by capillary. Next, apply a well-known substance («witness»). Air dry.

Place the plate in a chromatographic chamber with a solvent system: ETHYL ACETATE: ISOPROPYL ALCOHOL: WATER (100: 17: 13). After chromatography the chromatogram is dried under air in the draft.

Observe anthraquinones color stains (from yellow to red) with daylight and UV light. After processing the chromatogram with 5 % alkali alcoholic solution staining of the spots intensifies, acquiring red tones. Calculate Rf values for AD spots.

Draw the results of chromatography

	№ of spots	**Numeric value of Rf**	**Spots staining**

Conclusion: _____

_____

TASK № 2. Conduct quantitative determination of AD in Frangulae cortex and Rhei radix.

1. Acid hydrolysis. The exact sample is about 0,05 g of the powdered raw material is introduced into a flask with a 100 ml section and add 7.5 ml of glacial acetic acid, 1 ml of hydrochloric acid and boil on an electric plate with reflux for 15 min, avoiding burning.

2. Extraction of aglycons. After cooling, 15 ml of chloroform is added to the flask through the condenser and boiled in a water bath for 15 minutes. The extraction is cooled, filtered through cotton wool into a separatory funnel with a capacity of 200 ml. The cotton was washed with 15 ml of chloroform, transferred back to the flask, 15 ml of chloroform added and boiled for 10 minutes.

The cooled chloroform extraction is filtered through cotton wool into the same separatory funnel. The flask is rinsed twice with 10 ml of chloroform and filtered through the same cotton wool.

In the separatory funnel, the chloroform recovery is washed from the acid with 10 ml of water. The aqueous layer is discarded

3. Decontamination. To combine chloroform-acetic extracts, add carefully 15 ml of 300 g/l sodium hydroxide solution, 25 ml of alkaline-ammonia solution and gently shake them for 5–7 minutes, cooling the funnel under a stream of cold water. After complete stratification, the transparent red layer is poured into a 100-ml volumetric flask, and the chloroform layer is treated in portions of 20 ml of alkaline ammonia solution until the staining of the liquid stops. Merge together the colored solutions in the same volumetric flask, and adjust the solution volume in the flask with an alkaline-ammonia solution to the mark.

4. Oxidation of reduced forms and color stabilization. 25 ml of alkaline-ammoniated colored solution is placed in a flask and heated for 15 minutes in a boiling water bath under reflux. The solution is cooled to room temperature under a stream of cold water.

5. Measurement. Measure the optical density of the solution after 10 minutes at 530 nm in a cuvette. Alkaline-ammonia is the control solution.

If the color is too intense, the test solution should be diluted with an alkaline-ammonia solution. Determine, according to the calibration graph, the concentration of the sum of AD aglicones, expressed in isothine (1,8-dioxoanthraquinone).

Calculation of the AD content by formula:

$$X, \% = \frac{A \times V \times 100}{m \times (100 - W) \times E_{1см}^{1\%}} = \frac{A \times 111}{m \times E_{1см}^{1\%}},$$

where A — optical density of solution; V — initial volume of alkaline ammonia solution, ml (100); m — weight of raw materials, g (0.0500); W — weight loss during drying, % (10); $E_{1см}^{1\%}$ — specific absorption rate of glucofrangulin A equal to 204.

Quality control

On the basis of the analysis carried out, give an opinion on the AD content of in the raw material. Compare the result with RD.

Conclusion: _____

_____

TASK № 3. Carry out a macroscopic analysis and indicate the diagnostic signs of Hyperici herba.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, pubescence, size, color).	
2. Leaf arrangement.	
3. **Leaves.** Type of sheet (simple or complex).	
4. Petiole or sessile.	
5. Leaf blade shape.	
6. Dimensions of leaf or leaflets, petiole.	
7. Edge of the sheet.	
8. Venation pattern.	
9. Pubescence.	
10. Color from the outside	
and at the fracture.	
11. Smell.	
12. Arrangement of flowers on the stem.	
13. **Flowers.** Type of inflorescence or single flowers.	
14. Flower shape (actino- or zygomorphic).	
15. Sizes of inflorescence or flower.	

16. Absence or presence of peduncle (shape, size).	
17. Pubescence.	
18. Color.	
19. Smell.	
20. **Fruit.** Type of fruit (dry, juicy).	
21. Form.	
22. Dimensions (length, thickness, diameter).	
23. Character of the pericarp.	
24. Number of seeds or seeds, their shape and structure, surface structure.	
25. Color.	
26. Smell.	

Conclusion: _____

_____

TASK № 4. Carry out a macroscopic analysis and indicate the diagnostic signs of Frangulae alni cortex.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

b) Carry out qualitative reactions:

1. Apply a drop of alkali solution to the inner surface of the bark.

Observation: _____

Conclusions: _____

_____

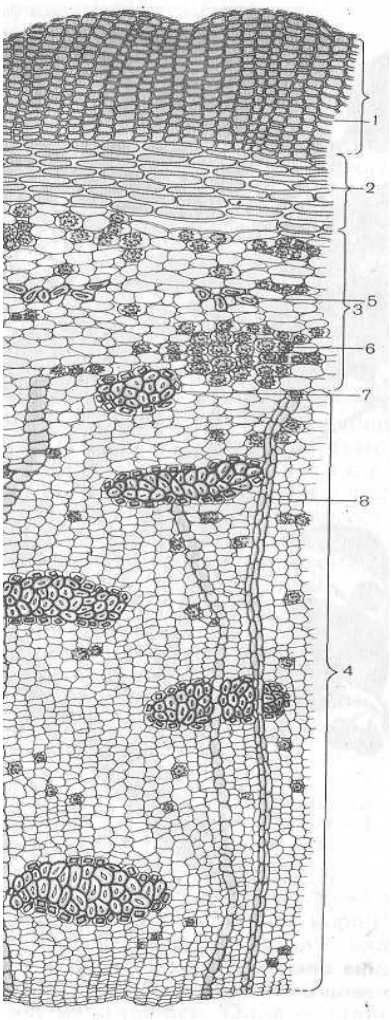
2. Moisten the inner surface of the bark with a drop of ferroammonium alum solution.

Observation: _____

Conclusions: _____

_____

c) Carry out a microscopic analysis and indicate the diagnostic signs of Frangulae alni cortex.



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To give an opinion about the identification and quality of the MPRM on macroscopic and microscopic diagnostic signs.

Conclusion: _____

_____

TASK № 5. Carry out a macroscopic analysis and indicate the diagnostic signs of Rhei radix.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	
7. Shape.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs.

Conclusion: _____

_____

DRM and MPRM containing anthracene derivatives

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Frangula alnus					
Rhamnus cathartica					
Cassia acutifolia					
Rheum palmatum					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Rumex confertus					
Aloe arborescens					
Rubia tinctorum					
Hypericum perforatum					
Hypericum maculatum					

Practice № 17
THE FINAL CLASS

Cardiac glycosides

1. Definition of the cardiac flicosides. Features of their chemical structure.
2. Classification. Physicochemical characteristics. Extraction.
3. Substances dispersal in the plant world, their localization in the plants.
4. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
5. Methods of qualitative detection and potency assay.
6. Biological standardization of MPRM and medicines containing CG.
7. Application in medicine.
8. DRM and MPRM containing cardiac glycosides: Digitalis purpurea Digitalis grandiflora Digitalis lanata Strophanthus kombe Adonis vernalis Convallaria majalis Erysimum diffusum.
9. Formulas: purpureoglycoside A and B, cymarin, digitoxygenin, konvalotoxin, lantosides A, B, C, adonitoxin, strobanthidine, K-strophanthin-β, K-strophanthoside.

Saponins

1. Definition of the saponins.
2. Dispersal of saponins in the plant world, their localization in organs and tissues, their role in the life of plants.
3. Chemical structure of saponins and their classification.
4. Physicochemical and biological properties.
5. Methods of saponins extraction and methods for their decontamination.
6. Methods of detection and potency assay.
7. MPRM and medicines application.
8. DRM and MPRM containing saponins: Oplopanax elatus, Aesculus hippocastanum, Glycyrrhiza glabra**,** Glycyrrhiza uralensis, Polemonium caeruleum, Aralia mandshurica, Panax ginseng, Tribulus terrestris, Primula veris, Hedera helix, Rhaponticum carthamoides, Dioscorea nipponica, Astragalus dasyanthus
9. Formulas: α- and β-amyrin, glycyrrhizic acid, diosgenin, dammaran.

Phenolic glycosides and lignans

1. Definition of the phenolic glycosides and lignans.
2. Chemical structure and physicochemical properties of PG and lignans.
3. Dispersal in the plant world.
4. Application of MPRM and medicines, containing PG and lignans.
5. DRM and MPRM containing phenolic glycosides: Arctostaphylos uva-ursi, Vaccinium vitis-idaea, Rhodiola rosea, Salix alba, Salix viminalis
6. DRM and MPRM containing lignans: Schisandra chinensis, Eleutherococcus senticosus, Silybum marianum, Podophyllum peltatum.
7. Formulas: arbutin, hydroquinone, salidroside, n-tyrosol.

Anthracene derivatives

1. Definition of the AD. Classification.
2. Physicochemical properties.
3. Extraction.
4. Dispersal in the plant world, their localization in organs and tissues, their role in the life of plants.
5. Methods of qualitative detection and potency assay**.** Pharmacopoeia method of AD potency assay in Frangula alnus cortex.
6. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage..
7. Application of MPRM and medicines, containing AD.
8. DRM and MPRM containing AD: Frangula alnus, Rhamnus cathartica, Cassia acutifolia, Rheum palmatum, Rumex confertus, Aloe arborescens, Rubia tinctorum,.
9. Formulas: anthracene, anthraquinone, anthranol, anthrone, oxyanthron, chrysacin, alizarin, rubritric acid, chrysofanol, sennoside A, frangulaemodine.

Practice № 18
COUMARINS AND CHROMONES.
DRM AND MPRM CONTAINING COUMARINS AND CHROMONES

Control questions:

1. Definition of the coumarins and chromones.
2. Classification. Physicochemical properties of coumarins.
3. Dispersal of coumarins in the plant world.
4. Methods for detecting of the coumarins in MPRM.
5. Coumarins extraction methods.
6. Methods of potency assay.
7. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
8. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
9. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
10. MPRM external signs.
11. Structural formulas of the coumarin, dihydrocoumarin, psoralen, angelitsin, isopimpinellin, bergapten, umbelliferon, kellin.
12. Chemical composition of MPRM and DRM and medicines.
13. Indicate the chemical composition, pharmacological activity and application of MPRM containing coumarins and chromones.

THE ALGORITHM OF LABORATORY WORK

Morphological and anatomical analysis of plant raw materials containing coumarines and chromones

TASK № 1. Carry out a macroscopic analysis and indicate the diagnostic signs of Pastinacae sativae fructus .

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of fruit (dry or fleshy).	
2. Shape.	
3. Size of the fruit (length, thickness, diameter)	
4. The features of the pericarp.	
5. The number of seeds, their shape and structure, the surface structure.	
6. Color.	
7. Smell.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs.

Conclusion: _____

_____

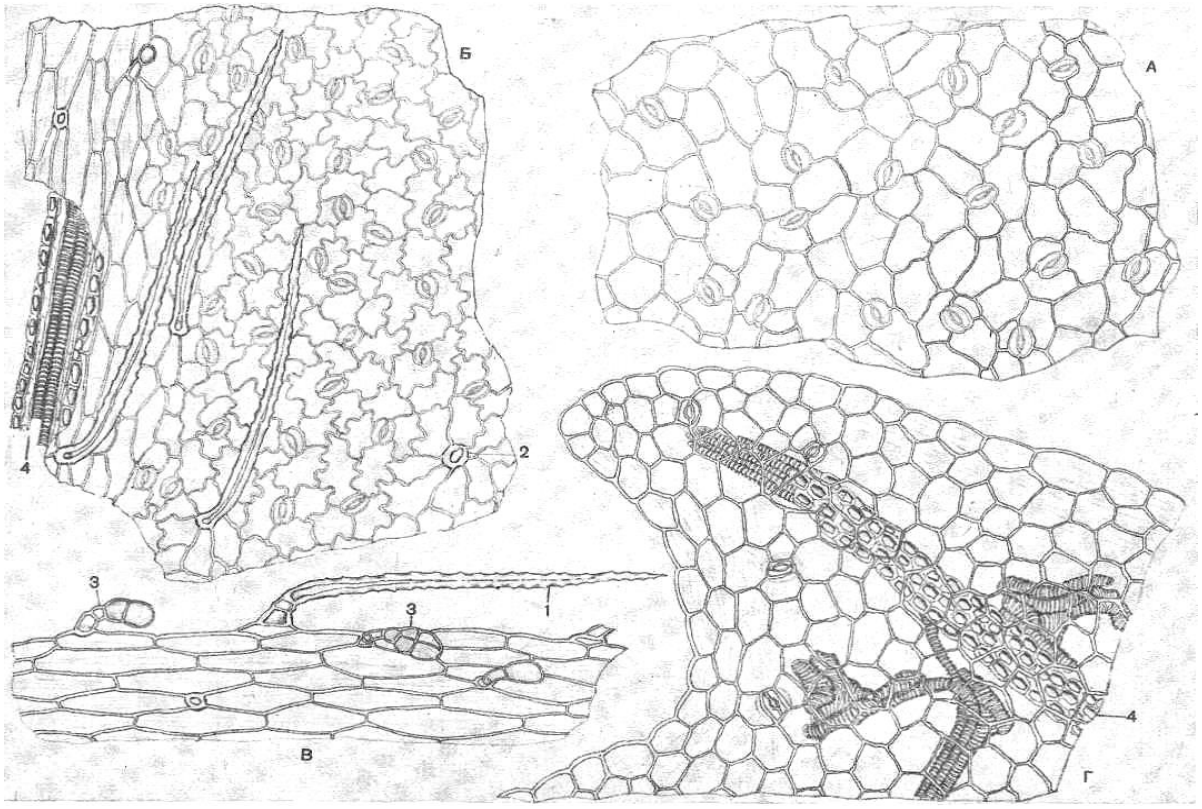
TASK № 2. Carry out a macroscopic analysis and indicate the diagnostic signs of Meliloti officinalis herba

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade.	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside.	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers.	
13. The shape of the flower (actino- or zygomorphic).	
14. Inflorescence or flower size.	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum.	
17. Color.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Meliloti officinalis folia.



To give an opinion about the identification and quality of the MPRM on macroscopic and microscopic diagnostic signs.

Conclusion: _____

Qualitative analysis of coumarins

1. Get the alcohol extract from the MPRM containing coumarins. Add 2.5 g of powdered raw material to the flask and 25 ml of 95 % alcohol and heat with reflux in a water bath until boiling. Maintain a boil for 15–20 minutes, then cool, filter and use for qualitative reactions and chromatography.

2. Determine the presence of coumarins in MPRM.

Lactone test. 2 ml of alcohol extract are poured into 2 test tubes and 0.5 ml of a 10 % alcohol solution of NaOH is added. One test tube is heated on an alcohol lamp to a boil and cooled, the other is left without heating. 4 ml of purified water is poured into each test tube. Compare the results in two test tubesThe solution obtained by heating with a 10 % alcohol solution of NaOH is poured into 2 parts.

The first part of the solution is acidified with a few drops of HCI (conc.).
The second part is left for the azo coupling reaction.

Observation: _____

Conclusions: _____

_____

Diazo test. In a test tube pour 1–2 ml of alcohol extraction and 3–4 ml of 10 % sodium hydroxide alcohol solution. The liquid is heated to boiling and cooled. Add 2–3 drops of freshly prepared diazotized sulfanilic acid.

Observation: _____

Conclusions: _____

_____

Conclusion about the presence of coumarins is made on the basis of positive 1 and 2 reactions provided that there are no flavonoids and anthraquinone derivatives in the raw material.

Qualitative chromatographic analysis of MPRM for the content of coumarins.

Apply to a chromatographic plate 1 drop of alcohol extracts containing coumarins and put in a solvent system: ETHYL ACETATE: BENZENE (1 : 2)

Observe the coumarin substances spots blue fluorescence in the UV light. Process 5 % alkali solution and observe the change in the fluorescence of the coumarin substances spots in UV light. Mark them on a chromatogram and calculate the value of Rf.

Draw the results of chromatography.

	№ of spots	**Numeric value of Rf**	**Spots staining**

Conclusion: _____

_____

DRM and MPRM containing coumarins and chromones

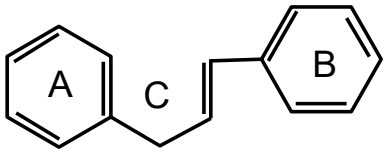
Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Melilotus officinalis					
Ammi majus					
Ammi visnaga					
Pastinaca sativa					
Phlojodicarpus sibiricus					

Practice № 19
FLAVONOIDS. ANALYSIS OF MPRM CONTAINING FLAVONOIDS

Control questions:

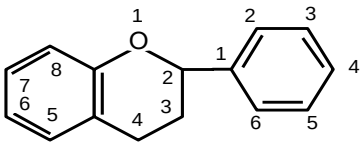
1. Classification and features of the flavonoid compounds structure.
2. Physico-chemical properties of flavonoids.
3. Dispersal of flavonoids in the plant world, localization in plants organs and tissues.
4. Methods for extraction, decontamination and separation of flavonoids.
5. Analysis (qualitative, quantitative, chromatographic) of MPRM containing flavonoids.
6. Formulas of flavone, flavonol, flavan, flavanone, flavanonol, isoflavone, anthocyanidin, catechin, chalcone, auron.
7. Biological activity of flavonoids. Ways of using MPRM containing flavonoids.

INFORMATION

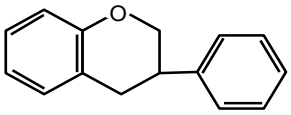


Flavonoids are phenolic compounds based on the diphenylpropane skeleton C6-C3-C6 structure. The variety of flavonoid compounds is due not only to the number, position and nature of substituents in A, B and C rings, but also structural features. These features are manifested in the fact that in the propane fragment B can be found in C-2,

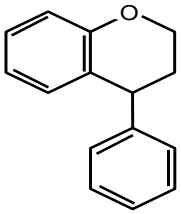
then this class is called **flavonoids (euphlavonoids)**, in C-3 is **isoflavonoids** and in C-4 is **neoflavonoids**, and the propane fragment can be in the form of an open chain or the form of a heterocycle (ring C) — five- or six-membered ring when it is condensed with ring A through an oxygen atom.



euphlavonoid



isoflavonoid



neoflavonoid

THE ALGORITHM OF LABORATORY WORK

Chemical analysis of MPRM containing flavonoids

TASK № 1. Carry out qualitative reactions for the detection of flavonoids in medicinal plants; Crataegi flores, Tanaceti flores, Helichrysi flores.

Extraction of flavonoids. Place 2.0 ml of crushed raw material into a 50 ml flask, add 30 ml of 70 % ethyl alcohol, heat in a water bath at 60°C for 5 minutes, cool and filter..

1. Coloring reactions

Cyanidin test (reduction of flavonols, flavones, flavanones to anthocyanidins).

Place 1 ml of extract in a test tube, add 3-4 drops of conc. HCl and 10–15 g (2–3 granules) magnesium or zinc metal. Heat in a boiling water bath.

Observation: _____

Conclusions: _____

Reaction with caustic alkali solution.

Add 2–3 drops of a 5 % alcohol solution of NaOH to 1 ml of extract.

Observation: _____

Conclusions: _____

Complexation reaction with aluminum chloride.

Add 2–3 drops of 2 % alcohol solution of aluminum chloride to 1 ml of extract.

Observation: _____

Conclusions: _____

Fluorescence under UV light: _____

_____

2. Precipitation reaction with a solution of basic lead acetate:

Add 3–5 drops of basic lead acetate solution to 1 ml of extract.

Observation: _____

Conclusion: _____

TASK № 2. To detect flavonoids in MPRM by thin layer chromatography.

Use the flavonoid extract remaining from qualitative reactions.

Apply the extract and «witness» (rutin, quercetin, hyperoside) to the starting line of the chromatographic plate. Place the plate in a chromatographic chamber with a solvent system: anhydrous formic acid-water-ethyl acetate (10:10:80).

Dry the chromatogram in air under air draft.

Note the color of the spots in visible and UV light.

Treat the chromatogram with a 2 % alcohol solution of aluminum chloride and dry in an oven at a temperature of 90–100 °C. Observe the color of spots in visible and UV light.

Calculate Rf values for flavonoids and compare them with «witnesses». Draw a chromatogram and record the results in the protocol.

	№ of spots	**Numeric value of Rf**	**Spots staining**

Conclusion: _____

_____

_____

TASK № 3. Conduct a quantitative determination of flavonoids in medicinal plants using a *spectrophotometric method*.

0.5 g of crushed raw material (1400) (2.9.12) is placed in a conical flask with a capacity of 250 or 100 ml, 75 ml of 96 % alcohol is added and refluxed in a water bath for 20 minutes. Cool to room temperature and filter through a paper filter pre-moistened with 96 % alcohol. Extraction using the above method is repeated one more time, using 50 ml of 96 % alcohol R. The filtrates are combined and diluted with 96 % alcohol R to a volume of 125 ml (solution A).

Test solution. To 2.0 ml of solution A add 1.0 ml of a solution of 20 g/l aluminum chloride R in 96 % alcohol R, 5 drops of diluted hydrochloric acid R and dilute with 96 % alcohol R to a volume of 25.0 ml.

Reference solution. 0,0125 g of rutin, dissolved in 20 ml of 96 % alcohol when heated in a water bath, cooled and brought to a volume of 25.0 ml with the same solvent (solution B). To 1.0 ml of solution B add 1.0 ml of a solution of aluminum chloride in 96 % alcohol, 5 drops of diluted hydrochloric acid R and dilute with 96 % alcohol R to a volume of 25.0 ml.

Compensation solution (a). To 2.0 solution A add diluted hydrochloric acid R and dilute with 96 % alcohol to a volume of 25.0 ml.

Compensation solution (b). To 1.0 ml of solution B add diluted hydrochloric acid R and dilute with 96 % alcohol to a volume of 25.0 ml.

After 40 minutes, measure the absorbance (2.2.25) of the test and reference solutions at 411 nm using compensation solutions (a) and (b), respectively..

The content of total flavonoids in terms of rutin as a percentage is calculated using the formula:

$$\frac{A \times m_0 \times P \times 0{,}625}{A_0 \times m},$$

where A — optical density of the test solution; A_0 — optical density of reference solution; m — weight of a sample of the tested raw material, g; m_0 — mass of rutin sample, g; P — rutin content in FSS (pharmacopoeial standard sample), %.

Conclusion: _____

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Practice № 20
FLAVONOIDS. DRM AND MPRM CONTAINING FLAVONOIDS

Control questions:

1. Definition of the flavonoids.
2. Dispersal of flavonoids in the plant world, localization of organs and tissues.
3. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
4. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
5. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
6. MPRM external signs.
7. Diagnostic microscopic signs of Polygonum persiaria and Polygonum hydropiper leaves.
8. Structural formulas of quercetin, luteolin, rutin, hyperoside, apigenin, naringenin.
9. Chemical composition, application of MPRM and medicines containing flavonoids.
10. Indicate the chemical composition, pharmacological activity and use of MPRM containing flavonoids.

THE ALGORITHM OF LABORATORY WORK

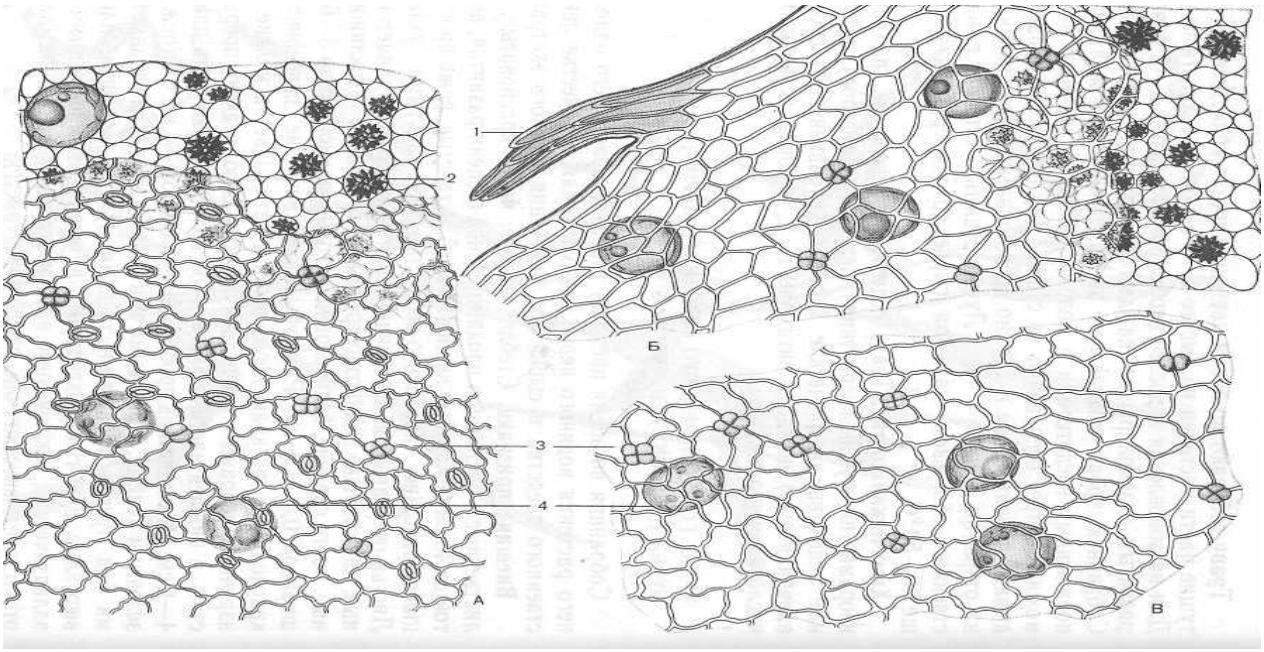
TASK № 1. Determine authenticity and quality of Polygoni hydropiperis herba.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers	
13. The shape of the flower (actino- or zygomorphic)	
14. Inflorescence or flower size	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum	
17. Color	
18. Smell	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Polygoni hydropiperis folium.



To give an opinion about the identification and quality of the MPRM on external diagnostic signs.

Conclusion:

TASK № 2. Determine authenticity and quality of Polygoni persicariae herba.

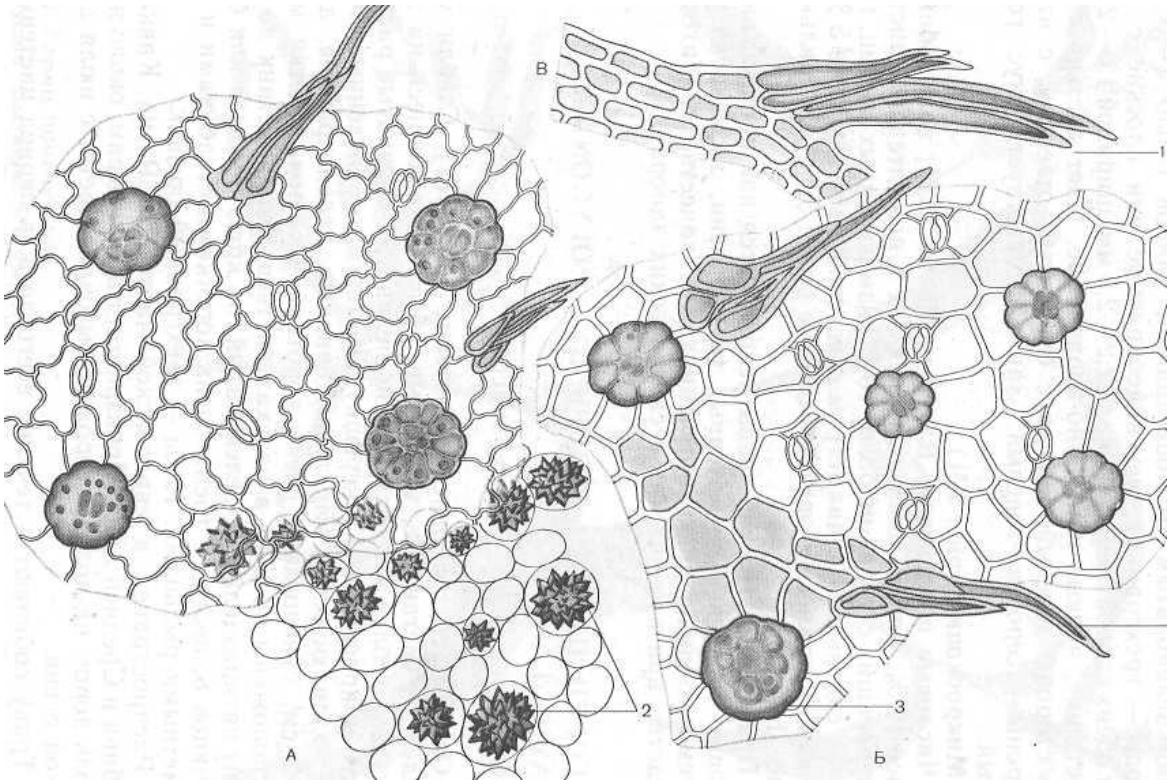
The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside	

11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers	
13. The shape of the flower (actino- or zygomorphic)	
14. Inflorescence or flower size	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum	
17. Color	
18. Smell	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Polygoni persicariae folium.



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To give an opinion about the identification and quality of the MPRM on external diagnostic signs.

Conclusion: _____

_____

TASK № 3. Carry out a macroscopic analysis and indicate the diagnostic signs of Helichrysi arenarii flores.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Type of an inflorescence or single flowers.	
2. The shape of the flower (actino- or zygo-morphic).	
3. Inflorescence or flower size.	
4. Absence or presence of peduncle (its shape and size).	
5. Indumentum.	
6. Color.	
7. Smell.	

To give an opinion about the identification and quality of the MPRM on external diagnostic signs.

Conclusion: _____

_____

TASK № 4. Determine authenticity and quality of Equiseti herba.

The name of MPRM	
The name of the plant	
Family	

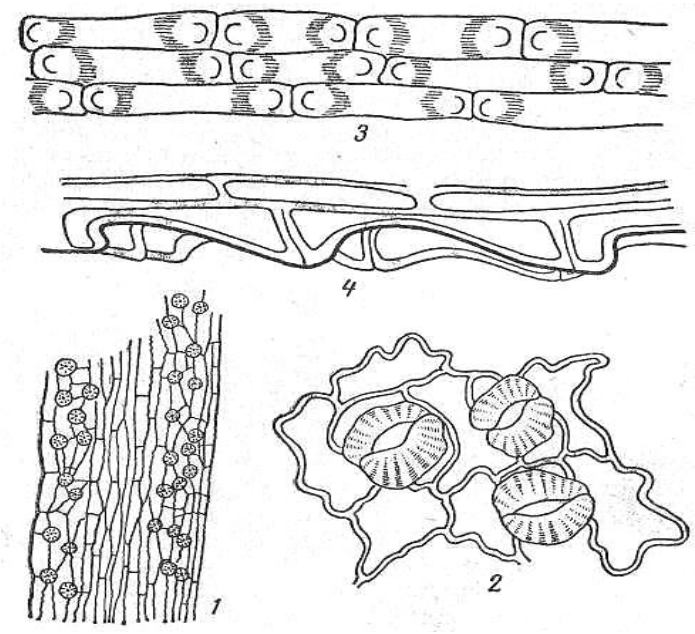
a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).).	
2. Characteristics of branches (direction, how many edges, hollow or without cavity)	
3. Characteristics of stem sheaths	
4. Form.	
5. Size	
6. Color.	
7. Cohesion of the vaginal teeth.	
8. Pubescence	
9. Smell.	

b) Study the appearance of possible impurities using herbarium specimens and indicate the distinctive features.

Equisetum sylvaticum	
Equisetum pratense	
Equisetum palustre	
Equisetum heleocharis	

c) Carry out a microscopic analysis and indicate the diagnostic signs of Equiseti arvensis herba.



To give an opinion about the identification and quality of the MPRM on external diagnostic signs.

Conclusion: _____

_____

DRM and MPRM containing flavonoids (flavons)

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Crataegus oxyacantha					
Crataegus sanguineae					
Tanacetum vulgare					
Polygonum hydropiper					
Polygonum persiaria					
Polygonum aviculare					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Helichrysum arenarium					
Gnaphalium uliqinosum					
Ginkgo biloba					
Equisetum arvense					
Fragaria vesca					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Sophora japonica					
Scutellaria baicalensis					
Filipendula ulmaria					
Potentilla alba					
Hypericum perforatum					
Hypericum maculatum					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Agastache rugosa					
Cynara					
Rudbeckia hirta					
Epilobium angustifolium					

Practice № 21
FLAVONOIDS. DRM AND MPRM CONTAINING FLAVONOIDS

Control questions:

1. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
2. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
3. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
4. MPRM external signs.
5. Diagnostic signs of Bidens tripartita and Equisetum arvense herb.
6. Chemical composition, structural formulas of rutin, quercetin, cyanidine.
7. Application of DRM and MPRM containing flavonoids.

THE ALGORITHM OF LABORATORY WORK

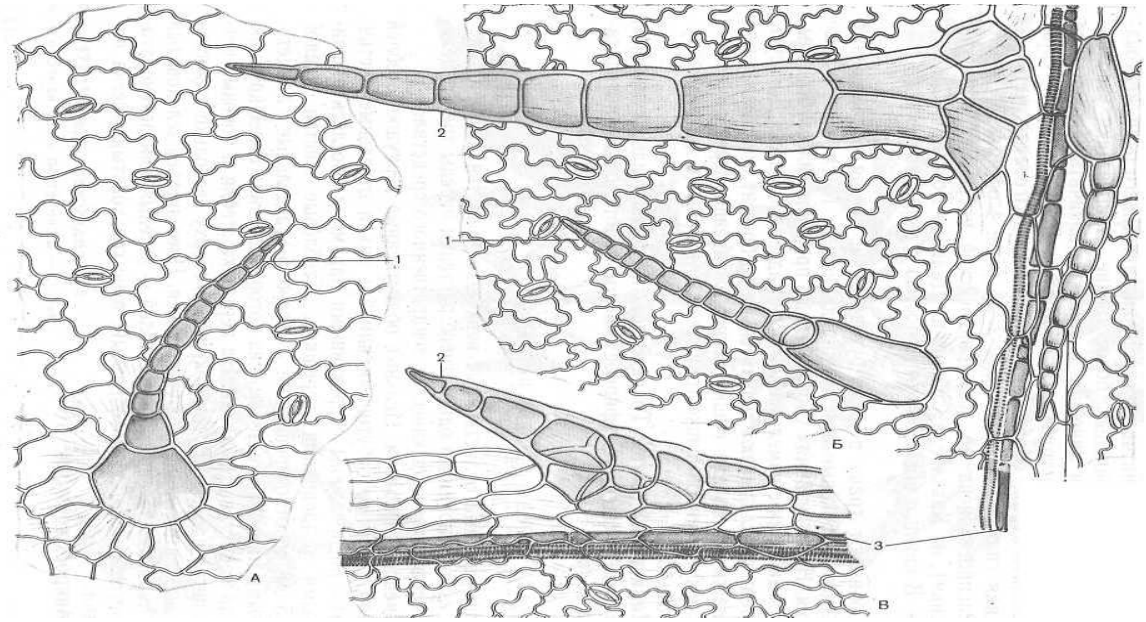
TASK № 1. Carry out a macroscopic analysis and indicate the diagnostic signs of Bidentis herba.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside.	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers.	
13. The shape of the flower (actino- or zygomorphic).	
14. Inflorescence or flower size.	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum.	
17. Color.	
18. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Bidentis folium.



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_____

To give an opinion about the identification and quality of the MPRM on microscopic and macroscopic diagnostic signs.

Conclusion: _____

_____

TASK № 2. Carry out a macroscopic analysis and indicate the diagnostic signs of Violae tricolor herba.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade.	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	

10. Color of upperside	
and underside.	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers.	
13. The shape of the flower (actino- or zygomorphic).	
14. Inflorescence or flower size.	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum.	
17. Color.	
18. Smell.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs.

Conclusion: _____

_____

TASK № 3. Detect flavonoids in medicinal plants (Violae tricolor herba) using thin layer chromatography.

2.0 g of medicinal plant, pour 10 ml of 70 % alcohol, heat in a water bath at 65 °C for 5 minutes with stirring. Cool and filter the extract.

Apply the extract and «witness» (rutin, hyperoside, caffeic acid) to the starting line of the chromatographic plate in the form of stripes). Place the plate in a chromatographic chamber with a solvent system: anhydrous formic acid-ice acetic acid-water-ethyl acetate (11:11:27:100). Mobile phase front not less than 12 cm. Dry the plate at a temperature of 100–105 °C.

Treat the hot plate with a solution of 10 g/l diphenylboronic acid aminoethyl ester in alcohol, and then with a solution of 50 g/l macragol 400 in alcohol.

Observe the color of the spots in visible and UV light 30 minutes after processing the plate.

Calculate Rf values for flavonoids and compare them with «witnesses». Draw the chromatogram and record the results in the protocol.

	№ of spots	**Numeric value of Rf**	**Spots staining**

Conclusion: _____

_____

DRM and MPRM containing flavonoids (flavans, chalcones, aurons, isoflavonoids)

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Viola tricolor					
Viola arvensis					
Bidens tripartita					
Centaurea cyanus					
Ononis arvensis					
Begonia erythrophylla					
Glycyrrhiza glabra					
Glycyrrhiza uralensis					

Practice № 22
TANNINS. DRM AND MPRM CONTAINING TANNINS

Control questions:

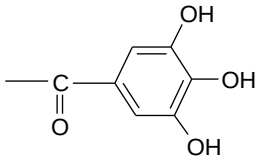
1. Definition of the tannins.
2. Classification. Physicochemical properties.
3. Chemical composition and structural formulas of tannin, gallic acid, ellagic acid, catechin, leucoanthocyanidin, pyrocatechrome, pyrogallol.
4. Dispersal of tannins in the plant world.
5. Methods of detection in MPRM.
6. Methods of extraction.
7. Methods of tannins potency.

INFORMATION

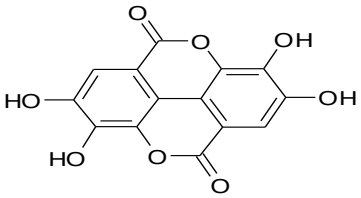
The term ***«tannins»*** unites the whole complex of plant polyphenols, tannides and flobafenes, genetically related to each other, having a tanning effect and astringent taste.

The tanides are very diverse in their structure. They can be divided into two main groups:

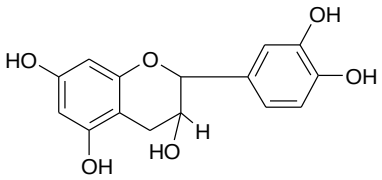
1. ***Hydrolyzed or gallotanides,*** which during hydrolysis give glucose, gallic, dicvalic and ellagic acids.
2. ***Condensed tannides,*** which are based on catechins, leucocyanidins, stilbene.



gallic acid



ellagic acid



catechin

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Carry out a chemical analysis of MPRM containing tannins.

_____

(Specify the MPRM to analyze)

Qualitative analysis

Extraction: 1.0 g of powdered raw material (1-3 mm) is placed in a 250 ml flask, add 50 ml of hot water and heat on a boiling water bath for 20 minutes. The extract is cooled and filtered through cotton wool.

a) Precipitation reactions:

1) To 2 ml of extract, add 1 % gelatin solution.

Observation: _____

2) To 2 ml of extract add 2 ml of 10 % acetic acid solution and 1 ml of 10 % normal lead acetate solution.

Observation: _____

b) Staining reaction:

To 2 ml of extract add 4 drops of 1 % iron ammonium alum solution.

Observation: _____

TASK № 2. Carry out a potency assay of tannins in samples of MPRM.

a) Spectrophotometric method using casein

Place 0.500 g of crushed raw material (180) into a round-bottomed flask with a capacity of 250 ml and add 150 ml of water. Heat in a water bath for 30 minutes. Cool under running water and transfer quantitatively into a 250 ml volumetric flask. Rinse the round-bottomed flask and pour the washing water into a volumetric flask, then dilute with water to a volume of 250.0 ml. Allow the solid particles to settle and filter the liquid through filter paper with a diameter of 125 mm. The first 50 ml of filtrate is discarded.

Total polyphenols. 5,0 ml of the filtrate is brought to a volume of 25.0 ml with water. 2.0 ml of the resulting solution is mixed with 1.0 ml of phosphomolybdenum-tungsten reagent and 10.0 ml of water and adjusted with a solution of 290 r/l sodium carbonate to a volume of 25.0 ml. After 30 minutes, measure the optical density at a wavelength of 760 nm (A1), using water as a compensation solution..

Polyphenols are not adsorbed by casein. Add 0.10 g of casein to 10.0 ml of filtrate and shake vigorously for 60 minutes. Filter and 5.0 ml of the resulting filtrate is diluted with water to a volume of 25.0 ml. 2.0 ml of the resulting solution is mixed with 1.0 ml of phosphomolybdenum-tungsten reagent and 10.0 ml of water and adjusted with a solution of 290 r/l sodium carbonate to a volume of 25.0 ml. After 30 minutes, measure the optical density at a wavelength of 760 nm (A2), using water as a compensation solution.

Standard. Immediately before use, dissolve 50.0 mg of gallic acid R in water and dilute to a volume of 100.0 ml with the same solvent. 5.0 ml of the resulting solution is diluted with water to a volume of 100.0 ml. 2.0 ml of the resulting solution is mixed with 1.0 ml of phosphomolybdenum-tungsten reagent and 10.0 ml of water and adjusted with a solution of 290 r/l sodium carbonate to a volume of 25.0 ml. After 30 minutes, measure the optical density at a wavelength of 760 nm (A3), using water as a compensation solution.

Calculations. Calculate the percentage of tannins (%) in terms of gallic acid by the formula:

$$X, \% = \frac{62{,}5 \times (A_1 - A_2) \times k \times m_2}{A_3 \times m_1},$$

where A_1 — optical density of extraction without added casein; A_2 — optical density of extraction after casein treatment; k — conversion factor (0,52); A_3 – optical density of standard solution; m_2 — weight of a sample of a standard sample (gallic acid); m_1 — weight of a sample of the tested raw material.

Raw matherial	Conversion factor
Quercus robur cortex	0,56
Potentilla erecta rhizoma	0,52

Evaluation of raw material quality

Based on the analysis carried out, a conclusion is made on the conformity of raw materials to RD requirements

Conclusion: _____

_____

b) Titrimetric method

Preparation of raw materials. 2.0 g (accurately weighed) of the powdered raw material is sieved through a sieve with a hole diameter of 3 mm, place into a flat-bottomed flask with a capacity of 500 ml.

Extraction. Pour the raw material into 250 ml of water heated to a boil and reflux on an electric stove for 30 minutes while stirring. Cool the liquid to room temperature, strain and add water to a volume of 250 ml.

Quantitation. Remove 25 ml of the extract and place in a 750 ml conical flask, add 500 ml of water, 25 ml of indigosulfonic acid and titrate with constant stirring with a solution of potassium permanganate (0.02 mol/l) to a golden yellow color.

In the control experiment, add 25 ml of indigosulfonic acid to 525 ml of water and titrate with potassium permanganate solution until golden yellow.

The content of tannins in % in terms of absolutely dry raw materials:

$$X = \frac{(V - V_1) \times 0{,}004157 \times 250 \times 100 \times 100}{m \times 25 \times (100 - W)},$$

where V — volume of potassium permanganate solution consumed for titration, ml; V_1 — volume of potassium permanganate solution consumed for titration in a control experiment, ml; 0,004157 — the amount of tannins corresponding to 1 ml potassium permanganate solution (0.02 mol/l) in terms of tannin, g; m — mass of raw materials, g; W — loss in mass when drying raw materials, 10 % (conditionally); 250 — total extraction volume, ml; 25 — volume of extraction, taken for titration, ml.

Evaluation of raw material quality

Based on the analysis carried out, a conclusion is made on the conformity of raw materials to RD requirements

Conclusion: _____

_____

_____

Practice № 23
TANNINS. DRM AND MPRM CONTAINING TANNINS

Control questions:

1. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
2. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
3. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
4. MPRM external signs**.**
5. Chemical composition and structural formulas of tannin, gallic acid, ellagic acid, catechin, leucoanthocyanidin, pyrocatechol, pyrogallol.
6. Drugs and use in medicine.
7. Indicate the chemical composition, pharmacological activity and use of MPRM containing tannins.

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Carry out a macroscopic analysis and indicate the diagnostic signs of Quercus cortex.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Shape.	
2. Size (thickness).	
3. The features of the outer surface.	
The features of the inner surface.	
4. Cork color and lenticle shape.	
5. The nature of the fracture.	
6. Smell.	

b) Carry out qualitative reactions: when wetting the inner surface of the oak bark with a drop of iron ammonium alum.

Observation: _____

_____

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs

Conclusion: _____

_____

TASK № 2. Carry out a macroscopic analysis and indicate the diagnostic signs of Potentillae erectae rhizomata.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs**.**

Conclusion: _____

_____

TASK № 3. Carry out a macroscopic analysis and indicate the diagnostic signs of Myrtilli fructus.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Type of fruit (dry or fleshy).	
2. Shape.	
3. Size of the fruit (length, thickness, diameter)	
4. The features of the pericarp.	
5. The number of seeds, their shape and structure, the surface structure.	
6. Color.	
7. Smell.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs.

Conclusion: _____

_____

TASK № 4. To detect tannins in Potentillae erectae rhizomata using thin layer chromatography.

Test solution. Add 10 ml of water R to 0.5 crushed raw material (355) (2.9.12), shake for 10 minutes and filter.

Reference solution 1.0 mg of catechin is dissolved in 1.0 ml of methanol.

Plate: TLC plate with silica gel layer.

Mobile phase: glacial acetic acid–ether–hexane-ethyl acetate (20:20:20:40).

Sample volume applied: 10 µl in strips.

Mobile phase front: at least 10 cm from the start line.

Drying: in air for 10-15 minutes.

Manifestation: the plate is sprayed with a freshly prepared solution of 5 g/l strong blue B. Reddish colored zones appear. The plate is kept in ammonia vapor. The zones acquire a more intense reddish-brown color. Viewed in daylight.

Calculate the Rf values for the zones and compare them with the standard. Draw the chromatogram and record the results in the protocol according to the form:

	№ of spots	**Numeric value of Rf**	**Spots staining**

Conclusion: _____

_____

DRM and MPRM containing tannins

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Quercus robur					
Cotinus coggygria					
Rhus coriaria					
Potentilla erecta					
Polygonum bistorta					
Sanguisorba officinalis					
Bergenia crassifolia					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Alnus glutinosa					
Alnus incana					
Vaccinium myrtillus					
Padus avium					
Camellia sinensis					
Comarum palustre					
Agrimonia eupatoria					

Practice № 24
THE FINAL CLASS

Coumarins and Chromones

1. Definition of the coumarins and chromones**.**
2. Classification of coumarins. The main groups of coumarins.
3. Dispersal of coumarins in the plant world. Their localization in plants. Examples.
4. Physical and chemical properties of coumarins. Extraction.
5. Methods for the qualitative detection of coumarins in raw materials.
6. Coumarins potency assay in MPRM.
7. Ways and methods of using raw materials containing coumarins and chromones.
8. Gathering, drying and storage of raw materials containing coumarins and chromones.
9. DRM and MPRM containing coumarins and chromones: Melilotus officinalis, Ammi majus, Ammi visnaga, Pastinaca sativa, Phlojodicarpus sibiricus**.**
10. Formulas: coumarin, umbelliferone, psoralen, angelitsin, bergapten, isopimpinellin, xanthotoxin, kellin.

Flavonoids

1. Definition of flavonoids. Features of the chemical structure.
2. Classification. Physicochemical properties. Extraction.
3. Dispersal of coumarins in the plant world. Their localization in plants. Examples.
4. Gathering, drying and storage of raw materials containing flavonoids.
5. Methods for the qualitative detection of flavonoids, their potency assay.
6. Using in medicines. Examples.
7. DRM and MPRM containing flavonoids: Crataegus, Sophora japonica, Aronia melanocarpa, Helichrysum arenarium, Tanacetum vulgare, Fragaria vesca, Polygonum**,** Ononis arvensis**,** Equisetum arvense, Scutellaria baicalensis**,** Centaurea cyanus**,** Bidens tripartite**,** Gnaphalium uliqinosum**,** Hypericum perforatum, Hypericum maculatum**,** Viola**,** Ginkgo biloba, Filipendula ulmaria**,** Cynara.
8. Formulas of rutin, quercetin, hyperoside, quercitin, apigenin, noringenin, cyanidin, catechin, luteolin.

Tannins

1. Definition of tannins. Classification.
2. Physicochemical properties. Extraction.
3. Dispersal of coumarins in the plant world. Their localization in plants. Examples.
4. Methods for the qualitative detection of tannins in raw materials.
5. Reactions of detection of hydrolysed and condensed tannins in their joint presence.
6. Tannins potency assay in MPRM.
7. The essence of the weighted single method of tannins determining.
8. Features of accumulation of tannins in plants.
9. Gathering, drying and storage of raw materials containing tannins. Use in medicines. Examples.
10. DRM and MPRM containing tannins: Cotinus coggygria, Rhus coriaria, Bergenia crassifolia, Quercus robur, Polygonum bistorta, Sanguisorba officinalis, Potentilla erecta, Padus avium, Vaccinium myrtillus, Alnus glutinosa, Alnus incana, Camellia sinensis.
11. Formulas of gallic, ellagic acid, pyrogallol, pyrocatechin, tannin, leucoanthocyanidin

Practice № 25
ANALYSIS OF MPRM CONTAINING ALKALOIDS

Control questions:

1. Concept and classification of alkaloids.
2. Distribution of alkaloids in plants, localization.
3. Physical and chemical properties of alkaloids.
4. Methods for detecting alkaloids in MPRM. Chemical reactions: general, group-specific. Chromatographic methods of analysis.
5. Extraction methods of alkaloids from raw materials, methods of decontamination and separation.
6. Methods for quantitative determination of alkaloids in MPRM.
7. The pharmacopoeia method of tropane alkaloids quantitative determination in Atropa belladonna leaves.
8. Ways of using raw materials containing alkaloids.

INFORMATION

Alkaloids are natural nitrogen-containing organic compounds having the basic character and exhibiting high pharmacological activity.

Alkaloids, formed in plants from amino acids and containing in their composition heterocycles with a nitrogen atom, are called true ***alkaloids***

Protoalkaloids are alkaloids formed in plants from amino acids, but contain a nitrogen atom in the side chain. They are also called biogenic amines (amino-alkaloids). ***Pseudo-alkaloids*** are (formed without the participation of amino acids) nitrogen compounds of terpenic and steroid structure.

Alkaloids show the properties of amines, therefore exist in two forms: in the form of salts and in the form of bases. Primary amines (mescaline), secondary amines (ephedrine), tertiary amines (atropine) and quaternary ammonium bases based compound are found. The group of tertiary amines is the most numerous. Alkaloids, as a rule, are mono-basic compounds. In plants they are in the form of salts of organic or mineral acids: citric, succinic, oxalic, acetic, sulfuric and others.

Alkaloids are differ in chemical structure, in the biosynthetic pathway of formation, and in pharmacological activity. The unified classification of alkaloids does not exist, but they can be divided, by some criterion. So Academician A. Orekhov proposed a ***chemical classification*** based on the nature of heterocycles.

Alkaloids can be classified by the name of amino acids (***biosynthetic classification***), from which alkaloids are formed. They can also be classified by ***pharmacological features***, combining alkaloids by pharmacological groups. ***The basis of phylogenetic classification*** is the principle of botanical relationship and the proximity of the chemical nature of alkaloids.

Classification of alkaloids

Classification of alkaloids

THE ALGORITHM OF LABORATORY WORK

TASK № 1. Carry out qualitative reactions with MPRM containing alkaloids.

1. *Extraction*: 1.0 powdered MPRM put in a flask with a capacity of 30 ml, add 10 ml of 2 % acetic acid solution and heat in a boiling water bath for 5 minutes. After cooling, the extraction is filtered into a test tube.

2. *Precipitation reactions*: On a glass slide apply 2 drops of extract and a drop of a reagent next to it with a glass rod. When merging the reagent and extracting drops, observe the appearance of turbidity or sediment. Mark the presence and color of the sediment.

Carry out qualitative reactions with the following reagents:

1. Bushard's Reagent ($KI * I_2$);

Observing: _____

2. Dragendorf's Reagent ($KI * BiI_3$);

Observing: _____

3. 10 % tannin solution;

Observing: _____

4. 1 % silicotungstic acid solution;

Observing: _____

5. 1 % phosphomolybdic acid solution;

Observing: _____

6. Picric acid solution.

Observing: _____

TASK № 2. To quantify the amount of tropane alkaloids in the MPRM.

_____

(specify the name of the analyzed MPRM)

1. Preparation of MPRM for analysis (powdering, sieving, taking a sample).

2. Extraction of alkaloids in the bases form. 5 g of powdered plant material (leaves, roots or rhizomes) passing through a sieve with a hole diameter of 1 mm are placed in a flask with a ground stopper, add 3.5 ml of concentrated ammonia solution, 75 ml of chloroform and shake vigorously. The plant material filled with the extractant is left overnight.

Chloroform extract is quickly filtered through cotton wool into a separatory funnel with a capacity of 200 ml. To the filtrate add 5 ml of water, vigorously shake and leave alone until complete stratification. Measure by graduated cylinder 50 ml of chloroform extraction, which is transferred to a separatory funnel. The cylinder is rinsed twice with chloroform in 5 ml portions, which are attached to the measured chloroform extraction.

3. Extraction decontamination:

a) Translation of bases alkaloids in salts.

From the chloroform extraction of the alkaloids, 10, 7, and 5 ml of 2 % acetic acid solution (pH of the water layer is 2–3) are sequentially removed until they are completely removed (sample with Mayer reagent), each time filtered through a water-wet filter into a 100-ml flask. The filter was washed twice with 2 % acetic acid solution per 5 ml, connecting the wash liquid to a common acid extract.

b) Transfer of salts alkaloids to the bases.

Acidic recovery from the flask is quantitatively transferred to a separatory funnel, basified with ammonia solution prior to an alkaline reaction by phenolphthalein, and alkaloids are extracted successively with 10, 7, 5 ml of chloroform. Each portion of the chloroform recovery is filtered through a paper filter onto which 3–4 g of anhydrous sodium sulfate moistened with chloroform are previously placed. Filtration is carried out in a dry flask with a 100 ml section. Chloroform is distilled off in a water bath to dryness (until the smell of chloroform disappears completely).

4. Potency assay of alkaloids: method of acid-base titration (reverse titration).

The evaporated residue (purified amount of alkaloid bases) is dissolved in 15 ml of 0.02 mol/l hydrochloric acid solution when heated in a water bath and the excess is titrated by the 0.02 mol/l NaOH solution until a yellow color appears (methyl red indicator) .

Calculation of alkaloids according to the formula:

$$X = \frac{(15 — V) \times 0{,}005780 \times 100 \times 100}{V_1 \times (100 — W)},$$

where V — olume of 0.02 mol/l solution of caustic soda, which went to titration, in ml; V_1 — a sample calculated from the measured volume of chloroform extraction, in grams; W — loss in mass during drying of raw materials, in %; 0,005780 — the amount of alkaloids in terms of hyoscyamine corresponding to 1 ml of HCl solution (0.02 mol/l)

On the basis of the analysis carried out, a conclusion must be made on the conformity of the raw materials sample to the requirements of regulatory documents on the content of alkaloids

Conclusion: _____

_____

_____

Practice № 26
DRM AND MPRM CONTAINING ALKALOIDS WITH NITROGEN IN THE LATERAL CHAIN, DERIVATIVES OF PYRROLIZIDINE AND TROPANE

Control questions:

1. Definition of alkaloids.
2. Classification of MPRM containing alkaloids.
3. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
4. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
5. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
6. MPRM external signs.
7. Chemical composition. Formulas of ephedrine, platifillin, hyoscyamine, scopolamine.
8. Ways of use and application in medicine of MPRM containing alkaloids.
9. Indicate the chemical composition, pharmacological activity and use of MPRM containing alkaloids.

THE ALGORITHM OF LABORATORY WORK

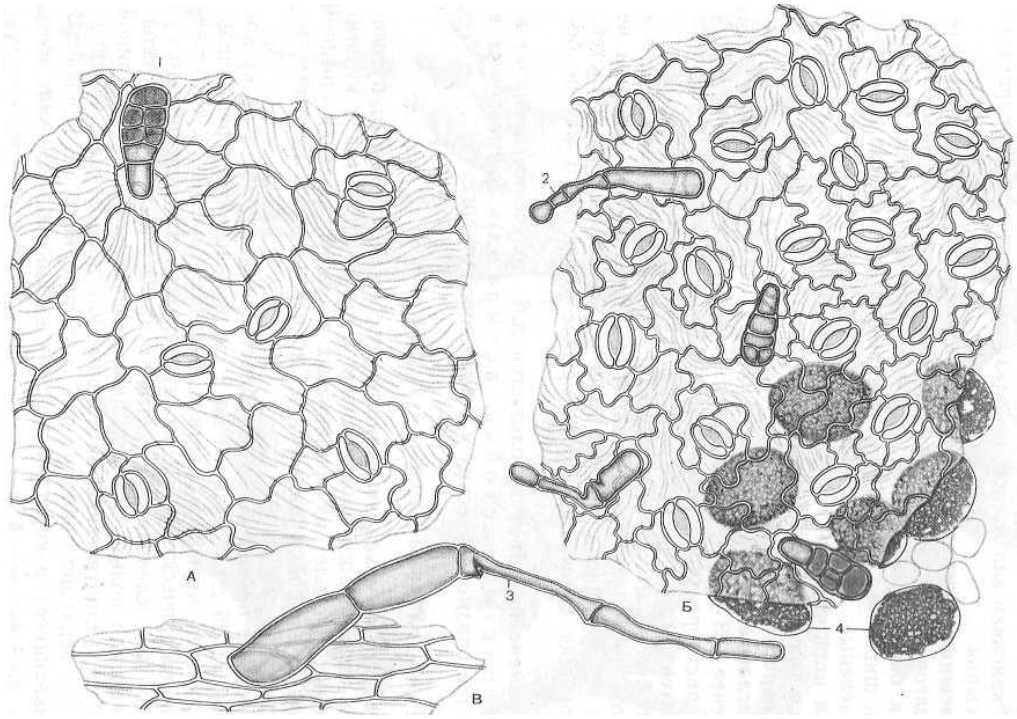
TASK № 1. Carry out a macroscopic analysis and indicate the diagnostic signs of Stramonii folium.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside	
9. Smell.	
10. Taste.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Stramonii folium.



To give an opinion about the identification and quality of the MPRM on macroscopic and microscopic diagnostic signs.

Conclusion: _____

_____

TASK № 2. Carry out a macroscopic analysis and indicate the diagnostic signs of Hyoscyami nigri foliae.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside.	
9. Smell.	

DRM and MPRM containing alkaloids with nitrogen in the side chain, derivatives of pyrrolizidine and tropane

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Ephedra equisetina					
Capsicum annuum					
Colchicum speciosum					
Senecio platyphylloides					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Atropa belladonna					
Hyoscyamus niger					
Datura stramonium					
Datura innoxia					

Practice № 27
CHINOLYSIDIN AND STEROIDAL ALKALOIDS (GLYCOALKALOIDS). DRM AND MPRM CONTAINING THESE GROUPS OF COMPOUNDS

Control questions:

1. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
2. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
3. Sustainable methods of collecting raw materials, MPRM primary processing, drying and storage.
4. MPRM external signs**.**
5. Chemical composition. Formulas: cytisine, pachycarpine, solasodine.
6. Ways of use and application in medicine of MPRM containing alkaloids.
7. Indicate the chemical composition, pharmacological activity and use of LBC containing alkaloids.

THE ALGORITHM OF LABORATORY WORK

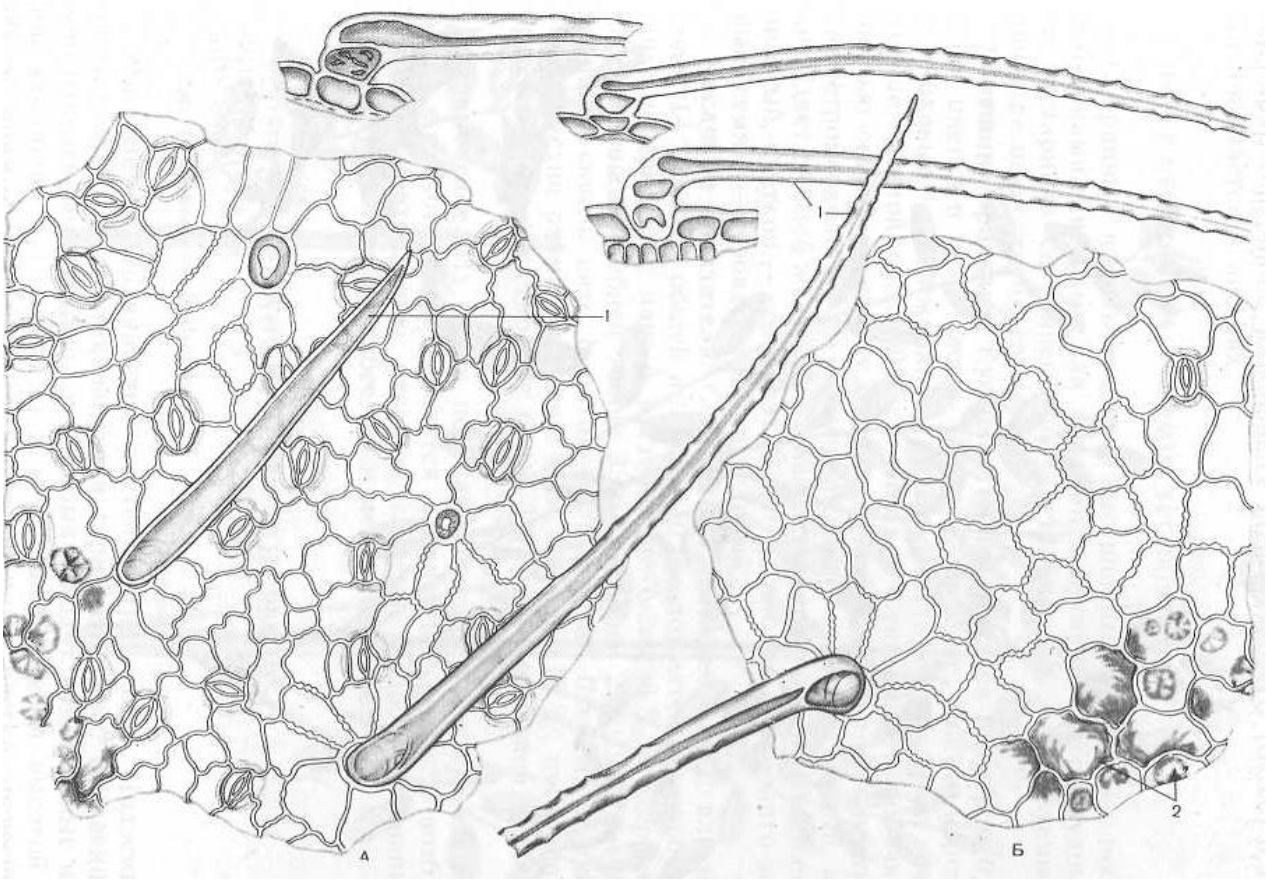
TASK № 1. Carry out a macroscopic analysis and indicate the diagnostic signs of Thermopsidis lanceolatae herba.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade.	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside.	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers.	
13. The shape of the flower (actino- or zygomorphic).	
14. Inflorescence or flower size.	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum.	
17. Color.	
18. Smell.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Thermopsidis lanceolatae folium.



To give an opinion about the identification and quality of the MPRM on macroscopic and microscopic diagnostic signs.

Conclusion: _____

TASK № 2. Carry out a macroscopic analysis and indicate the diagnostic signs of Tinctura rhizomatum cum radicibus Veratri lobelliani.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs.

Conclusion: _____

_____

TASK № 3. Carry out a macroscopic analysis and indicate the diagnostic signs of Huperziae selaginis herba.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside	
11. Colour.	
12. Smell.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs.

Conclusion: _____

_____

DRM and MPRM containing quinolizidine and steroidal alkaloids (glycoalkaloids)

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Thermopsis lanceolate					
Thermopsis alterniflora					
Sophora pachycarpa					
Nuphar lutea					
Huperzia selago					
Securinega suffruticosa					
Cinchona					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Solanum laciniatum					
Veratrum lobelianum					
Coffea					
Theobroma cacao					
Camellia sinensis					
Pilocarpus jaborandi					

Practice № 28
ISOQUINOLINE AND INDOLE DERIVATIVE ALKALOIDS. DRM AND MPRM CONTAINING THESE GROUPS OF COMPOUNDS

Control questions:

1. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
2. Morphological characteristics of plants, their areals, habitats, areas of cultivation.
3. Sustainable methods of raw materials collecting.
4. MPRM primary processing, drying and storage.
5. MPRM external signs.
6. Chemical composition and structural formulas of glaucin and berberine.
7. Application in medicine.
8. Indicate the chemical composition, pharmacological activity and use of MPRM containing alkaloids

THE ALGORITHM OF LABORATORY WORK

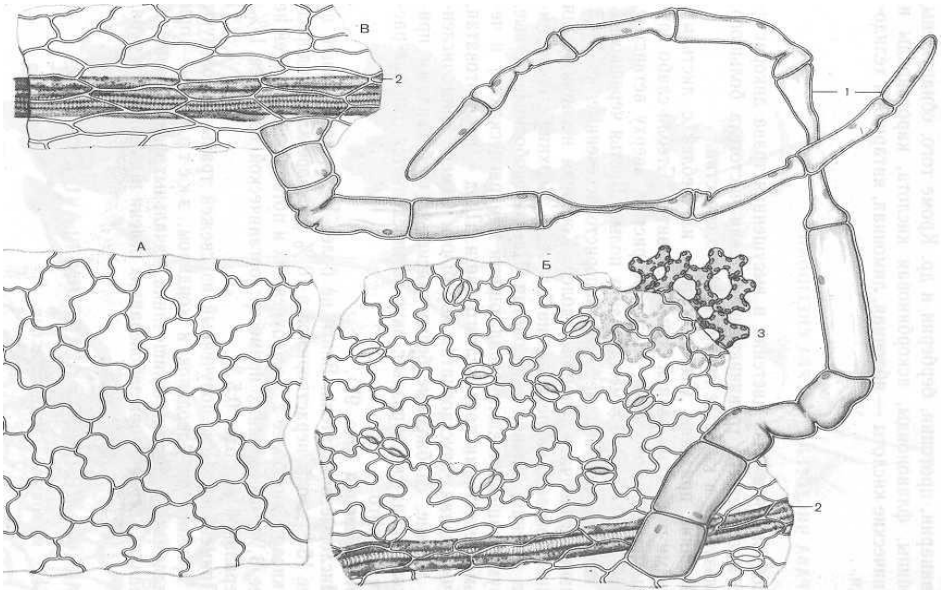
TASK № 1. Carry out tests for the authenticity of Chelidonii majoris herba.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Structure of the stem (shape, branching, indumentum, size, colour).	
2. Leaf position.	
3. **The leaves.** Type of a leaf (simple or complex).	
4. Petiolate or sessile leaf.	
5. Shape of the leaf blade.	
6. Size of the leaf and petiole.	
7. The blade edge.	
8. Type of the leaf venation.	
9. Indumentum.	
10. Color of upperside	
and underside.	
11. Flower arrangement on the stem.	
12. **Flower.** Type of an inflorescence or single flowers.	
13. The shape of the flower (actino- or zygomorphic).	
14. Inflorescence or flower size.	
15. Absence or presence of peduncle (its shape and size).	
16. Indumentum.	
17. Color.	

b) Carry out a microscopic analysis and indicate the diagnostic signs of Chelidonii majoris folium.



To give an opinion about the identification and quality of the MPRM on macroscopic and microscopic diagnostic signs.

Conclusion: _____

TASK № 2. Carry out a macroscopic analysis and indicate the diagnostic signs of Berberidis vulgaris radices.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs.

Conclusion: _____

TASK № 3. Carry out a macroscopic analysis and indicate the diagnostic signs of Macleayae herba.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. Color at the fracture.	
5. Smell.	

To give an opinion about the identification and quality of the MPRM on macroscopic diagnostic signs..

Conclusion: _____

_____

TASK № 4. MPRM chromatography.

1. Prepare the extraction from MPRM (herba Chelidonii, herba Macleayae): 0.5 g of the powdered raw material is placed in a test tube, add 2-5 ml of ethanol, heated in a water bath to a boil and insist for 10-15 minutes.

2. On the starting line of the chromatographic plate, draw the extract and the witnesses (solution of berberine, hyoscyamine alkaloids, sums of sanguinarine and chelerythrin) by the capillary in the form of a point or strip 1-1.5 cm in length.

3. Place the chromatogram in the chamber with the solvent system:

anhydrous formic acid–water–propanol (1:9:90) [Chelidonium].

toluene–ethyl alcohol–concentrated ammonia (10:2:0.05) [macleya].

After passing the solvent system at a distance of 10–12 cm, remove the chromatogram and dry it under traction.

4. Observe the coloration of the alkaloids sorption zones in visible light, in UV light, then treat the chromatogram with Dragandorf reagent. Mark the orange color spots (alkaloids) on a yellow background, calculate the Rf value for each spot.

	№ of spots	**Numeric value of Rf**	**Spots staining**

Conclusion: _____

_____

DRM and MPRM containing alkaloids (derivatives of isoquinoline and indole)

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Glaucium flavum					
Chelidonium majus					
Macleaya cordata					
Macleaya microcarpa					
Berberis vulgaris					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Stephania glabra					
Claviceps purpurea					
Strychnos nux-vomica					
Rauvolfia serpentina					

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Vinca minor					
Catharanthus roseus					
Passiflora incarnata					

Practice № 29
DRM AND MPRM CONTAINING VARIOUS GROUPS OF BIOLOGICALLY ACTIVE SUBSTANCES

Control questions:

1. Latin names of DRM and MPRM, producing plants and families of all plants of the training subject.
2. Morphological characteristics of plants, their areals, habitats, areas of cultivation**.**
3. Sustainable methods of raw materials collecting, MPRM primary processing, drying and storage.
4. MPRM external signs**.**
5. Chemical make-up.
6. Drugs and applications in medicine.
7. Indicate the chemical composition, pharmacological activity and use of MPRM containing BAS.

THE ALGORITHM OF LABORATORY WORK

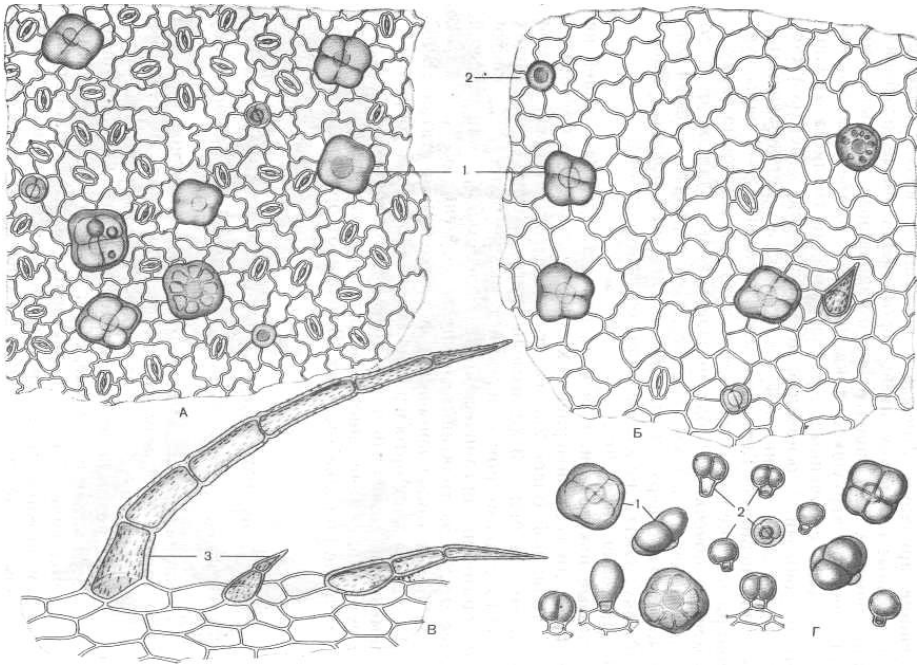
TASK № 1. Determine authenticity and quality of Orthosiphonis folium.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside	
9. Smell.	

b) Examine the microscopic signs of Orthosiphonis folium.



Orthosiphon stamineus microslide: A — epidermis of the underside; B — epidermis of the upper side.

To give an opinion about the identification and quality of the MPRM on external diagnostic signs.

Conclusion: _____

TASK № 2. Determine authenticity and quality of Inonotus obliquus.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
4. The nature of the fracture.	
5. Color from the outside	
and at the fracture.	
6. Smell.	

To give an opinion about the identification and quality of the MPRM on external diagnostic signs.

Conclusion: _____

TASK № 3. Determine authenticity and quality of Leguminis fructum Phaseoli vulgaris.

The name of MPRM	
The name of the plant	
Family	

Examine the external signs of raw materials.

1. Shape.	
2. Size.	
3. The surface structure.	
The nature of the inner surface	
4. Color	
5. Smell.	

To give an opinion about the identification and quality of the MPRM on external diagnostic signs.

Conclusion: _____

_____

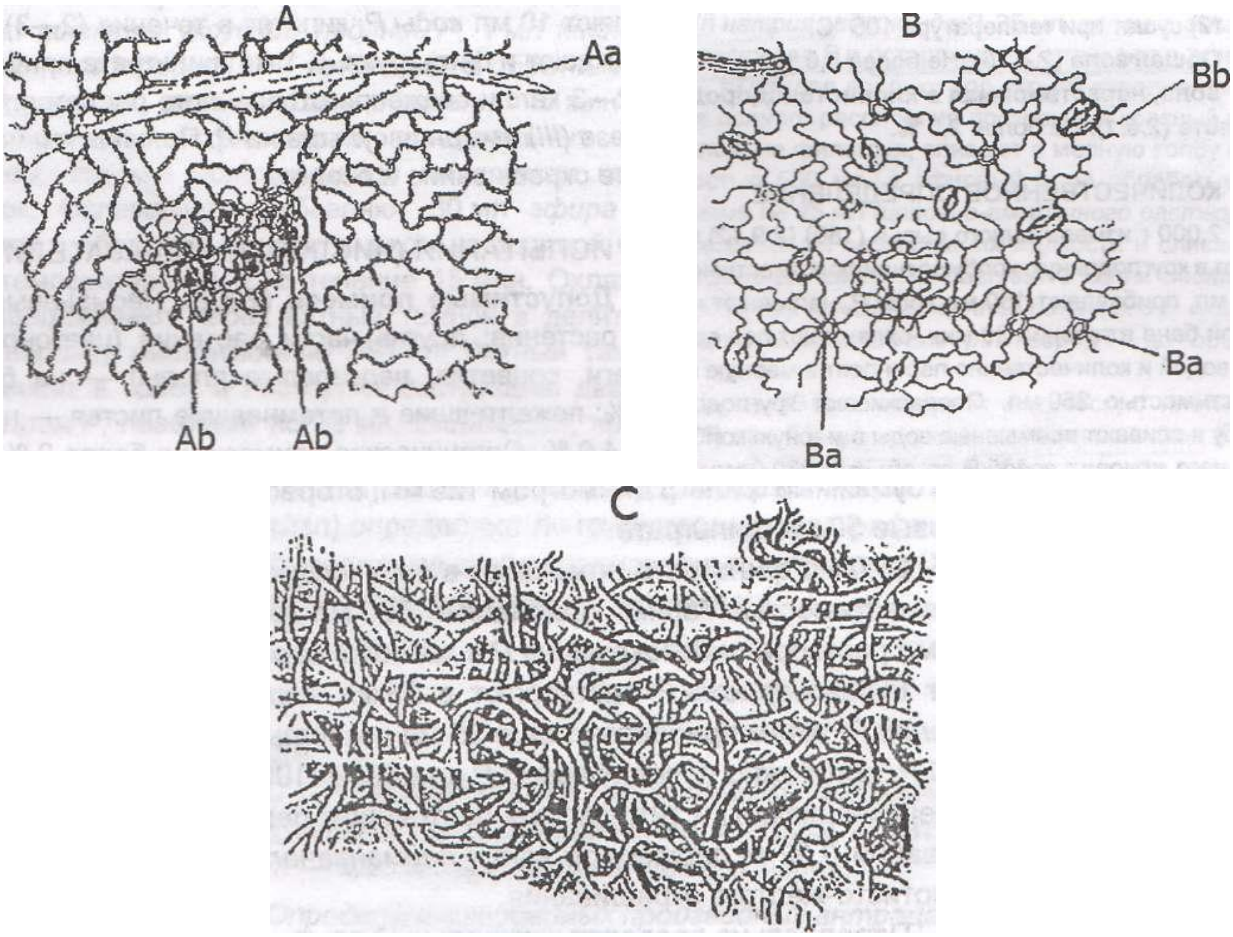
TASK № 4. Determine authenticity and quality of Rubi idaei folia.

The name of MPRM	
The name of the plant	
Family	

a) Examine the external signs of raw materials.

1. Type of a leaf (simple or complex).	
2. Petiolate or sessile leaf.	
3. Shape of the leaf blade.	
4. Size of the leaf and petiole.	
5. The blade edge.	
6. Type of the leaf venation.	
7. Indumentum.	
8. Color of upperside	
and underside.	
9. Smell.	

b) Examine the microscopic signs.



Rubi idaei folia microslide: A — epidermis of the underside; B — epidermis of the upper side C — tomentose indumentums.

To give an opinion about the identification and quality of the MPRM on external diagnostic signs.

Conclusion: _____

TASK № 5. Conduct quantitative determination using *HPLC method*.

MPRM: _____

Preparation of the test solution: _____

Reference solution:

Chromatography conditions:

Formula, calculations:

Conclusion:

DRM and MPRM containing various groups of biologically active substances

Name of DRM	Latin name of DRM, MPRM and family	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration
Kalanchoe pinnata					
Orthosiphon stamineus					
Rubus idaeus					
Echinacea purpurea					
Sambucus nigra					
Fungus betulinus					
Cucurbita					
Phaseolus vulgaris					
Allium sativum					

Practice № 30
ANIMAL ORIGIN MEDICINAL PRODUCTS AND NATURAL PRODUCTS

Control questions:

1. Drug raw materials of animal origin and natural products: snakes venoms.
2. Products of honey bee vital activity.
3. Medical leech.
4. Drug raw materials of animal origin and natural products: antlers of young Siberian stag.
5. Drug raw materials of animal origin and natural products: Mumiyo (Shilajit).
6. Drug raw materials of animal origin and natural products: spongilla.
7. Drug raw materials of animal origin and natural products: castoreum.

INFORMATION

***Snake venom*-**the some species of snakes poisonous glands release: Vipera berus, Naja oxiana, Macrovipera lebetina, etc. Pharmacological actions are an analgesic, anti-inflammatory agent that stimulates the receptors of the mucous membranes, skin and subcutaneous tissues**.**

Honey is produced by honey bees (Apis mellifica) from the nectar of flowers or honey-fall, processing them in special honey sac.

The ***leeches*** salivary glands secret has anticoagulant, anti-inflammatory, antithrombotic, thrombolytic, hypotensive, immunostimulating, bacteriostatic, analgesic and other actions on the body.

Antlers are young, growing, uncoated horns of Siberian stag.

Mumiyo (Shilajit) is a natural resin-like product of mineral and biological origin.

DRM of animal origin

Name of DRM (BAS)	Chemical composition	MF and DF	Indications for use	Manufacturer/ Registration

Practice № 31
THE FINAL CLASS

1. Definition of alkaloids.
2. Classification of alkaloids.
3. Physico-chemical properties.
4. Dispersal in the plant world.
5. Localisation in organs and tissues.
6. Influence of ontogenetic factors and environmental conditions on accumulation of alkaloids in plants.
7. Methods of extraction.
8. MPRM analysis (qualitative, chromatography, quantitative).
9. Pharmacopoeia method of tropane alkaloids quantitative determination in the Atropa belladonna leaves.
10. Sustainable methods of raw materials collecting, MPRM containing alkaloids primary processing, drying and storage.
11. MPRM and drugs ways of use and application in medicine.
12. Characteristics of DRM and MPRM containing:

– *alkaloids:* Senecio platyphylloides, Berberis vulgaris, Atropa belladonna, Stephania glabra, Hyoscyamus niger, Claviceps purpurea, Datura stramonium, Strychnos nux-vomica, Datura innoxia, Rauwolfia serpentina, Thermopsis lanceolata, Huperzia selago, Thermopsis alterniflora, Catharanthus roseus, Sophora pachycarpa, Passiflora incarnata, Securinega suffruticosa, Solanum laciniatum, Nuphar lutea, Veratrum lobelianum, Glaucium flavum, Ephedra equisetina, Chelidonium majus, Macleaya microcarpa, Macleaya cordata, Capsicum annuum, Colchicum speciosum.

Different groups of BAS: Kalanchoe pinnata, Orthosiphon stamineus, Rubus idaeus, Echinacea purpurea, Sambucus nigra, Cucurbita, Fungus betulinus, Phaseolus vulgaris.

13. Latin names of DRM and MPRM, producing plants and families.
14. Brief botanical characteristic.
15. Inhabitation.
16. Geographical dispersal and cultivated areas of plants.
17. Sustainable methods of raw materials collecting, MPRM primary processing, drying and storage.
18. Chemical composition and structural formulas of platyphylline, berberine, scopolamine, cytisine, glaucin, ephedrine, solasodine, pachycarpine, atropine.
19. Drugs, MPRM application in medicine.
20. Medicinal raw materials of animal origin and natural products.

Practice № 32
ANALYSIS OF CUT MEDICINAL PLANT MATERIALS.
ANALYSIS OF POWDERED AND CUT-PRESSED MPRM.
ANALYSIS OF HERBAL COLLECTIONS

Control questions:
1. Herbal medicine: definition, features, rules and principles.
2. Biorhythmological characteristics of the MPRM.
3. Fees: definition, classification, production.
4. Indicators of authenticity and quality. Principles for drawing up fees.
5. Indicators of authenticity and quality of herbal teas.

THE ALGORITHM OF LABORATORY WORK

TASK №1. Identify the authenticity of 1 samples of cut MPRM different morphological groups.

Macroscopic analysis of selected collection components:
Description of external features of component №1::
Description of external features of component №2:
Description of external features of component №3:

Microscopic analysis of selected collection components:
Description of external features of component №1:
Description of external features of component №2:

Latin names of raw materials	Main group of biologically active substances
Latin name of the component №1:	
Latin name of the component №2:	
Latin name of the component №3:	

Pharmacological properties of selected components of the collection:
Pharmacological properties of the component №1:
Pharmacological properties of the component №2:
Pharmacological properties of the component №3:
Pharmacological properties of the component:
Indications for use of the collection:

Conclusion: _____

_____

Practice № 33
PASS THE PRACTICAL SKILLS

1. Determine the authenticity and quality of whole medicinal plant raw materials using macroscopic and microscopic methods according to the State Pharmacopoeia of the Republic of Belarus.
2. Determine the authenticity of medicinal plant raw materials using thin-layer chromatography according to the State Pharmacopoeia of the Republic of Belarus.
3. Determine the quality of medicinal plant raw materials by quantitative determination: the main group of biologically active substances according to the section of the «Regulatory Document on Quality».
4. Determine the quality of medicinal plant raw materials by quantitative determination: permissible impurities according to the State Pharmacopoeia of the Republic of Belarus.

Practice № 34
COURSE WORK

Course work requirements

In accordance with the curriculum of the educational institution in the specialty 1-79 01 08 «Pharmacy», 36 hours are allotted for course work.

Defense of coursework is carried out in the 6th semester (full-time education), in the 7th semester (correspondence education).

The purpose of the course work: deepening and expanding theoretical knowledge, mastering the techniques of independent work with scientific literature, logically consistent presentation of the material, developing the ability to draw conclusions and document the results (writing a term paper, preparing a report and presentation), as well as acquiring the skills of publicly defending the work performed (report, answers to questions).

Completing coursework includes the following steps:

1. Familiarization with methodological recommendations for completing coursework.
2. Selecting a topic from the list proposed by the department, coordinating it with the supervisor, completing assignments for course work.
3. Selection and study of literature on the chosen topic.
4. Drawing up a work plan and work schedule.
5. Collection and processing of factual material.
6. Adjustment of the work plan and its agreement with the supervisor.
7. Writing sections of the work, formulating conclusions, conclusions and generalizations based on its results.
8. Technical design of course work in accordance with established requirements.
9. Submitting the work to the supervisor for verification.
10. Receiving written feedback from the supervisor and eliminating the shortcomings noted by him.
11. Obtaining admission to defend coursework and defending it.

CHEMICAL FORMULAS OF SUBSTANCES

Ascorbic acid	Dehydroascorbic acid	Geraniol
Lilanool	Citral	Thymol
Carvacrol	Anethole	Menthol
Cineole	Ledol	Thujon

Karvon	Camphor	Borneol
α-pinene	Bisabolene	Matricin
Alantolactone	Loganin	Valeopatriate(s)
Hamazulene	Sweroside	Aucubin

Purpureaglycoside a	Purpureaglycoside B	Lantoside A
Digitoxigenin	K-strophanthoside	Cymarin
Convallotoxin	Diosgenin	Scopolamine
K-strophanthin-β	Adonitoxin	Dammaran

Ursolic acid	α-amyrin	β-amyrin
Oleanolic acid	Glycyrrhizic acid	Hydroquinone
Arbutin	p-tyrosol	Anthracene
Antranol	Antron	Oxiantron

Anthraquinone	Chrysacine	Frangula-emodin
Aloe-emodin	Chrysophanol	Alizarin
Sennoside A	Caffeine	Hyoscyamine
Rubyerythrinic acid	Diantron	Coumarin

Dihydrocoumarin	Umbeliferon	Kellyn
Psoralen	Bergapten	Isopimpenellin
Angelicin	Flavan	Flavone
Flavonone	Flavonol	Flavonononol

Solasodine	Glaucine	Isoflavone
Berberine	Katekhin	Leucoanthocyanidin
Anthocyanidin	Epicatechin	Halcon
Auron	Quercetin	Hyperoside

Rutin	Luteolin	Apigenin
Naringenin	Cyanidin	Gallic acid
Tannin	Pyrocatechol	Pyrogallol
Ellagic acid	Ephedrine	Platyfillin

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CONTENT

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ФАРМАКОГНОЗИЯ

PHARMACOGNOSY

Практикум
для студентов медицинского факультета иностранных учащихся

На английском языке

Ответственная за выпуск О. В. Мушкина
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