MINISTRY OF HEALTH OF UKRAINE
BOGOMOLETS NATIONAL MEDICAL UNIVERSITY

HANDBOOK ON PHARMACOGNOSY
for auditory and independent students work
Laboratory handbook
PART I

Discipline: Pharmacognosy
Direction: second (master's) level of higher education
Specialty: 226 "Pharmacy, industrial pharmacy"
Department: Pharmacognosy and botany

Name _____
Course _____
Group _____

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This handbook covers the established methodologies for morphological, anatomical, and chemical analysis of medicinal plants that can successfully teach the theoretical and practical course of pharmacognosy in accordance with the "Program of Pharmacognosy."

For students of higher educational establishments of level III-IV pharmaceutical accreditation full-time and part-time training in the specialty "Pharmacy, industrial pharmacy"

INTRODUCTION

The educational discipline "Pharmacognosy" is a profile for students majoring in "226 Pharmacy, industrial pharmacy". According to the training plan for specialists in this specialty of teaching the educational discipline, pharmacognosy is conducted in 5-6 semesters of the III year for a total of 240 hours, of which 30 hours are lectures, 120 hours are practical classes, and 90 hours are independent work of students.

The purpose of teaching pharmacognosy is to teach students: to find and identify medicinal plants in natural habitats; to know the periods, terms and rational methods of harvesting, primary processing, drying conditions and storage rules of medicinal plant raw materials (LPR); to carry out commodity analysis, macroscopic, microscopic, phytochemical analysis of LRS, its processing products and raw materials of animal origin, which is necessary in the future professional activity of a pharmacist.

The practical part of the educational discipline involves the development and assimilation of skills and abilities to identify medicinal plants in the natural environment and herbarium state; harvesting, drying, storage of LRS, its identification based on macroscopic and microscopic analysis; thin-layer chromatography methods, establishing its benign quality by determining the qualitative composition and quantitative content of active substances (BAR groups) and basic numerical indicators (moisture, ash content, presence of impurities, etc.).

In the study guide, the structure of each practical lesson is developed in detail, diagrams, the content of the tasks are given, and the methodology of their implementation is outlined. In order to study theoretical questions, acquire practical abilities and skills, provided by the program of the academic discipline, at each practical session, the student must have not only the recommended educational - methodical and reference literature, but also a completed work journal. By checking the class protocols filled out by the students in the work journals, the teacher assesses the readiness of each of them for the class, independent extracurricular work, and the quality of practical tasks. The presence of this work journal and its neat filling during homework and at each practical session is mandatory for students and is a guarantee of mastering the program requirements of the discipline and, as a result, successful completion of the final control in pharmacognosy.

CONTENTS

Handbook on Pharmacognosy. Part 1.

Notation conventions

MP – medicinal plant

MPM – medicinal plant material

BAS – biologically active substances

SPhU – State Pharmacopoeia of Ukraine

PhEur – European Pharmacopoeia

QCM – Quality Control Methods

TLC – thin-layer chromatography

PC – paper chromatography

SAMPLE OF FILLING OUT THE HANDBOOK

Sample 1. **Marshmallow root**

	Latin name	English name
MPM	Althaeae radices	Marshmallow root
MP	Althaea officinalis L.	Marshmallow
Family	Malvaceae	

Dissemination of MP	It grows throughout the territory of Ukraine (except the mountainous regions of the Carpathians and the Crimea), Europe and South America
Harvesting time	Raw materials are harvested in early spring or autumn, after the above-ground part has died
Drying conditions	Raw materials are dried at a temperature of 45-50^{0}C
Storage conditions	According to the general list
Basic group of BAS, %	Polysaccharides (mucilage, starch, pectin substances, sugars)
Other substances	Fatty oil, tannins, steroids, betaine, mineral salts
Standardization by content of BAS	The content of polysaccharides in terms of dry raw materials is not less than 14%

Macroscopic analysis of marshmallow root:

whole, cut, ground, or pulverized	Unrefined whole raw material
shape	Cylindrical, somewhat twisted
surface	With deep longitudinal grooves and numerous scars from the roots
characteristic of fracture	Fracture is fibrous on the outside, rough and granular on the inside
presence of core	-
colour of fracture surface	Fracture is white or yellowish-white
colour of external surface	The cleaned raw material has a grayish-white fine-fibrous outer surface
odour	Not specific
taste	Mucilaginous, sweet

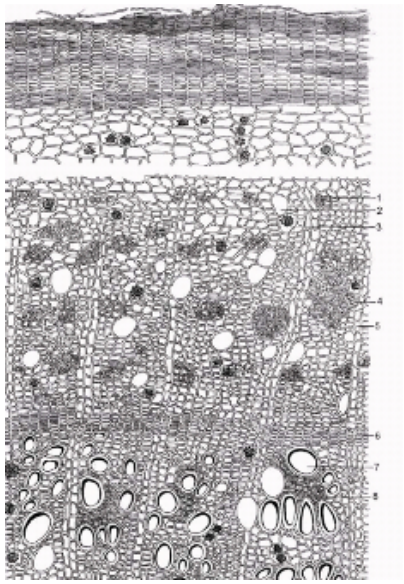
Adulteration (English and Latin names):

1. *Lavatera thuringiaca*
2. *Malva sylvestris*

Histochemical reactions:

Name of reaction	Methods	Observation
Reaction for mucilage	Add a drop of 5% sodium hydroxide solution on a fracture of marshmallow root.	A yellow color appears
Conclusions: this reaction indicates the presence of mucilage		
Reaction for starch	Add a drop of Lugol's solution on a fracture of marshmallow root.	Starch grains are colored blue (blue-violet).
Conclusions: this reaction indicates the presence of starch		
The reaction for lignin (Wiesner test)	Place a cut of pre-softened root on a glass slide with 1% alcohol phloroglucinol solution, and add 1 drop of concentrated hydrochloric acid. After 1 min remove the excess reagent with filter paper and add 1 drop of chloral hydrate, cover with a glass-slide cover, and examine under a microscope at magnification 100.	Lignified cell membranes acquire a crimson or cherry color
Conclusions: this reaction indicates the presence of lignin		
Dual-colour reaction	Place a cut of root on a glass slide in a solution of iron (III) chloride for 20 min. Remove reagent with filter paper, add a drop of methylene blue, then wash with water and cover with a glass-slide cover; examine under a microscope at magnification 100.	Cells with mucilage are colored yellow; bast fibers - in blue color; wood vessels - green
Conclusions: this reaction indicates the presence of mucilage		

Microscopic analysis of marshmallow root



The main diagnostic microscopic features of MPM:

1. thin-walled parenchyma with starch grains;
2. cells with mucilage in the parenchyma of bark and wood;
3. calcium oxalate druses;
4. groups of bast fibers, mostly non-woody with thickened walls;
5. core rays single-rowed, rarely double-rowed;
6. cambium;
7. xylem vessels;
8. tracheids

Use in medicine Expectorant, enveloping, anti-inflammatory action in acute and chronic respiratory diseases;

TOPIC: DETERMINATION OF IDENTITY OF MEDICINAL PLANT MATERIAL

Aim: to determine the identity of MPM using macroscopic, microscopic, and histochemical methods of analysis, identifying and highlighting the common morphological and anatomical features and specific characteristics that are inherent in the test object, and distinguishing it from other kinds of MPM.

I. Macroscopic analysis of MPM

Task 1. Do a macroscopic analysis of MPM of different morphological groups (leaves, flowers, herbs, fruits, seeds, bark, roots, rhizomes) using Appendix 1 as a reference. Compare the established morphological signs of the observed MPM with descriptions in the pharmacopoeia monographs SPhU and make a conclusion based on identifying and analyzing the features.

Sample 1. For this analysis you have this MPM: leaves
Macroscopic analysis of MPM:

general appearance	
shape of leaf	
division of the blade	
attachment of leaf to stem; presence of petiole	
leaf base	
leaf apex	
leaf edge	
type of venation	
leaf pubescence	
size of a leaf blade and petiole	
colour of upper and of lower side of leaf blade	
odour	
taste	

Determination of the identity of this MPM:

_____

_____

Conclusion:_____

_____

	Latin name	**English name**
MPM		
MP		
Family		

Sample 2. For this analysis you have this MPM: flowers
Macroscopic analysis of MPM:

general appearance	
type of inflorescence	
pedicel, cm	
bract, cm	
shape and size of the receptacle	
type of perianth	
symmetry	
shape and colour of calyx	
shape and colour of corolla	
dimensions	
odour	
taste	

Determination of the identity of this MPM:

_____

_____

Conclusion:_____

_____

	Latin name	**English name**
MPM		
MP		
Family		

Sample 3. For this analysis you have this MPM: fruits
Macroscopic analysis of MPM:

general appearance	
type of fruit	
shape	
type of surface	
number, shape, and size of seeds	
dimensions	
colour	
odour	
taste	

Determination of the identity of this MPM:

_____

_____

Conclusion:_____

	Latin name	English name
MPM		
MP		
Family		

Sample 4. For this analysis you have this MPM: seeds
Macroscopic analysis of MPM:

general appearance	
shape	
type of surface	
colour	
dimensions	
odour	
taste	

Determination of the identity of this MPM:

_____

_____

Conclusion:_____

_____

	Latin name	English name
MPM		
MP		
Family		

Sample 5. For this analysis you have this MPM: bark
Macroscopic analysis of MPM:

shape	
characteristics of outer surface	
characteristics of inner surface	
colour of outer surface	
colour of inner surface	
characteristic of fracture	
dimensions	
odour	

taste	

Determination of the identity of this MPM:

_____

_____

Conclusion:_____

_____

	Latin name	**English name**
MPM		
MP		
Family		

Sample 6. For this analysis you have this MPM: underground organs

Macroscopic analysis of MPM:

general appearance	
type of underground organs	
shape	
characteristics of surface	
characteristic of fracture	
dimensions	
colour of external surface	
colour of fracture surface	
odour	
taste	

Determination of the identity of this MPM:

_____

_____

Conclusion:_____

_____

	Latin name	**English name**
MPM		
MP		
Family		

Sample 7. For this analysis you have this MPM: herb
Macroscopic analysis of MPM:

general appearance	
form of stem in cross section	
size of stem	
colour of stem	
shape of leaf	
attachment of leaf to stem, presence of petiole	
leaf base	
leaf apex	
leaf edge	
type of venation	
leaf pubescence	
size of leaf blade and petiole	
color of upper and of lower side of leaf blade	
location of flowers on stem, type of inflorescence	
pedicel, cm	
bract, cm	
shape and size of receptacle	
type of perianth	
symmetry	
shape and colour of calyx	
shape and colour of corolla	
dimensions	
flower pubescence	
odour	
taste	

Determination of the identity of this MPM:

_____

_____

Conclusion:_____

_____

	Latin name	**English name**
MPM		
MP		
Family		

II. Microscopic analysis of MPM

Task 1. Do a microscopic analysis of MPM of different morphological groups (leaves, bark, roots, rhizomes) using Appendix 2. Compare the established anatomical characteristics of the given MPM with the discriptions in the pharmacopoeia monographs SPhU or GPh XI, and make a conclusion on the identity of the MPM that was received for analysis. Draw, label, and describe the microscopic features.

Method of preparation for microscopic analysis: for dry raw materials, soften and clarify by boiling in 5% solution of NaOH. To study anatomical features of leaf specimens, take the prepared surface and separate the upper and lower epidermis. To study anatomical features of bark, roots, and rhizomes, prepare cross sections of the softened materials. To see details of raw materials in the objective lenses you further need to heat the materials gently in a solution of chloral hydrate. Temporary preaparations can be viewed in a light microscope at low and high magnifications.

Sample 1. For this analysis you have this MPM: leaves

Microscopic analysis of MPM:

Upper epidermis	1. Cells of upper epidermis_____ _____ 2. Cells of lower epidermis _____ _____ 3. Type of stomatal complex _____ 4. Simple trichomes_____ _____ 5. Glandular hairs_____ 6. Glandules_____ 7. Inclusions: type_____ _____ shape_____ _____ 8. Secretory structures_____
Lower epidermis	

Determination of the identity of this MPM:

_____

_____

Conclusion:_____

	Latin name	English name
MPM		
MP		
Family		

Sample 2. For this analysis you have this MPM: leaves
Microscopic analysis of MPM:

Upper epidermis	1. Cells of upper epidermis_____ _____ 2. Cells of lower epidermis _____ _____ 3. Type of stomatal complex _____ 4. Simple trichomes_____ _____
Lower epidermis	5. Glandular hairs_____ 6. Glandules_____ 7. Inclusions: type_____ _____ shape_____ _____ 8. Secretory structures_____

Determination of the identity of this MPM:
_____
_____

Conclusion:_____

	Latin name	**English name**
MPM		
MP		
Family		

Sample 3. For this analysis you have this MPM: leaves
Microscopic analysis of MPM:

Upper epidermis	1. Cells of upper epidermis_____ _____ 2. Cells of lower epidermis _____ _____ 3. Type of stomatal complex _____ 4. Simple trichomes_____ _____
Lower epidermis	5. Glandular hairs_____ 6. Glandules_____ 7. Inclusions: type_____ _____ shape_____ _____ 8. Secretory structures_____

Determination of the identity of this MPM:
_____
_____
_____

Conclusion:_____
_____

	Latin name	English name
MPM		
MP		
Family		

Sample 4. For this analysis you have this MPM: bark
Microscopic analysis of MPM:

Fragment of a cross-section of a cortex	1.Cork: colour_____ number of layers _____ 2. Characteristics of parenchyma form of cells _____ _____ 3. Medullary rays _____ 4. Mechanical elements: type:_____ arrangement: _____ _____ 5. Crystalline inclusions:_____

Determination of the identity of this MPM:
_____
_____
Conclusion: _____

	Latin name	**English name**
MPM		
MP		
Family		

Sample 4. For this analysis you have this MPM: roots or rhizome
Microscopic analysis of MPM:

A fragment of a cross-section of a root or a rhizome	1. Covering tissue_____ 2. Vascular tissues_____ _____ 3. Medullary rays _____ _____ 4. Main parenchyma _____ _____ 5. Secretory structures_____ _____ 6. Crystalline inclusions _____ _____ 7. Stored substances_____ _____

Determination of the identity of this MPM:

_____

_____

Conclusion: _____

	Latin name	**English name**
MPM		
MP		
Family		

III. Histochemical analysis of MPM

Task 1. Do the histochemical reactions with the MPM.

Name of reaction	Methods of reaction	Observation	Conclusions
Reaction for lignified cell membrane	A cut marshmallow root is placed on a glass slide in 1% solution of phloroglucinol in alcohol; reagent is removed by filter paper; a drop of concentrated hydrochloric acid is put on the cut.		
Reaction for starch	A cut marshmallow root is placed on a glass slide in a drop of Lugol's solution.		
Reaction for mucilage	A cut marshmallow root is placed for a few minutes in an alcohol solution of methylene blue (1: 5000) and then is put into glycerin, covered with a glass-slide cover, and observed under a microscope.		
	A cut marshmallow root is placed in a saturated solution of copper sulfate for 5-10 minutes; then it is washed with water and transferred to a 50% solution of potassium hydroxide, covered with glass-slide cover, and observed under a microscope.		
	Flax-seed powder is placed on a glass slide in a drop of freshly prepared solution of ink (1:10) and stirred with a needle, then covered by a glass-slide cover and observed under a microscope		
Reaction for inulin (Molish's test)	A cross-cut of dandelion root or elecampane is placed in 1-2 drops of alcohol solution of α-naphthol (or thymol) and a drop of concentrated sulfuric acid is added.		
Reaction for essential oil	A cross-cut of rhizomes of Acorus is placed for a few minutes in a solution of Sudan III, and a drop of water or glycerin is added, covered with a glass-slide cover and observed under a microscope.		
Reaction for fatty acid	A cross-cut of a Ricinus seed is placed for a few minutes in a solution of Sudan III, and a drop of water or glycerin is added, covered with a glass-slide cover and observed under a microscope.		
Reaction for anthracene derivatives	A piece of buckthorn bark is placed in a drop of 5% potassium hydroxide on a glass slide.		
Reaction for tannins	A piece of oak bark is placed on a glass slide in a drop of 1% ferric (III) chloride or 1% solution of ferric ammonium alum.		

Teacher signature _____

TOPIC: CARBOHYDRATES. GLYCOSIDES

Aim: to establish the identity of MPM containing polysaccharides according to macroscopic and microscopic characteristics, and to determine the qualitative composition and quantitative content of BAS using methods of phytochemical analysis.
Objects for laboratory work: marshmallow root, marshmallow herb, common plantain leaves, psyllium seeds, coltsfoot leaves, common flax seeds, laminaria thallus.
Objects for independent study: *mucilage sources*: psyllium herb, tilia flowers, raspberry fruits, iceland moss thallus; *cellulose sources*: cotton plant bolls; *pectine sources*: apple-tree fruits, beet roots; *gum sources*: astragalus species, apricot tree; *inulin sources:* jerusalem artichoke tubers, coneflower rhizomes and roots, chicory roots, dandelion roots, elecampane rhizomes and roots; *starch sources:* potato tubers, wheat caryopses, corn caryopses, rice caryopses.
Structural formulas of main BAS: α- glucose, β- glucose**,** D- fructose, D- glucuronic acid, D-galacturonic acid, amylose, amylopectin, inulin, cellulose.

I. Phytochemical analysis of MPM containing polysaccharides

Task 1. Fill in Appendix 2 for this topic.
Task 2. Extract polysaccharides from MPM.

Method. Place about 10.0 g of accurately weighed, powdered, air-dried MPM into a 250 ml flask. Add 200 ml water. Attach a reflux condenser to the flask. Boil it on a hotplate for 30 min. Extraction is repeated twice: for the first time 200 ml of water is used, for the second time 100 ml. The water extracts are combined and centrifuged (speed of rotation 5000 rev), decanted into a volumetric flask through 5 layers of gauze laid in a glass funnel of diameter 55 mm. The filter is washed with water, and the flask is then filled to the mark by water (solution A). 25 ml of solution A is placed in a 200 ml flask, 75 ml of 96% alcohol is added, mixed, and heated in a water bath for 3 min, for better coagulation of the precipitate. The precipitate is filtered through filter paper. After filtration, do this further work with the precipitate obtained:

Task 3. Conduct quality reactions on mono- and polysaccharides**.**

Name of reaction	Methods	Observation
Reaction of identification of reducted (neutral) monosaccharides		
Reaction with Fehling's solution	Half of the precipitate (from Task 2) is transferred to a 25 ml flask, mixed with 5 ml of 10% hydrochloric acid and boiled for 30 min. Cool the hydrolysate, then add 10 ml of Fehling's solution and boil again.	
Reaction to identify acidic monosaccharides		
Reaction with carbazole	Take the other half of the precipitate (from Task 2) and transfer it into a 50 ml flask, add 1 ml of water, 0.25 ml of 0.5% carbazole solution, and 5 ml of concentrated sulfuric acid. Mix and heat in a water bath.	
Qualitative reaction for starch		
Formation of paste	Place 1 g of starch in a 100 ml flask; add 50 ml of water; heat for 5 min.	
Reaction with iodine solution	Add a drop of Lugol's solution to 2 ml of cooled starch paste.	

	The colored solution is heated to 100 ^{0}C and then cooled to room temperature.	
Conclusions		
Reaction with Fehling's solution	Add 2 drops of Fehling's solution (water solution of copper (II) sulfate (solution A) and 2 drops of alkaline solution of Rochelle salt (solution B)) to 2 ml of the starch paste. Mixture is heated in a water bath.	
Chemistry of the reaction:		
Conclusions		
Acid hydrolysis of starch	Place 1 ml of the paste and 10 drops of 10% sulfuric acid solution into a test tube and heat in a water bath for 20 min.	
	A drop of the hydrolysate is placed on a glass slide and mixed with a drop of iodine in potassium iodide solution.	
	Add 2 drops of Fehling's solution (water solution of copper (II) sulfate (solution A) and 2 drops of alkaline solution of Rochelle salt (solution B)) to 2 ml of the hydrolysate. Mixture is heated in a water bath.	
Chemistry of the reaction (acid hydrolysis of starch):		
Conclusions		
Qualitative reaction for dextrin		
Reaction with alkali solution	Dissolve 0.1 g of dextrin in 10% sodium hydroxide solution.	
Reaction with Fehling's solution	Add 1 ml of Fehling's solution to the alkaline dextrin solution and heat in a water bath.	
Interaction with alcohol	Add 5 ml of 95% alcohol to 0.5 ml of 5% dextrin solution.	
Conclusions		
Qualitative reaction for cellulose		
Reaction with iodine solution	Add a drop of iodine solution on cellulose powder.	
Reaction with iodine in solution of zinc chloride and potassium iodide	Place a pinch of cellulose powder on a glass slide and add the reagent.	

Conclusions		
Qualitative reaction for inulin		
Reaction with α-naphthol (Molish's test)	On the cross section of the cut raw material (chicory, dandelion, echinacea, or elecampane root; or earth apple tuber), add a drop of 20% α-naphthol alcohol solution and a drop of concentrated sulfuric acid.	
Conclusions		
Qualitative reaction for mucilage		
Reaction with alkali solution	Place 2 drops of sodium hydroxide solution on a cross section of marshmallow root.	
Reaction with concentrated hydrochloric acid	Place 1 ml of 10% marshmallow root infusion (from Task 2) in a test tube and add 2-3 drops of concentrated hydrochloric acid.	
	Add 2 ml of alcohol to the coloured solution.	
Reaction with lead acetate solution	Add 2 ml of lead acetate solution to 2 ml of 10% marshmallow root infusion.	
Conclusions		
Determination of the swelling index of mucilage-containing plant material	Place 1.0 g of raw material containing mucilage (flax seeds) into a 25 ml graduated cylinder with a scale division of 0.5 ml. Moisten the raw material with 1 ml of alcohol; fill with water to the mark, shake the mixture every 10 min for 1 h for uniform wetting of the raw material. After 2 h measure the volume of raw material with the swollen mucilage. Repeat 3-5 times and calculate the average swelling index.	Calculation:
Conclusions		

Task 4. Quantitative determination of polysaccharides by the gravimetric method (SPhU 2.0 T.3).

Method. Place about 5.0 g of accurately weighed, powdered, air-dried MPM (1000) (2.9.12) into a 250 ml flask. Add 75 ml water P. Attach a reflux condenser to the flask. Boil it on a hotplate for 30 min. The water extract centrifuged (speed of rotation 5000 rev), decanted into a volumetric flask 250 ml through 5 layers of gauze laid in a glass funnel of diameter 55 mm. The gauze laid is washed with water P. Extraction is repeated 3 times: 50 ml of water is used each time. Then add 25 ml of water P for the last extraction. Each extract centrifuged (speed of rotation 5000 rev), decanted into a volumetric flask. The filter is washed with 10 ml of ethanol (96%) P and the flask is then filled to the mark by water.

25 ml of obtained solution is placed in centrifuge test-tube, 50 ml of ethanol (96%) P added, mixed, heated on water bath (30 °C) for 5 min, leaved for 1 h, centrifuged (speed of rotation 5000 rev) for 30 min. The supernatant liquid is filtered. The precipitate is quantitatively transferred to the

filter with the help of 15 ml of a mixture of water P - ethanol (96%) P (1:2) and washed with 10 ml of ethanol (96%) P. The filter with the precipitate is dried in air, then dried to a constant mass at a temperature (100-105)°C.

The content of polysaccharides, in terms of dry raw materials, in percent, is calculated according to the formula:

$$(m_2 - m_1) \times 100000 / m \times (100 - W)$$

where: m - the weight of the tested raw material, g, m_1 — mass of the filter, g, m_2 — filter mass with residue, g, W — loss on during, g

II. Macro- and microscopic analysis of MPM containing polysaccharides

Sample 1. **Marshmallow root**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by content of BAS	

Macroscopic analysis of marshmallow root:

whole, cut, ground, or pulverized	
shape	
surface	
characteristic of fracture	
presence of core	
colour of fracture surface	
colour of external surface	
odour	
taste	

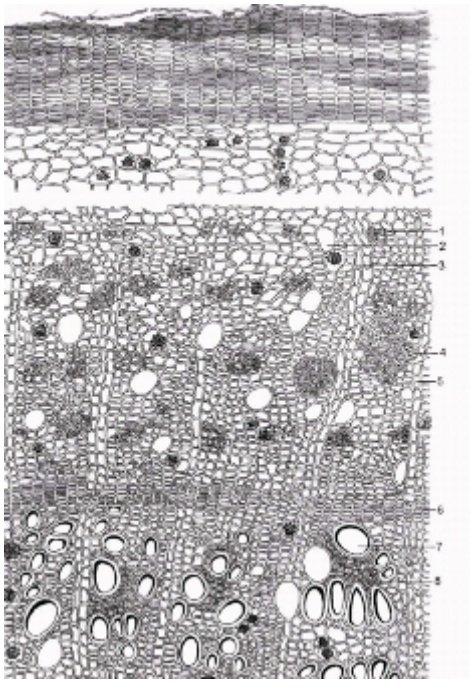
Adulteration (English and Latin names):

1. _____
2. _____

Histochemical reactions:

Name of reaction	Methods	Observation
Reaction for mucilage	Add a drop of 5% sodium hydroxide solution on a fracture of marshmallow root.	
Conclusions		
Reaction for starch	Add a drop of Lugol's solution on a fracture of marshmallow root.	
Conclusions		
The reaction for lignin (Wiesner test)	Place a cut of pre-softened root on a glass slide with 1% alcohol phloroglucinol solution, and add 1 drop of concentrated hydrochloric acid. After 1 min remove the excess reagent with filter paper and add 1 drop of chloral hydrate, cover with a glass-slide cover, and examine under a microscope at magnification 100.	
Conclusions		
Dual-colour reaction	Place a cut of root on a glass slide in a solution of iron (III) chloride for 20 min. Remove reagent with filter paper, add a drop of methylene blue, then wash with water and cover with a glass-slide cover; examine under a microscope at magnification 100.	
Conclusions		

Microscopic analysis of marshmallow root



The main diagnostic microscopic features of MPM:

1.

2.

3.

4.

5.

6.

7.

8.

Use in medicine _____

_____

_____

_____

Sample 2. **Marshmallow herb**

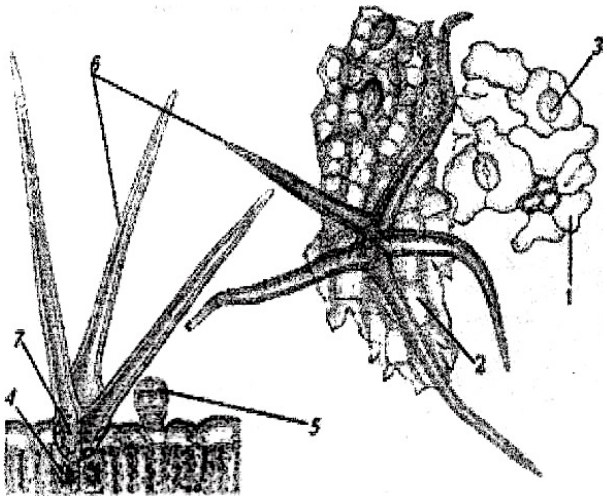
	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of marshmallow herb:

whole, cut, ground, or pulverized		leaf size	
stem form in cross section		leaf colour	
stem diameter		location of flowers on the stem, type of inflorescence	
stem colour		flower size	
shape of leaf		flower color	
presence of petiole		leaf pubescence	
leaf edge		odour when crushed	
type of venation		taste	
specific characteristics			
flower: calyx		colour of petals	
petals		colour of anthers	

Microscopic analysis of marshmallow herb

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6. 7.

Use in medicine _____

_____

_____

_____

Sample 3. Common plantain leaves

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of common plantain leaves:

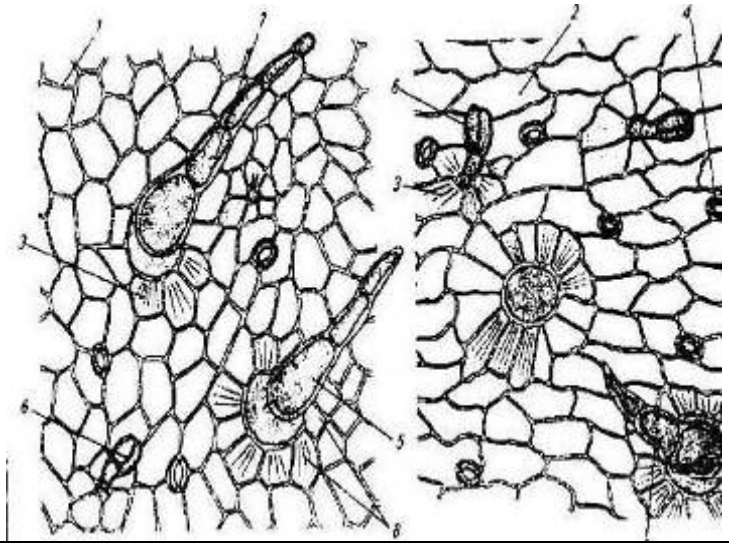
whole, cut, ground, or pulverized		leaf edge	
shape of leaf		type of venation	
leaf blade division		leaf pubescence	
attachment of leaf to stem, presence of		size of a leaf blade and petiole	

petiole			
leaf base		colour of upper and lower surface of leaf blade	
leaf apex		odour when crushed	

Adulteration (English and Latine names):

1. _____
2. _____
3. _____

Microscopic analysis of common plantain leaves



The main diagnostic microscopic features of MPM:

1.
2.
3.
4.
5.
6.
7.
8

Use in medicine _____

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Sample 4. Psyllium seeds

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	

Other substances	
Standardization by the content of BAS	

Macroscopic analysis of psyllium seeds:

whole, cut, ground, or pulverized	
shape	
surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____

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Sample 5. **Coltsfoot leaves**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of coltsfoot leaves:

whole, cut, ground, or pulverized		type of venation	
shape of leaf		leaf pubescence	
leaf blade division		size of a leaf blade and petiole	
attachment of leaf to stem, presence of		colour of upper surface of leaf blade	

petiole			
leaf base		colour of lower surface of leaf blade	
leaf apex		odour when crushed	
leaf edge		taste	

Use in medicine _____

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Sample 6. Linseeds

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of linseeds:

whole, cut, ground, or pulverized	
shape	
surface	
colour	
dimensions	
location of rib	
odour when crushed	
taste	

Use in medicine _____

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Sample 7. Laminaria thallus

	Latin name	**English name**
MPM		

The name of source plants:

MP	1. Laminaria saccharina 2. Laminaria japonica	
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of laminaria thallus:

whole, cut, ground, or pulverized	
shape	
surface	
colour	
dimensions	
character of thin coat on surface	
odour when crushed	
taste	

Use in medicine _____

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Signature of teacher_____

INDEPENDENT STUDENTS WORK

Sample 1. **Psyllium herb**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

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Sample 2. **Tilia flowers**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

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Sample 3. **Raspberry fruits**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____
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Sample 4. Iceland moss thallus

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____
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Sample 5. Cotton plant bolls

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 6. Apple-tree fruits

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 7. Beet roots

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 8. Astragalus species

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 9. Apricot tree

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 10. Jerusalem artichoke tubers

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 11. Coneflower rhizomes and roots

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 12. Chicory roots

	Latin name	English name
MPM		

MP		
Family		

Use in medicine _____

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Sample 13. Dandelion roots

	Latin name	**English name**
MPM		
MP		
Family		

Use in medicine _____

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Sample 14. Elecampane rhizomes and roots

	Latin name	**English name**
MPM		
MP		
Family		

Use in medicine _____

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Sample 15. Potato tubers

	Latin name	**English name**
MPM		
MP		
Family		

Use in medicine _____
_____
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Sample 16. Wheat caryopses

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____
_____
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Sample 17. Corn caryopses

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____
_____
_____

Sample 17. Rice caryopses

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____
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Signature of teacher_____

TOPIC: LIPIDS AND LIPOIDS

Aim: to establish the identity of MPM containing lipids according to macroscopic and microscopic characteristics, and to determine the qualitative composition and quantitative content of BAS using methods of phytochemical analysis.
Objects for laboratory work: olive oil, almond oil, peach kernel oil, castor oil, sunflower oil, linseed oil, cod-liver oil, cocoa butter, waxes, lanolin, spermaceti, products of soybean (oil, protein, phospholipids).
Objects for independent study: pumpkin seed oil, peanut oil, wheat germ oil; corn germ oil, coconut oil, palm oil, walnut oil, animal fats.
Structural formulas of main BAS: glycerin, general formula of triacylglycerides, general formula of phospholipids

I. Phytochemical analysis of MPM which contain lipids

Task 1. Fill in Appendix 2 for the topic of this lesson.
Task 2. Determine the quantitative content of lipids in MPM. Calculate the percentage of lipids (X) in MPM.

Method. Weigh 5.0 g of MPM containing lipids on the analytical scales and wrap the sample in filter paper. Place the package into the extractor. Before connecting the device, it is also necessary to weigh the receiving flask on the analytical scales.

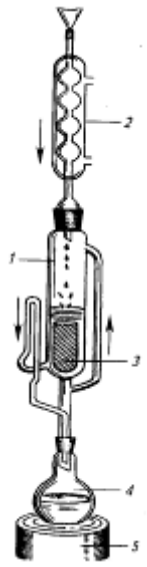
After connecting all parts of the device, pour solvent through the condenser until the liquid is just about to overflow through the siphon into the flask; also add solvent to the extractor to approximately 1/3 the volume.

The flask with solvent is heated in a boiling water bath. The steam from the solvent rises in the backflow condenser, condenses, and flows down to the extractor through the package with the MP. When the extractor is filled with liquid to the height of the siphon, the liquid flows down into the receiving flask. The whole process continues until the fatty oil is fully extracted.

Extraction must be conducted carefully to avoid overheating the solvent higher than 60°C. It must boil evenly, because solvent vapors that are heated too strongly will not condense properly.

The fats have been properly extracted when there is no longer any grease spot on the filter paper when you add a few drops of the extract.

After achieving full extraction, the solvent is removed; the receiving flask is dried in a drying box at 90-95°C. Knowing the weight of the empty receiving flask and of the flask with oil, the percentage of lipids (X) in MPM can be calculated.

 **Soxhlet apparatus**	Write down the basic parts of the Soxhlet apparatus 1. 2. 3. 4. 5.

Calculate the percentage of lipids using this formula:

$$X= (A – B)\cdot 100/C,$$

where A is the weight of receiving flask with oil, g; B is the weight of empty flask, g; and C is the weight of MPM, g.

Conclusions: _____

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Task 3. Analyze the oil sample.

Name of parameter	Methods	Observation
Description	Describe color, odour, taste, consistency. Put a drop of the oil on filter paper using a glass rod and heat the paper above a hotplate.	
Solubility	Place 1.0 g of oil into the solvent and continuously shake for 10 minuies at room temperature 20 ± 2 °C.	
Admixtures (paraffin, beeswax, resins)	1 ml of oil is heated with 10 ml of 0.5 N potassium hydroxide alcohol solution with continuous shaking. Then 25 ml of water is added.	
Peroxides and aldehydes (Kreis test)	Shake 1 ml of oil for 1 min with 1 ml of concentrated hydrochloric acid; add 1 ml of phloroglucinol ether solution (1:1000) and mix.	
Soaps	*For fatty oils, used for the preparation of injection solutions:* 5 ml of oil are put into a porcelain crucible and ignited. To the residue add 1 ml of freshly boiled water, dissolve in mild heat and add 2 drops of phenolphthalein. *For fatty oils, not used for the preparation of injection solutions*: Mix 50 ml of water with 10 drops of phenolphthalein solution, boil in a conical 250 ml flask for 1 min. The mixture should remain colorless. Then pour 5.0 g of oil into this hot water and boil for 5 min. The liquid is cooled to room temperature, placed against white paper, and 10 more drops of phenolphthalein are added.	
Elaidic test	2.0 g of oil is shaken in a test tube (closed by a ground glass stopper) with 1 ml of concentrated nitrous acid. Dilute with 1 ml of water and let set for 4-6 h.	
Acrolein test	1.0 g of oil is was placed in a test tube; 2.0 g of potassium bisulfate are added and heated (under a fume hood!).	
Conclusions		

Task 4. Determine authenticity of castor oil by solubility and find extraneous oils in it.

Name of reaction	Methods	Observation
Authenticity of castor oil	Add 2 ml of petroleum-ether into a test tube. Add 4 ml of castor oil and mix for 10 min.	
Admixtures of extraneous oils	Mix equal volumes of castor oil and 96% alcohol in a test tube at 20 °C.	

Conclusions

Task 5. Do reactions on seed oils and drupe oils.

Name of reaction	Methods	Observation
Reaction on seed oils (Bellier reaction)	Put 2 ml of the oil into a test tube, carefully layer 1 ml solution of nitric acid and 0.15 % resorcinol in benzene. Mix vigorously.	
Conclusions		
Reaction on drupe oils (Bieber's reagent)	Place 2.5 ml of oil into a test tube, then carefully add 1 ml of a mixture of equal volumes of water and sulphuric and nitric concentrated acids.	
Conclusions		

Task 6. Do reactions on cod-liver oil and lanolin.

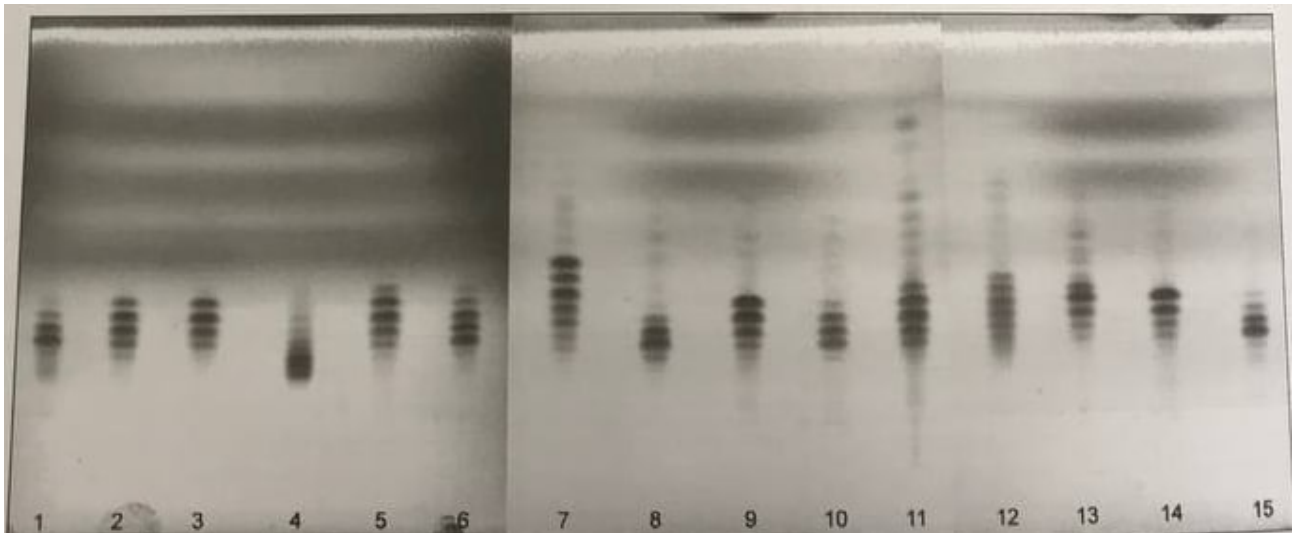
Name of reaction	Methods	Observation
Reaction on cod-liver oil	Dissolve 0.1 g cod-liver oil in 1 ml chloroform and add 5 ml Antimony(III) chloride solution.	
Reaction on cod-liver oil	Shake 1 drop of oil into 1 ml chloroform; add 1 drop of concentrated sulfuric acid.	
Conclusions		
Reaction on lanolin	Dissolve 0.1 g lanolin into 5 ml chloroform and carefully layer onto 5 ml concentrated sulfuric acid in a test-tube.	
Conclusions		

Task 7. Chromatographic analysis of a fatty oil sample by thin-layer chromatography (SPhU 2.2)

Test solution. Unless otherwise indicated, about 20 mg (1 drop) of fatty oil is dissolved in 3 ml of methylene chloride P.
Comparison solution. Dissolve about 20 mg (1 drop) of corn oil P in 3 ml of methylene chloride P.
Plates: suitable octadecylsilyl silica gel is used as a thin layer for high-performance thin-layer chromatography.
Mobile phase:

- *mobile phase:A: ether P;*
- *mobile phase B:* methylene chloride *P – glacial acetic acid P – acetone P* (20:40:50).

Causing: 1 µl.
The distance that the mobile phase has to travel: the plate is chromatographed twice at a distance of 0.5 cm from the start line using mobile phase A, then chromatographed twice at a distance of 8 cm from the start line using mobile phase B.
Drying: in the air
Detection: spray with a solution of 100 g/l phosphoromolybdic acid R in ethanol (96%) R, heat at a temperature of 120˚C for 3 minutes and view in daylight.
A typical chromatogram for the identification of fatty oils is shown in the figure.



1 – peanut oil; 2 – sesame oil; 3 – corn oil; 4 – rapeseed oil; 5 – soybean oil; 6 – rapeseed oil (free from erucic acid); 7 – linseed oil; 8 – olive oil; 9 – sunflower oil; 10 – almond oil; 11- wheat germ oil; 12 – cucumber oil; 13- evening primrose oil; 14 – safflower oil (type I); 15 – safflower oil (type II).

Fig. A typical chromatogram for the identification of fatty oils

Conclusions:_____

Task 8. Determine oil quality indexes and compare with Table 1.1, Appendix 3.

1. *Determination of acid number.*

Method. Dissolve 10 g (exact weight) of oil in 50 ml of solution of equal volumes of alcohol and ether, preliminarly neutralized with phenolphthalein solution and 0.1 mole/1 potassium hydroxide. Add 3-5 drops of phenolphthalein and titrate with 0.1 mole/1 potassium hydroxide solution, with constant stirring, until the pink colour appears and does not vanish over 15 seconds.

1 ml of 0.1 M potassium hydroxide solution corresponds to 5.61 mg of potassium hydroxide.

If the volume of 0.1 M solution of potassium hydroxide required for titration is less than 2 ml, increase the weight of sample tested substances appropriately or use more dilute titrant (for the latter making corresponding changes to the formula).

The acid number is calculated by formula:

$$I_A = 5.61 \cdot n/m,$$

where: n is volume of 0.1 mole/l potassium hydroxide solution used for titration, in ml; m is the mass of oil, g.

Conclusions:_____

2. *Determination of saponification number.*

Method. Place the oil (for exact weight, see Table 1.2) in a 200-250 ml flask and mix it with 25 ml of 0.5 mole/1 potassium hydroxide alcohol solution.

Connect the flask to the backflow condenser and heat gently in the water bath for 30 min. The end of saponification is determined by the formation of a completely transparent and homogeneous solution that does not change its clarity when diluted with water. The control experiment is conducted simultaneously: 25 ml of potassium hydroxide alcohol solution is heated in another flask (without oil).

Then, after heating add 25 ml of hot water and 5 drops of phenolphthalein to both flasks and titrate with 0.5 mole/1 hydrochloric acid solution until there is no more colour. From the number of ml of solution in the control experiment deduct the number of ml of

Table 1.2

The choice of sample to determine the saponification number

The value of expected saponification number	Mass of sample, g
3-10	12-15
10-40	8-12
40-60	5-8
60-100	3-5
100-200	2.5-3
200-300	1-2
300-400	0.5-1

Table 1.4

The choice of sample to determine the hydroxyl value

The value of expected	Mass of sample, g	Volume of acetic

hydrochloric acid solution expended during titration.1 ml of 0.5 Mole potassium hydroxide solution corresponds to 28.05 mgs of potassium hydroxide.

The saponification number is calculated using the formula:

$$I_S = 28.05 \cdot (n_2 - n_1)/m,$$

where: n_1 is the volume of 0.5 mole/1 hydrochloric acid solution, used to titrate in the control experiment, ml; n_2 is the volume of 0.5 mole/1 hydrochloric acid solution, used to titrate the examined sample, ml; and m is the mass of the oil, g.

hydroxyde number		anhydride, ml
10-100	2.0	5.0
100-150	1.5	5.0
150-200	1.0	5.0
200-250	0.75	5.0
250-300	0.60 or 1.20	5.0 or 10.0
300-350	1.0	10.0
350-700	0.75	15.0
700-950	0.5	15.0

Conclusions

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3. *Determination of ether number.*

Ether number (I_E) is calculated using the formula:

$$I_E = I_S - I_A,$$

where: I_S is the saponification number and I_A is the acid number.

Conclusions: _____

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Table 1.3

The choice of sample to determine the iodine number

The value of expected iodine number	Mass of sample, g
< 20	1.0
20-60	0.5-0.25
60-100	0.25-0.15
> 100	0.15-0.10

4. *Determination of iodine number.*

Method. Place the oil (for exact weight, see Table 1.3) in a 250 ml flask, add 15 ml chloroform (unless there is another standard for this substance). Slowly add 25 ml iodine bromide solution.

A flask is stoppered and stored in a dark place with frequent stirring for 30 min (unless there is another standard for this substance). Add 10 ml of 100 g/1 potassium iodide solution. Take 100 ml of water and titrate with 0.1 mole/1 sodium thiosulphate solution with intensive stirring until there is light-yellow colour, then add 5 ml of starch solution and titrate with 0.1 mole/1 sodium thiosulphate solution until the colour disappears.

Carry out the control test at the same time.

Iodine number (I_I) is calculate using a formula:

$$I_I = 1.269 \cdot (n_1 - n_2)/m,$$

where n_1 is the volume of 0.1 mole/1 sodium thiosulphate solution, used for titrating the control test, ml;
n_2 is the volume of 0.1 mole/1 sodium thiosulphate solution, used for titratingthe examined solution, ml: and m is the mass of oil, g.

Conclusions: _____

5. *Determination of hydroxyl value.*

Method. Place the oil (for exact weight see Table 1.4) into a 150 ml flask. Add the volume of solution of acetic anhydride as shown in Table 1.4.

Connect the flask to a backflow condenser and heat gently in the water bath for 1 h, keeping water level in the bath to 2.5 cm above the level of liquid in the flask.

Then through the top end of the condenser add 5 ml of water. If the solution becomes cloudy, stir in pyridine until the turbidity disappears; measure the volume. Place the flask in a boiling water bath for 10 min, then cool to room temperature. Wash the condenser and the wall of the flask washed with 5 ml of alcohol, previously neutralized using phenolphthalein solution.

The resulting solution is titrated with alcoholic solution of potassium hydroxide 0.5 mole/l, using 0.2 ml of phenolphthalein solution.

Carry out the control test in the same way.

The hydroxyl value (I_{OH}) is calculated using the formula:

$$I_{OH} = 28.05 \cdot (n_2 - n_1)/m + I_A,$$

where: n_1 is the volume of potassium hydroxide 0.5 Mole/l used for titrating in the examined solution, ml; n_2 is the volume of potassium hydroxide 0.5 Mole/l used for titrating the control test, ml; m is the mass of oil, g; 28,05 is the amount of potassium hydroxide, corresponding to 1 ml of 0.5 mole/l solution of potassium hydroxide, mg; and I_A is the acid number.

Conclusions: _____

6. *Determination of peroxide value.*

Method. Place 5.0 g (exact sample) of oil into a 250 ml conical flask with ground glass stopper, add 30 ml mixture of chloroform and glacial acetic acid (2:3). Shake the flask to dissolve the substances, then add 0.5 ml of saturated solution of potassium iodide; stir for 1 min, and add 30 ml water. The resulting solution is titrated by a solution of sodium thiosulphate 0.01 mole/l, adding the titrant slowly with continuous stirring until there is almost complete disappearance of colour. Then add 5 ml of starch solution and continue to titrate, intensively stirring until colour disappears.

Carry out the control test in the same way.

The amount of sodium thiosulphate 0.01 mole/l used in the control titration experiment should not exceed 0.1 ml.

Peroxide value I_p is calculate using the formula:

$$I_p = 10 \cdot (n_1 - n_2)/m,$$

where: n_1 is the volume of sodium thiosulphate 0.01 mole/l, used for titrating the examined solution, ml; n_2 is the amount of sodium thiosulphate 0.01 mole/l used for titrating the control test, ml; and m is the mass of oil, g.

Conclusions:_____

II. Macroscopic analysis of MPM containing fatty oils

Medicinal plant material – source of nondrying fatty oils

Sample 1. Olive fruits

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of olive fruits:

whole, cut, ground or	

pulverized	
type of fruit	
shape	
surface	
presence of seeds and number	
dimensions	
colour	
odour when crushed	
taste	

Use in medicine _____
_____
_____
_____

Sample 2. Almond seeds

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of almond seeds:

whole, cut, ground or pulverized	
shape	
surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____
_____

Sample 3. Peach kernels

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of peach kernels:

whole, cut, ground or pulverized	
shape	
surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____

_____

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Medicinal plant material – a source of nondrying fatty oils with specific acids

Sample 4. Castor beans (the MPM is poisonous!)

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of castor bean seeds:

whole, cut, ground or pulverized	
shape	
surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____
_____
_____

Medicinal plant material – a source of semi-drying fatty oils

Sample 5. Sunflower seeds

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of sunflower seeds:

whole, cut, ground or pulverized	
shape	
surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____

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Medicinal plant material – a source of drying fatty oils

Sample 6. Linseeds

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of linseeds:

whole, cut, ground or pulverized	
shape	
surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____

_____

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Medicinal plant material – a source of solid vegetable fats

Sample 7. Cacao seeds

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

_____

_____

Animal fats

Sample 12. Cod-liver oil

Latin name	
Appearance	
Source	
Chemical composition	
Biological action and use in medicine	

III. Analysis of lipoids. MPM containing lipoids.

Medicinal plant material – a source of phospholipids

Sample 8. Soybean seeds

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	

Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of soybean seeds:

whole, cut, ground or pulverized	
shape	
surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____

_____

Natural waxes

Sample 9. Lanolin

Latin name	
Appearance	
Source	
Chemical composition	
Biological action and use in medicine	

Sample 10. Spermaceti

Latin name	
Appearance	
Source	
Chemical composition	
Biological action and use in medicine	

Signature of teacher _____

INDEPENDENT STUDENTS WORK

Medicinal plant material – a source of semi-drying fatty oils

Sample 1. Pumpkin seeds

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

_____

_____

_____

Sample 2. Peanut seeds

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

_____

_____

_____

Sample 3. Corn germs

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

_____

_____

Sample 4. Walnut kernels

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

_____

_____

_____

Medicinal plant material – a source of phospholipids

Sample 5. Wheat germ

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

_____

_____

Medicinal plant material – a source of solid vegetable fats

Sample 6. Coconut kernel

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

_____

_____

Sample 7. Palm fruits

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

_____

_____

Signature of teacher _____

TOPIC: VITAMINS, MACRO- AND MICROELEMENTS, ORGANIC ACIDS.

Aim: to establish the identity of MPM containing vitamins, macro- and microelements, and organic acids according to macroscopic and microscopic characteristics, and to determine the qualitative composition and quantitative content of BAS using methods of phytochemical analysis.
Objects for laboratory work: dog rose, common nettle leaves, Shepherd's purse herb, maize (corn) silks, calendula flowers, sea-buckthorn fruits, hibiscus flowers, pomegranate fruits, barberry fruits, grape berries, raspberry fuits, horsetail herb.
Objects for independent study: blackcurrant leaves and fruits, pumpkin fruits, carrot root, primrose leaves, citrus fruits.
Structural formulas of main BAS: L-ascorbic acid, β-carotene, tocopherol, phylloquinone.

I. Phytochemical analysis of MPM containing vitamins, macro- amd microelements, and organic acids

Task 1. Fill in Appendix 2 on the topic of this lesson.
Task 2. Do the chromatographic determination of ascorbic acid and carotenoids in dog rose fruits according to SPhU (2.0 V.3).

Test solution. Place 0.5 g of powdered plant material in a 100 ml flask. Add 25 ml ofethanol 96% stir, infuse for 30 min, and filter.

Comparison solution. 10 mg of ascorbic acid R is dissolved in 5.0 ml of ethanol (60%, v/v) P.

Plate: TLC plate with a layer of silica gel F_{254} P.

Mobile phase: acetone P - glacial acetic acid P - methanol P - toluene P (5:5:20:70).

Sample volume: 20 µl of the test solution and 2 µl of the reference solution. Distance to be covered by the moving phase: 15 cm from the starting line.

Drying: in the air.

Detection A: viewed in UV light at a wavelength of 254 nm.

Results A: the chromatogram of the test solution shows an absorption zone at the level of the main zone in the chromatogram of the reference solution.

Detection B: spray with a solution of 0.2 g/l dichlorophenolindophenol sodium salt R in ethanol (96%) R, view in daylight.

Results B: the chromatogram of the test solution reveals a white zone on a pink background (ascorbic acid) at the level of the main zone on the chromatogram of the comparison solution, corresponding to it in color. The chromatogram of the tested solution also shows an intense orange-yellow zone near the solvent front and a yellow zone in the upper third (carotenoids).

Conclusions:_____

Task 3. Determine the content of ascorbic acid in dog rose fruits according to monograph SPhU (2.0 V.3) "Dogrose"

Method. Test solution. Place 0.500 g of freshly ground raw material (2.9.12) in a round-bottomed flask, add a solution of 1.0 g of oxalic acid R in 50.0 ml of methanol R, boil under reflux for 10 min, cool in an ice bath to a temperature of (15-20) °C and filtered.

2.0 ml of the filtrate is transferred to a conical flask with a capacity of 50 ml, successively added, shaking gently after each addition, 2.0 ml of dichlorophenolindophenol standard solution P, then, after exactly 60 s, 0.5 ml of a solution of 100 g/l thiourea P in ethanol (50%, vol. /vol) P and 0.7 ml of dinitrophenylhydrazine-sulphuric acid solution P, heated under reflux at a temperature of 50°C for 75 min and immediately placed in an ice bath for 5 min. Add dropwise 5.0 ml of a mixture of 12 ml of water P and 50 ml of sulfuric acid P, adding over a period of not less than 90 s and not more than 120 s, vigorously shaking the flask in an ice bath. Keep for 30 min at room temperature and measure the optical density (2.2.25) at a wavelength of 520 nm, using solution A as a compensating liquid.

Solution A. 2.0 ml of the filtrate obtained during the preparation of the test solution is treated as described above, adding dinitrophenylhydrazine sulfuric acid solution P immediately before measuring the optical density.

Comparison solution. 40.0 mg of ascorbic acid R is dissolved in a freshly prepared solution of 20 g/l oxalic acid R in methanol R, the volume of the solution is brought to 100.0 ml with the same solvent. 5.0 ml of the resulting

solution is brought up to 100.0 ml with a freshly prepared solution of 20 g/l oxalic acid R in methanol R. 2.0 ml of the resulting solution is treated as described above for the filtrate obtained during the preparation of the test solution

The optical density (2.2.25) is measured at a wavelength of 520 nm, using solution B as a compensating liquid.

Solution B. 2.0 ml of the comparison solution is processed as described above for solution A. The content of ascorbic acid, in percent, is calculated according to the formula:

$$2{,}5x\ A_1x\ m_2 / A_2x\ m_1$$

where: A_1 — optical density of the tested solution; A_2 — optical density of the reference solution; m_1 — the weight of the tested raw material, g; m_2 — weight of ascorbic acid, g.

Conclusions:_____

Task 4. Determine the content of organic acids in medicinal plant material to monograph SPhU (2.1) "Dogrose fruits^{N}"

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Method. Put 20.0 g of crushed raw material (exact weight), which has passed through a sieve with gradation 2 mm, into a 250 ml flask. Add 200 ml of water, heat in a water bath for 2 h, then let cool; transfer the mixture to a 250 ml volumetric flask, fill to the mark with water, and stir. Place 10 ml of filtrate in a 500 ml flask, add 200 ml of boiled water, 1 drop of 1% alcohol solution of phenolphthalein, and 2 drops of 0.1% solution of methylene blue.Titrate with sodium hydroxide solution (0.1 mole/l) until purple-red colour appears.

The percentage content (X) of organic acids in terms of malic acid contained in the absolutely dry raw material is calculated using this formula:

$$X = \frac{V \cdot 0.0067 \cdot 250 \cdot 100 \cdot 100}{M \cdot 10 \cdot (100-W)}$$

where: 0.0067 is the amount of malic acid that corresponds to 1 ml of sodium hydroxide solution (0.1 mole/l), g; V is the amount of sodium hydroxide solution (0.1 mole/l) used for titration, ml; m is the mass of raw material, g; and W is the loss in weight during the drying of plant material, percentage.

Conclusions: _____

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II. Macro- and microscopic analysis of MPM containing vitamins

Sample 1. **Dog rose fruits**

	Latin name	**English name**
MPM		

Name of plant source:

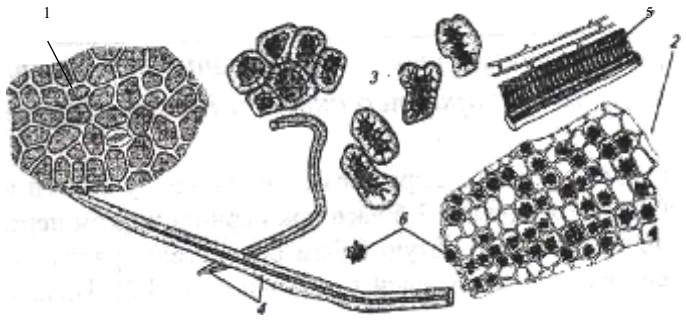
MP	1. Rosa canina 2. Rosa cinnamomea	
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Standardization by the content of BAS		*Ascorbic acid*

Macroscopic analysis of dog rose fruits:

whole, cut, ground or pulverized		number, shape, and size of seeds	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	

Microscopic analysis of dog rose fruits

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6.

Use in medicine _____

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Sample 2. Common nettle leaves

	Latin name	**English name**
MPM		
MP		
Family		

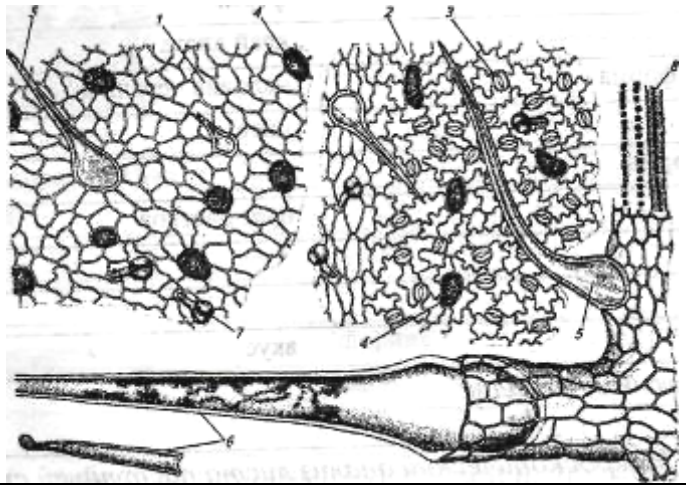
Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		

Basic group of BAS, %		
Standardization by the content of BAS		*Vitamin K*

Macroscopic analysis of common nettle leaves:

whole, cut, ground or pulverized		type of venation	
shape of leaf		leaf protuberance	
leaf blade division		size of a leaf blade and petiole	
attachment of leaf to stem, presence of petiole		color of upper surface of a leaf blade	
leaf base		color of lower surface of a leaf blade	
leaf apex		odour when crushed	
leaf edge		taste	

Microscopic analysis of common nettle leaves

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6. 7. 8.

Use in medicine _____

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Sample 3. Shepherd's purse herb

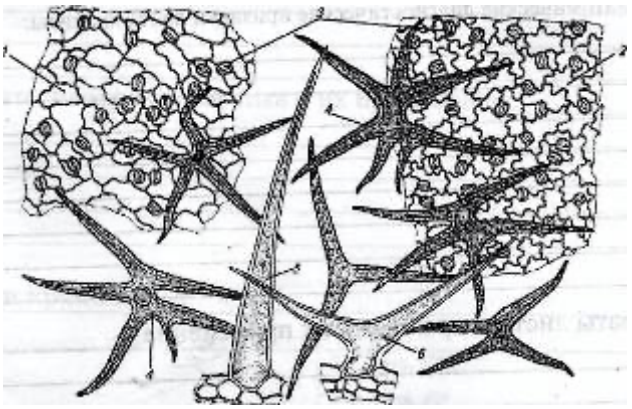
	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Standardization by the content of BAS	

Macroscopic analysis of shepherd's purse herb:

whole, cut, ground or pulverized		flower size	
form of stem in cross section		flower colour	
surface of stem		leaf pubescence	
stem colour		odour when crushed	
location of flowers on the stem, type of inflorescence		taste	
specific characteristics			
basal leaves: type		shape	
shape		edge	
edge		colour	
colour		presence of petiole	
presence of petiole		top leaves: shape	
stem leaves: type		colour	

Microscopic analysis of of shepherd's purse herb

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6.

Use in medicine _____

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Sample 4. Maize (corn) silks

	Latin name	**English name**
MPM		

MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Standardization by the content of BAS	

Macroscopic analysis of maize (corn) silks:

whole, cut, ground or pulverized	
shape	
size	
colour	
pubescence on silks	
odour when crushed	
taste	

Use in medicine _____
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Sample 5. Calendula flowers

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	

Storage conditions	
Basic group of BAS, %	
Standardization by the content of BAS	

Macroscopic analysis of calendula flowers:

whole, cut, ground or pulverized		shape of calyx	
type of inflorescence		colour of calyx	
pedicle, cm		shape of corolla	
type of perianth		colour of corolla	
symmetry		odour when crushed	
dimensions		taste	

Use in medicine _____
_____
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Sample 6. Sea-buckthorn fruits

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Standardization by the content of BAS	

Macroscopic analysis of sea-buckthorn fruits:

whole, cut, ground or pulverized		number, shape, and size of seeds	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	

Use in medicine _____

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III. Macro- and microscopic analysis of MPM containing organic acids

Sample 1. Raspberry fruits

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Standardization by the content of BAS	

Macroscopic analysis of raspberry fruits:

whole, cut, ground or pulverized		number, shape, and size of seeds	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	

Use in medicine _____

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Sample 2. Hibiscus flowers

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	

Storage conditions	
Basic group of BAS, %	
Standardization by the content of BAS	

Macroscopic analysis of hibiscus flowers:

whole, cut, ground or pulverized		shape of calyx	
type of inflorescence		colour of calyx	
pedicle, cm		shape of corolla	
type of perianth		colour of corolla	
symmetry		odour when crushed	
dimensions		taste	

Use in medicine _____

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Sample 3. **Barberry fruits**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	

Macroscopic analysis of barberry fruits:

whole, cut, ground or pulverized		number, shape, and size of seeds	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	

Use in medicine _____

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Sample 4. Pomegranate fruits

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	

Macroscopic analysis of pomegranate fruits:

whole, cut, ground or pulverized		number, shape, and size of seeds	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	

Use in medicine _____
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Sample 5. Grape berries

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	

Macroscopic analysis of vine fruits:

whole, cut, ground or pulverized		number, shape, and size of seeds	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	
whole, cut, ground or pulverized		amount of seeds	

Use in medicine _____
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IV. Macro- and microscopic analysis of MPM containing silicates

Sample 6. Common horsetail herb

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Standardization by the content of BAS	

Use in medicine _____
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Signature of teacher_____

INDEPENDENT STUDENTS WORK

Sample 1. Blackcurrant leaves

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Standardization by the content of BAS	

Use in medicine _____

_____

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Sample 2. Blackcurrant fruits

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 3. Pumpkin fruits

	Latin name	English name
MPM		
MP		

Family		

Use in medicine _____
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Sample 4. Carrot roots

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____
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Sample 5. Primrose leaves

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____
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Sample 6. Citrus fruits

	Latin name	English name
MPM		
MP		

Family		

Use in medicine _____
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Signature of teacher_____

TOPIC: PROTEINS AND ENZYMES

Aim: to establish the identity of MPM containing proteins and enzymes according to macroscopic and microscopic characteristics, and to determine the qualitative composition and quantitative content of BAS using methods of phytochemical analysis.
Objects for laboratory work: common mistletoe shoots, love-in-a-mist seeds, papaya fruits, pineapple fruits, watermelon seeds.
Objects for independent study: alfalfa herb, soybean seeds, kiwi fruits, mumiyo.

I. Phytochemical analysis of MPM containing proteins and enzymes

Task 1. Do an extraction of lectins (proteins) from MPM.
Method: Preparation of lectin-containing extract

Lectin-containing extract is prepared by weighing a 1 g sample of plant material which is then transferred into a porcelain mortar. 10 ml of physiological sodium chloride solution (0.9 %) is added and the plant material is ground for 5 min to attain a homogeneous state; this is filtered through a double-layer gauze filter and centrifuged at 3000 rpm for 15 min. Lectin activity is then tested using the supernatant that contained the lectin extract.

Task 2. Determinating agglutinating activity

Method: A 0.1 ml erythrocyte suspension is added to a vial dispenser with 5 ml of saline solution and shaken gently. The reaction of hemagglutination is performed on an immunological plate with U-shaped apertures. In each of the 8 holes of the vertical row, 0.05 ml of buffered saline solution was added (consisting of 1 l of water, 8 g sodium chloride, 0.2 g potassium chloride and 1.0 g of disodium phosphate). The solution is adjusted to pH 7.4 using 1N HCl solution.

In order to avoid unacceptable artifacts related to inadequate removal of citrate blood plasma and to hemolysis of erythrocytes. Make a control to find the spontaneous deposit of washed erythrocytes. Fort this control do not inject lectin-containing extract into the test-system; instead double the amount of saline solution to 0.1 ml with 0.05 ml of the suspension of rat erythrocytes (into a single vertical row of holes). To prepare a series of successive two-fold dilutions of lectin-containing extract, 0.05 ml of lectin-containing extract is added in the first hole of the above-mentioned vertical row, stirred, and 0.05 ml is collected, which is then transferred to the next hole, number 2, stirred and 0.05 ml is collected, and then transferred to the following hole and so on, until reached hole number 8, where 0.05 ml is also removed and discarded. Then, 0.05 ml of erythrocyte suspension is added to each hole and then the test system is left standing for 60 – 90 min at 25 °C. This testing of lectin substances from the plant extract iss carried out three times.

Conclusions _____

II. Macro- and microscopic analysis of MPM containing proteins and enzymes

Sample 1. Common mistletoe shoots

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	

Type of life form	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of common mistletoe shoots:

whole, cut, ground or pulverized		location of leaves on shoots	
surface of branches		shape of leaf	
colour of branches		leaf colour	
shape of branches		leaf edge	
type for branching		venation	
character of nodes		odour when crushed	

Use in medicine _____

_____

_____

Sample 2. **Love-in-a-mist seeds**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of love-in-a-mist seeds:

whole, cut, ground or pulverized	
shape	
surface	

colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____
_____
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Sample 3. **Watermelon seeds**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of watermelon seeds:

whole, cut, ground or pulverized	
shape	
surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____
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_____
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Sample 4. **Papaya fruits**

	Latin name	**English name**
MPM		

MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of papaya fruits:

whole, cut, ground or pulverized		amount of seeds	
type of fruit		size and shape of seeds	
shape		colour	
surface		odour when crushed	
presence of seeds		taste	

Use in medicine _____
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_____
_____

Signature of teacher_____

INDEPENDENT STUDENTS WORK

Sample 1. Alfalfa herb

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	

Basic group of BAS, %	
Other substances	

Use in medicine _____
_____
_____

Sample 2. Soybean seeds

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	

Use in medicine _____
_____
_____

Sample 3. Kiwi fruits

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	

Use in medicine _____
_____
_____

Signature of teacher_____

TOPIC: PHARMACEUTICAL RAW MATERIALS OF ANIMAL ORIGIN

Aim: to analyze raw material of animal origin, its chemical composition, mechanism of action, and use in medicine.
Objects for laboratory work: bee products: bee pollen, honey, wax, beebread, royal jelly, propolis**.** Snake and bee venom. Leech, antler velvet, freshwater sponge (Spongilla lacustris**)**, lanolin, cod liver oil.
Objects for independent study: spirulina, vitreous body**,** sea horse.

Task 1. Please describe the medicinal raw material of animal origin as outlined below.
Sample 1. Bee pollen

Latin name	
Mechanism of formation	
Description	
Composition	
Storage rules	
Biological action and use in medicine	

Sample 2. Bee wax (yellow and white)

Latin name	
Mechanism of formation	
Description	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 3. Beebread

Latin name	
Mechanism of formation	

Description	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 4. Honey

Latin name	
Mechanism of formation	
Description	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Establishment of honey quality

Name of reaction	Methods	Observation
Reaction for hydroxymethylfurfural		
Selivanov's reaction	In a mortar mix 5 g of honey rubbed with some ether. The ether solution is filtered into a cup, evaporated to dry consistency, then 2-3 drops of freshly made solution of resorcinol in concentrated hydrochloric acid is added to the residue.	
Podobedov-Molish's test	Add 1-2 drops of 10% alcohol solution of α-naphthol and 4-6 drops of conc. H_2SO_4 to 1 cm^3 of honey. (Work very carefully.)	
Conclusions:		
Reaction for a sugar syrup		
With silver nitrate solution	Add a few drops of silver nitrate solution to 5 ml of 5-10% aqueous solution of honey.	
With lead acetate solution	Add 2.5 g of lead acetate solution and 22.5 ml of methanol to 5 ml of 20% aqueous solution of honey.	

Conclusions:		
Reaction for starch syrup		
With ethanol	Add 1/4 volume of 96% ethanol to 1 part of honey mixed with 2-3 parts of water, and shake.	
With tartaric alcohol solution	Add 2 drops of concentrated hydrochloric acid and 20 ml of 95% tartaric alcohol to 2 ml of solution of 1 part of honey and 2 parts of water.	
Conclusions:		
Reaction to determinine presence of diastase		
With starch solution	To 10 ml of an aqueous solution of honey (1:2), add a few drops of 1% solution of starch. Shake and place the mixture for 1 h in a water bath with a temperature of 45°C, then cool and add 1-2 drops of iodine solution.	
Conclusions:		
Reaction for gelatin		
With tannin solution	Add 5-10 drops of 5% tannin solution to 5 ml of aqueous solution of honey (1:2).	
Conclusions:		

Sample 5. Propolis

Latin name	
Mechanism of formation	
Description	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 6. Bee venom

Latin name	
How obtained	
Description	

Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 7. Royal jelly

Latin name	
Mechanism of formation	
Description	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 8. Snake venom

Latin name	
Classification by the toxic effect on the human body and by the source	
How obtained	
Description	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 9. Leeches

Latin name	
Systematic affiliation (type, class) and description	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 10. Antler velvet

Latin name	
Source and how obtained	
Description	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 11. Freshwater sponge (Spongilla lacustris)

Latin name	
Systematic affiliation (type, class) and description	
How obtained	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Signature of teacher_____

INDEPENDENT STUDENTS WORK

Sample 1. Spirulina

Latin name	
Systematic affiliation (type, class) and discription	
How obtained	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 2. Vitreous body

Latin name	
How obtained	
Discription	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Sample 3. Sea horse

Latin name	
Systematic affiliation (type, class) and discription	
How obtained	
Composition (main components)	
Storage rules	
Biological action and use in medicine	

Signature of teacher_____

TOPIC: TERPENOIDS. IRIDOIDS

Aim: learn to establish the identity of MPM containing iridoids according to macroscopic and microscopic characteristics, and to determine the qualitative composition and quantitative content of BAS using methods of phytochemical analysis
Objects for laboratory work: gentian roots, buckbean leaves, dandelion roots, high bush cranberry bark, hop strobiles.
Objects for independent study: valeriana roots, centaury herb, species of plantain,
Structural formulas of main BAS: cyclopentane pyrane, aucubin, catalpol, loganin, sveroside, gentiopicroside, valtrate, dihydrovaltrate.

I. Phytochemical analysis of MPM containing iridoids

Task 1. Fill an Appendix 2 on the topic of the lesson.
Task 2. Contuct the selection of iridoids from a MPM.

Place 0.5 g of coarsely powdered plant material in a glass conical flask, add 15 ml of 96% alcohol. Attach a reflux condenser to the flask and boil on a hotplate for 20 min. Folter solution through a paper filter. The filtrate is used for the quality reactions and chromatographic analysis.

Task 3. Conduct quality reactions for iridoids.

Name of reaction	Methods	Observation
Stahl's reaction	place 2 ml of the obtained extract in the test tube. Add 0.5 ml of Stahl's reagent; heat on a water bath for 1-2 minutes.	
Trim-Hill's reaction	place 2 ml of the obtained extract in the test tube. Add 0.5 ml of Trim-Hill's reagent; heat on a water bath for 1-2 minutes.	
Conclusions:		

Task 4. Carry out the chromatographic determination of iridoids in a plant extract (SPhU 2.0).

Test solution. 10 ml of methanol is added to 1.0 g of raw materials crushed into powder, heated with shaking in a water bath at a temperature of 60°C for 5 min, cooled and filtered. Evaporate to dryness under reduced pressure in a water bath at a temperature of 60°C. The obtained residue is dissolved in 2.0 ml of methanol. Comparison solution. 5 mg of loganin is dissolved in 15 ml of methanol.

Plate: TLC plate with a layer of silica gel R.

Mobile phase: water P - methanol P - ethyl acetate P (8:15:77).

Sample volume: 30 µl, strips.

Distance to be covered by the moving phase: 15 cm from the starting line.

Drying: in the air.

Detection: spray vanillin with reagent P, heat it at a temperature of (100-105)°C for 10 minutes and examine it in daylight.

Results: Below is the sequence of zones on the chromatograms of the reference solution and the test solution. Other zones may also be detected on the chromatogram of the tested solution.

The upper part of the plate	
Loganin: grayish-purple zone	purple zone intense blue zone **zone from purple to grayish-purple color** zone from gray to grayish-blue color brown zone
Comparison solution	Test solution

II. Macro- and microscopic analysis MPM containing iridoids

Sample 1. **Gentian root**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*gentiopicroside*

Macroscopic analysis of gentian roots:

whole, cut, ground or pulverized	
shape	
surface	
fracture character	

presence of core	
colour on fracture	
colour of external surface	
dimension	
taste	

Reaction of sublimation

Methods	Observation	Conclusions
Place the powder of gentian root into a test tube and heat on a spirit lamp		

Use in medicine _____

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Sample 2. Buckbean leaves

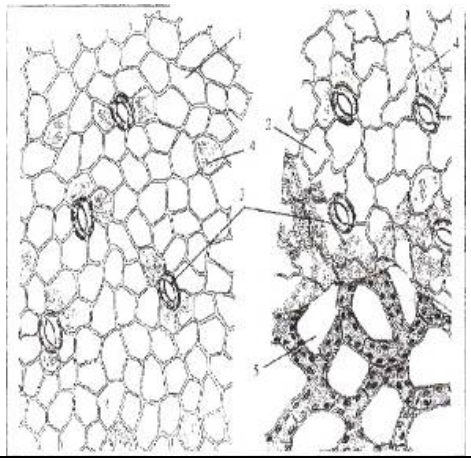
	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*sveroside*

Macroscopic analysis of buckbean leaves:

whole, cut, ground or pulverized		leaf edge	
shape of leaf		type of venation	
character of leaf blade		leaf pubescence	
leaf blade division		dimensions of a leaf blade and a petiole	
attaching a leaf to a stem, the presence of petiole		colour of upper and lower surface of a leaf blade	
leaf base		odour when crushed	
leaf apex		taste	

Microscopic analysis of buckbean leaves

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5.

Use in medicine _____

_____

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Sample 3. **High bush cranberry bark**

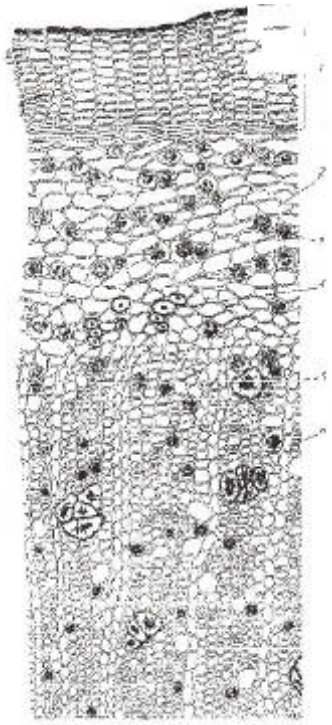
	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of high bush cranberry bark:

shape		color of inner surface	
external surface		characteristic of fracture	
inner surface		taste	
colour of external surface		odour	

Microscopic analysis of high bush cranberry bark

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6.

Use in medicine _____

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Sample 4. **Dandelion root**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of dandelion root:

whole, cut, ground or pulverized		colour of fracture	
shape		color of external surface	
surface		dimensions	
characteristic of fracture		taste	
presence of core		odour	

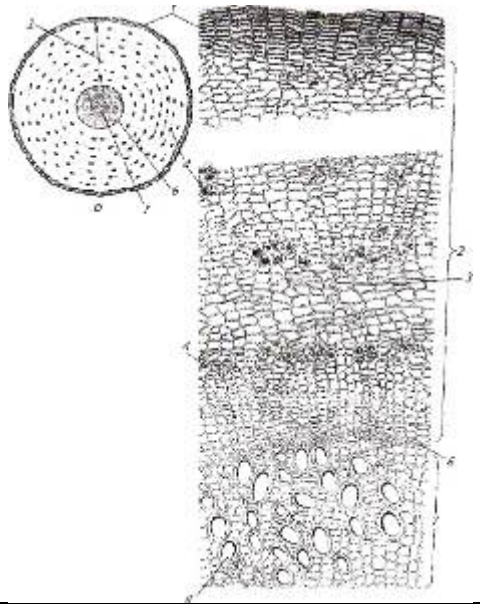
Adulteration (English and Latine names):

1. _____
2. _____

Histochemical reactions

Name of reaction	Methods	Observation
Reaction with iodine solution	add a drop of iodine solution on a fracture of dandelion root	
Reaction with α-naphthol	add a drop of 20 % α-naphthol alcoholic solution and a drop of sulfuric acid concebtrated on a fracture of dandelion root	
Conclusions:		

Microscopic analysis of dandelion root

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6. 7. 8.

Use in medicine _____

_____

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Sample 5. Hops strobiles

	Latin name	English name
MPM		
MP		

Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of hops strobiles:

whole, cut, ground or pulverized		**specific characteristics**	
type of infructescence		type of fruit	
shape		character of rod	
surface			
presence of seeds and its' number		flake colour	
dimensions		colour of flakes tip	
colour			
odour when crushed		glands presence	
taste		colour of glands	

Use in medicine _____

_____

_____

Signature of teacher_____

INDEPENDENT STUDENTS WORK

Sample 1. Valerian rhizomes with roots

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting term		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*Valtrate and dihydrovaltrate*

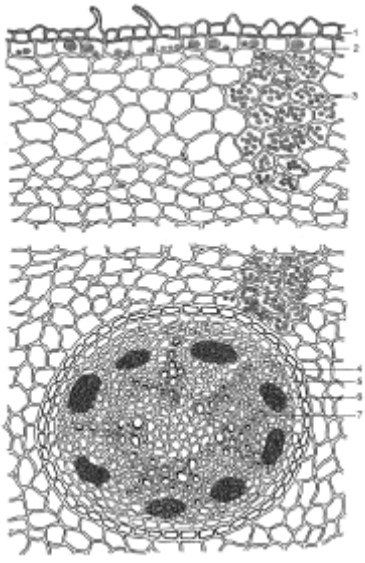
Macroscopic analysis of valerian roots:

whole, cut, ground or pulverized		characteristic of core	
shape		colour of fracture	
surface		color of external surface	
characteristic of fracture		dimensions	
		odour	
presence of core		taste	

Adulteration (English and Latine names):

1. _____
2. _____

Microscopic analysis of valerian roots

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6. 7.

Use in medicine _____

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_____

Sample 2. Centaury herb

	Latin name	**English name**
MPM		

MP		
Family		

Use in medicine _____
_____
_____

Sample 3. Common plantain leaves

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____
_____
_____

Signature of teacher_____

TOPIC: VOLATILE OILS

Aim: to establish the identity of MPM containin volatile oils according to macroscopic and microscopic characteristics, and to determine the qualitative composition and quantitative content of BAS using methods of phytochemical analysis.

Objects for laboratory work: *monoterpenoids***:** coriander fruits, lemon balm herb, peppermint leaves, sage leaves, eucalyptus leaves, valerian rhizomes with roots, juniper berries (fruits); *sesquiterpenoids*: calamus rhizome, elecampane rhizomes and roots, matricaria flowers, roman chamomile flowers, wormwood herb, yarrow herb, marsh Labrador tea herb*; aromatic compounds*: aniseed, fennel fruits, common thyme herb, wild thyme herb, oregano herb

Objects for independent study: *monoterpenoids***:** lavender flowers, caraway fruits, rosemary leaves; *sesquiterpenoids*: arnica flowers, ginger rhizomes, silver birch buds; *; aromatic compounds:* star anise friuts, cinnamon bark, clove flower buds

Structural formulas of main BAS: citral, linalool, menthol, cineole, camphor, borneol, pinen, chamazulene, alantolactone, thymol, anethole, vanillin, carvacrol

I. Phytochemical analysis of MPM containing volatile oils

Task 1. Fill in Appendix 2 on the topic of this lesson.

Task 2. Determine the content of volatile oils in MPM:

Method: Place 10 g of powdered plant material in a glass round-bottom flask with a capacity of 1000 ml. Add 300 ml of water and shake. Place the receiver (calibrated in 0.025 ml) on the upper part of flask. The receiver has a bent tube with diameter of 0.5-2 cm. The knee of the tube is bent down. The flask with MPM is heated to boiling and maintained at low boil for a time that is specified in the documentation of the MPM. Water vapor and volatile oils condense in the condenser, and the liquid flows into the receiver. Oil settles into the graduated knee of the receiver tube, and water flows back into the flask. After distillation and cooling, the volume of volatile oils is measured, and the content in the MPM is determined by the following:

1. Content of volatile oils in terms of weight by volume (X), for air-dry raw material, is calculated by using the formula:

$$X = \frac{A \times 100}{B}$$

where: A is the volume of volatile oil, ml; B is the mass of plant material, g.

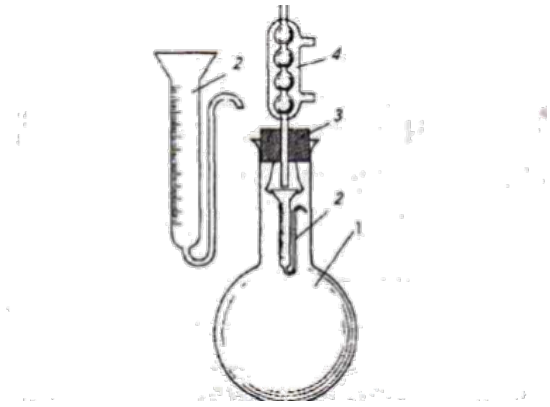
2. The mass fraction % is obtained when X is multiplied by the density of the volatile oil.

3. Content of volatile oils in terms of weight by volume (X), for absolutely dry raw materia,l is calculated by using the formula:

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

where: V is the volume of volatile oil, ml; m is the mass of plant material, g; W is the loss in weight when drying the plant material, %.

The apparatus for determination of content of volatile oils:

	Note the main parts of apparatus: 1. 2. 3. 4.

Task 3. Conduct the organoleptic analysis of volatile oil compared to standard:

Name of reaction	Methods	Observation
Colour and transparency	Place 10 ml of volatile oil in a cylinder 2-3 cm in diameter and observe the colour and transparency.	
Odour	Put 2 drops of oil on a strip of filter paper 12 cm long and 5 cm wide; try not to moisten paper edges. Compare odour of the tested oil and the standard every 15 min. After 1 h the smell of the tested oil and the standard must be identical.	
Taste	Mix 1 drop of volatile oil and 1 g of sugar and taste it.	
Solubility in alcohol	Put 1 ml of volatile oil in a graduated cylinder with capacity of 10 ml and add 0.1 ml of alcohol of certain concentration (indicated in the stardard), adding very gradually from the burette with careful shaking at 20^0C, until there is complete dissolution of the oil.	
Content of water	Mix 10 drops of volatile oil and 1 ml of carbon disulfide.	
Content of fatty oil and resins	Put 1 drop of volatile oil on a strip of filter paper and leave for 2 h.	
Content of foreign ester	Mix 1 ml of volatile oil and 3 ml of possassium hydroxide solution in alcohol and heat in a water bath for 2 min.	
Conclusions:		

Task 4. Determine the purity of a volatile oil sample (content of alcohol, fatty and mineral oils):

Name of reaction	Methods	Observation
Alcohol	1) Put 2 drops of volatile oil on water in a watch glass.	
	2) Put 1 ml of volatile oil in a test tube and plug with cotton; then put a crystal of fuchsine into the cotton and heat test tube until boiling.	
Fatty and mineral oils	Shake 1 ml of volatile oil and 10 ml of alcohol together in a test tube.	
Conclusions:		

Task 5. Determine the physical indexes of volatile oil (index of refraction):

Name of reaction	Methods	Observation
Index of refraction	The index of refraction is determined by using a refractometer. Before every test the refractometer must be checked by distilled water, which has an index of refraction n=1.3330 at 20^0C. The refractometer has two prisms, one of which is raised. To prepare the measurement, put 2 drops of oil on the lower prism, then lower the upper prism and press firmly. Looking through the eyepiece, connect the boundaries of light and dark areas of the field. The index of refraction is measured by the scale on the side of the light.	n=

Task 6. Determination the chemical quality indexes of a sample of volatile oil sample (see Appendix 4):

1. *Determination of acid number.*

Method: Dissolve 10 g of volatle oil sample in 50 ml of alcohol which is preliminarily neutralized by potassium hydroxide (0.1 mole/l) solution. 0.5 ml of phenolphthalein solution is used as the indicator. After dissolving the sample, the solution is titrated by potassium hydroxide solution (0.1 mole/l) until the pink colour appears and does not vanish after 15 sec.

The acid number is calculated using the formula:

$$I_A = 5.61 \cdot n/m,$$

where: n is the amount of 0.1 M potassium hydroxide that is used for titration, ml; m is the mass of oil, g; 5.61 is the amount of potassium hydroxide contained in 1 ml of 0.1 M potassium hydroxide solution, mg.

Conclusions:_____

2. *Determination of ether number.*

Method: The ether number can be found once the acid number has been determined. Add 20 ml of 0.5 mole/l potassium hydroxide solution to the previous solution (before titration), and heat in a flask with backflow condenser in a water bath for 1 h. After the end of saponification, dilute the solution by 100 ml of water. The excess of potassium hydroxide is titrated by 0.5 mole/l sulphuric acid (indicator is phenolphthalein). The control experiment (without oil) is conducted simultaneously.

The ether number is calculated using the formula:

$$I_E = 28.05 \cdot (V - V_1)/m,$$

where: V_1 is the volume of potassium hydroxide solution used for saponification of ethers, ml; V is the volume of 0.5 mole/l potassium hydroxide solution used for titration in the control experiment, ml; m is the mass of oil, g; 28.05 is the amount of potassium hydroxide solution contained in 1 ml of 0.5 mole/l potassium hydroxide solution, mg.

Conclusions:_____

3. Determination of hydroxylic number:

Method: Place the oil (for exact weight see Table 1.4) into a 150 ml flask. Add the volume of solution of acetic anhydride as shown in Table 1.4. Connect the flask to a backflow condenser and heat gently in the water bath for 1 h, keeping water level in the bath to 2.5 cm above the level of liquid in the flask.

Then through the top end of the condenser add 5 ml of water. If the solution becomes cloudy, stir in pyridine until the turbidity disappears; measure the volume. Place the flask in a boiling water bath for 10 min, then cool to room temperature. Wash the condenser and the wall of the flask with 5 ml of alcohol, previously neutralized using phenolphthalein solution.

Table 1.4

The choice of sample to determine the hydroxyl number

The value of expected hydroxyde number	Mass of sample, g	Volume of acetic anhydride, ml
10-100	2.0	5.0
100-150	1.5	5.0
150-200	1.0	5.0
200-250	0.75	5.0
250-300	0.60 or 1.20	5.0 or 10.0
300-350	1.0	10.0
350-700	0.75	15.0
700-950	0.5	15.0

The resulting solution is titrated with alcoholic solution of potassium hydroxide 0.5 mole/l, using 0.2 ml of phenolphthalein solution. Carry out the control test (without the oil) in the same way.

The hydroxyl number (I_{OH}) is calculated using the formula:

$$I_{OH} = 28.05 \cdot (n_2 - n_1)/m + I_A,$$

where: n1 is the volume of potassium hydroxide solution 0.5 mole/l used for titrating the examined solution, ml; n2 is the volume of potassium hydroxide 0.5 mole/l used for titrating the control test, ml; m is the mass of oil, g; 28.05 is the amount of potassium hydroxide contained in 1 ml of 0.5 mole/l solution of potassium hydroxide, mg; and IA is the acid number.

Conclusions:_____

Task 7. Conduct qualitative reactions on components of volatile oils:

Name of reaction	Methods	Observation
Reactions for aldehydes and ketones		
Formation of oximes	Add 3 drops of hydroxylamine chloride alcohol solution (15 g of hydroxylamine chloride in 100 ml of 80% alcohol) to 2 drops of volatile oil and some drops of methyl orange.	
Nitroprusside test	Mix 5-10 drops of volatile oil with the same amount of sodium nitroprusside solution and with 3 drops of 5% alkali solution.	
Conclusions:		
Reaction for phenols		
Reaction with iron III chloride	Add 3-4 drops of iron III chloride solution to 1 ml of concentrated alcohol solution of volatile oil.	
Reaction of formation of azodyes	Add 3-4 ml of 25% sodium hydroxide solution solution to 1 ml of volatile oil. Then add 1-2 drops of diazotized sulfanilic acid.	
Conclusions:		
Reactions for azulenogens		
Ehrlich-Mueller reaction	Mix 5 drops of volatile oil with 1 ml of reagent and heat on a water bath.	
Reaction with bromine	Dissolve 5-10 drops of volatile oil in 1-2 ml of chloroform and add by drops 0.1-1 ml of 5% solution of bromine in chloroform.	
Conclusions:		

II. Macro and microscopic analysis of MPM containing monoterpenoids

Sample 1. Coriander fruits

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*linalool*

Macroscopic analysis of coriander fruits:

whole, cut, ground or pulverized		dimensions	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	
specific characteristics			
shape of inner side of mericarp		shape of external side of mericarp	
number of ribs		shape of ribs	
character of surface		features of top of fruit	

Use in medicine _____

_____

_____

Sample 2. Lemon balm herb

	Latin name	English name
MPM		
MP		

Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*citral*

Macroscopic analysis of lemon balm herb:

whole, cut, ground or pulverized		leaf size	
stem shape in cross section		leaf colour	
stem diameter		location of flowers on the stem, inflorescence	
stem colour		flower dimensions	
shape of leaf		flower colour	
presence of petiole		pubescence	
leaf edge		odour when crushed	
type of venation		taste	
specific characteristics			
calyx: shape		corolla: structure	
length		shape of upper lip	
number of teeth on sepals		number of stamens	

Use in medicine _____

_____

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Sample 3. **Peppermint leaves**

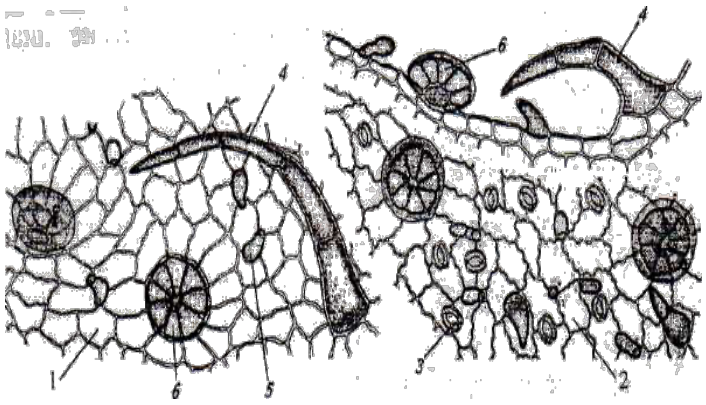
	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*menthol*

Macroscopic analysis of peppermint leaves:

whole, cut, ground or pulverized		type of venation	
shape of leaf		leaf pubecsence	
leaf blade division		size of a leaf blade and petiole	
attachment of leaf to stem, presence of petiole		colour of upper surface of leaf blade	
leaf base		colour of lower surface of leaf blade	
leaf apex		odour when crushed	
leaf edge		taste	
specific characteristics (under magnifying glass)			
presence of glands		colour of glands	

Microscopic analysis of peppermint leaves



The main diagnostic microscopic features of MPM:

1.

2.

3.

4.

5.

6.

Use in medicine _____

_____

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Sample 4. Sage leaves

	Latin name	**English name**
MPM		

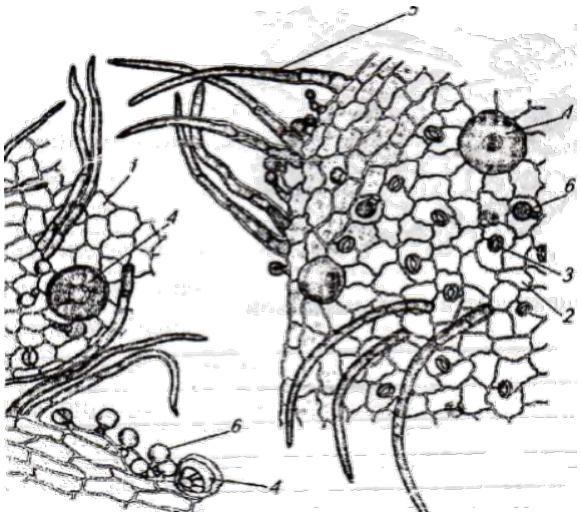
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*1,4-cineole*

Macroscopic analysis of sage leaves:

whole, cut, ground or pulverized		type of venation	
leaf blade division		size of leaf blade and petiole	
attaching a leaf to stem, presence of petiole		colour of upper and lower surface of a leaf blade	
leaf base		leaf pubescense	
leaf apex		odour when crushed	
leaf edge		taste	
specific characteristics			
shape of top leaves		shape of lower leaves	

Microscopic analysis of sage leaves

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6.

Use in medicine _____

_____

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Sample 5. Eucalyptus leaves

	Latin name	English name
MPM		

The name of source plants:

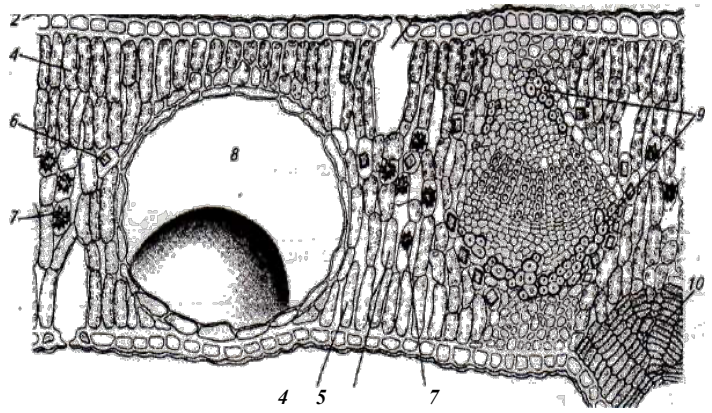
MP	1. Eucalyptus globulus 2. Eucalyptus viminalis 3. Eucalyptus cinerea	
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		
		1,8-cineole

Macroscopic analysis of eucalyptus leaves:

whole, cut, ground or pulverized		type of venation	
adult leaves: shape		leaf pubescense	
leaf blade division		size of leaf blade and petiole	
attachment of leaf to stem, presence of petiole		colour of upper surface	
leaf base		colour of lower surface	
leaf apex		odour when crushed	
leaf edge		taste	
specific characteristics			
dark spots -		leaf apex	
light spots -		leaf edge	
juvenile leaves: shape		colour of upper surface	
attachment of leaf to stem, presence of petiole		colour of lower surface	
leaf base		leaf pubescense	

Microscopic analysis of eucalyptus leaves

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6. 7. 8. 9. 10.

Use in medicine _____

_____

_____

Sample 6. Valerian rhizomes with roots

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting term	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of valerian rhizomes with roots:

whole, cut, ground or pulverized		characteristic of core	
shape		colour of fracture surface	
surface		colour of external surface	
characreristic of fracture		dimensions	
		odour	
presence of core		taste	

Adulteration (English and Latine names):

1. _____
2. _____

Use in medicine _____

_____

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Sample 7. Juniper berries (fruits)

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*pinen*

Macroscopic analysis of juniper berry (fruits):

whole, cut, ground or pulverized		dimensions	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	
specific characteristics			
colour of epicuticular wax bloom		dimensions	
colour of flesh		colour	
seeds: number		essential oil cavities: number	
shape		location	
characteristic of skin			

Use in medicine _____

_____

_____

Signature of teacher_____

III. Macro- and microscopic analysis of MPM, which contain sesquiterpenoids and sesquiterpene lactones

Sample 8. Calamus rhizome

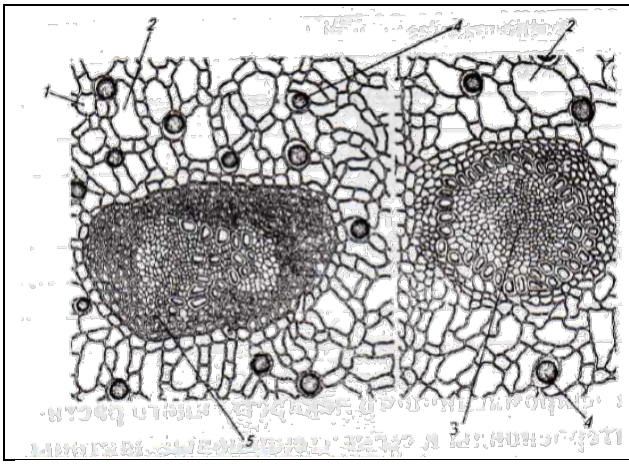
	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of calamus rhizomes:

whole, cut, ground or pulverized		colour of fracture	
shape		colour of external surface	
type of surface		dimensions	
characteristic of fracture		taste	
presence of core		odour	
specific characteristics			
character of upper surface		character of lower surface	

Microscopic analysis of calamus rhizomes

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5.

Adulteration (English and Latine names):

1. _____
2. _____

Use in medicine _____

_____

_____

Sample 9. Elecampane rhizomes and roots

	Latin name	**English name**
MPM		
MP		
Family		

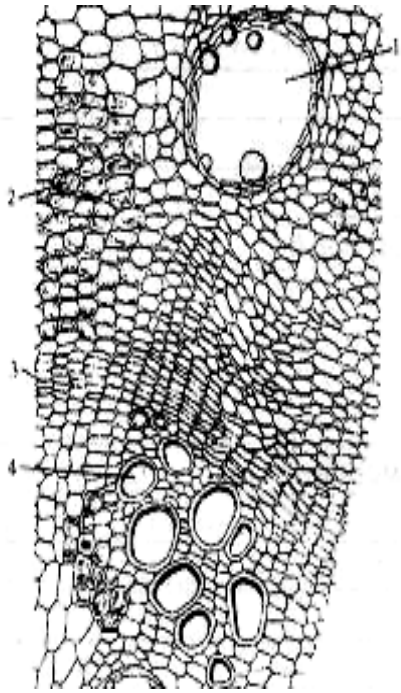
Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*alantolactone*

Macroscopic analysis of elecampane rhizomes and roots:

whole, cut, ground or pulverized		colour of fracture	
shape		colour of external surface	
surface		dimensions	

characteristic of fracture		taste	
presence of core		odour	
specific characteristics			
essential oil cavity location		colour of essential oil cavity	

Microscopic analysis of elecampane rhizomes and roots

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4.

Use in medicine _____

_____

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Sample 10. Matricaria flowers

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		

Other substances		
Standardization by the content of BAS		*chamazulene*

Macroscopic analysis of matricaria flowers:

whole, cut, ground or pulverized		shape of corolla	
type of inflorescence		dimensions	
pedicle, см		colour of flower parts	
type of perianth		odour when crushed	
symmetry		taste	
shape of calyx			
specific characteristics			
inforescence: shape		character of inner part	
dimensions		involucre: structure	
receptacle: form		charecteristics of phyllary	
character of surface			
Draw receptacle of chamomile and its cross section			

Adulteration (English and Latine names):

1._____

2. _____

Use in medicine _____

_____

_____

Sample 11. **Roman chamomile flowers**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		

Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*chamazulene*

Macroscopic analysis of roman chamomile flowers:

whole, cut, ground or pulverized		shape of corolla	
type of inflorescence		dimensions	
pedicle, см		colour of flower parts	
type of perianth		odour when crushed	
symmetry		taste	
shape of calyx			
specific characteristics			
inforescence: shape		character of inner part	
dimensions		involucre: structure	
receptacle: form		charecteristics of phyllary	
character of surface			

Use in medicine _____

_____

_____

Sample 12. Wormwood herb

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	

Other substances	
Standardization by the content of BAS	

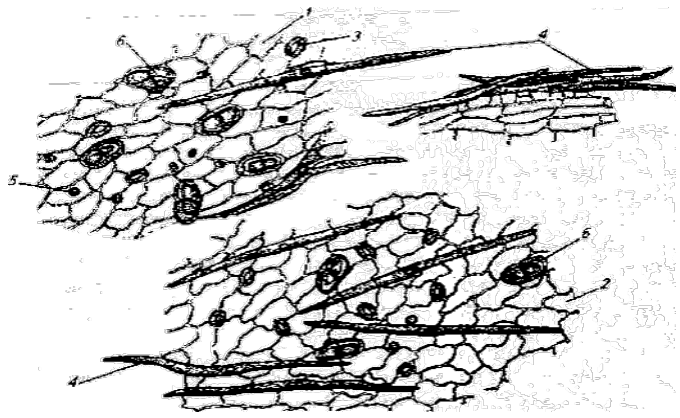
Macroscopic analysis of wormwood herb:

whole, cut, ground or pulverized		**specific characteristics**
stem: shape of cross section		lower leaves: type
dimensions		the presence of petiole
colour		shape
upper leaves: type		colour of lower surface
shape		colour upper surface
attaching a leaf to a stem, the presence of petiole		inflorescence: type
leaf edge		receptacle: shape
leaf dimensions		characteristic of surface
leaf colour		disc flowers
flower: location of flowers on the stem		ray flowers
dimensions		spathe: phyllaries location
colour		external spathe
flower pubescense		inner spathe
odour when crushed		
taste		

Adulteration (English and Latin names):

1. _____
2. _____

Microscopic analysis of wormwood herb

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6.

Use in medicine _____

_____

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Sample 13. Common yarrow herb

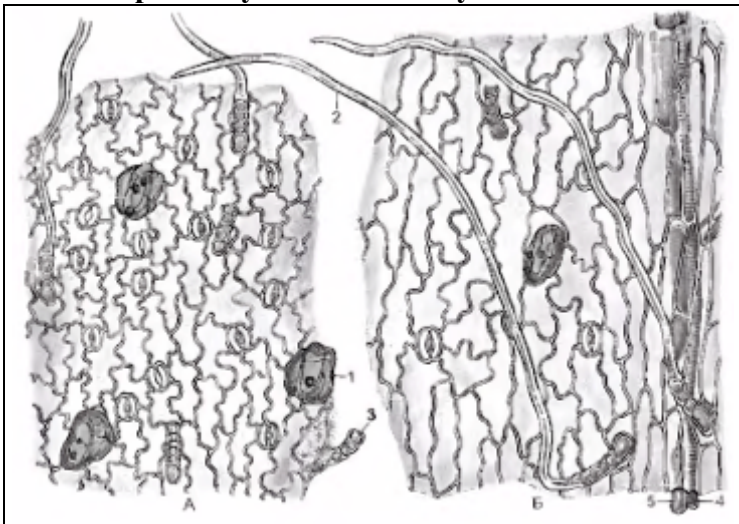
	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of common yarrow herb:

whole, cut, ground or pulverized		leaf dimensions	
stem shape		leaf colour	
stem diameter		location of flowers on the stem, inflorescence	
stem colour		flower dimensions	
shape of leaf		flower colour	
the presence of petiole		flower pubescence	
leaf edge		smell when crushed	
type of venation		taste	
specific characteristics			
inflorescence: shape		presence of bracts	
spathe: shape		ray flowers: type	
edge		disc flowers: type	

Microscopic analysis of common yarrow herb

	The main diagnostic microscopic features of MPM: A. B. 1. 2. 3. 4. 5.

Use in medicine _____

_____

_____

Sample 14. Marsh Labrador tea herb

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*ledol*

Macroscopic analysis of Marsh Labrador tea herb:

whole, cut, ground or pulverized		leaf dimensions	
stem shape		leaf colour	
stem diameter		location of flowers on the stem, inflorescence	
stem colour		flower dimensions	
shape of leaf		flower colour	

attaching a leaf to a stem		flower pubescense	
leaf edge		odour when crushed	
type of venation		taste	

Use in medicine _____

_____

_____

IV. Macro- and microscopic analysis of MPM containing aromatic compounds

Sample 15. Aniseed fruits

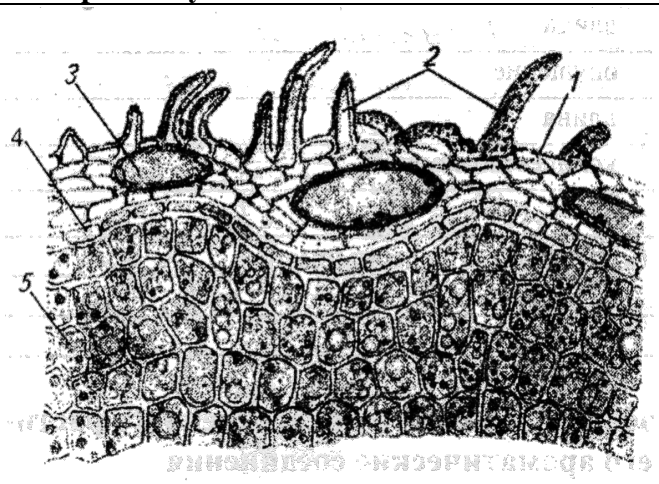
	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*anethole*

Macroscopic analysis of aniseeds fruits:

whole, cut, ground or pulverized		dimensions	
type of fruit		colour	
shape		odour when crushed	
type of surface		taste	
specific characteristics			
shape of inner part of mericarp		shape of external part of mericarp	
number of ridges		shape of ridges	

Microscopic analysis of aniseeds fruits

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5.

Use in medicine _____
_____
_____

Sample 16. Fennel fruits

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*fenchone*

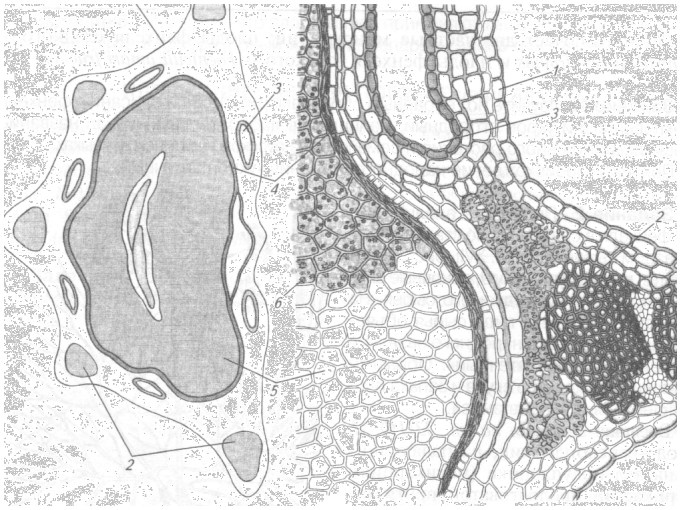
Adulteration (English and Latine names):
1._____
2. _____

Macroscopic analysis of fennel fruits:

whole, cut, ground or pulverized		dimensions	

type of fruit		colour	
shape		odour when crushed	
type of surface		taste	
specific characteristics			
shape of inner part of mericarp		shape of external part of mericarp	
number of ridges		shape of ridges	

Microscopic analysis of fennel fruits



The main diagnostic microscopic features of MPM:

1.
2.
3.
4.
5.
6.

Use in medicine _____

_____

_____

Sample 17. Common thyme herb

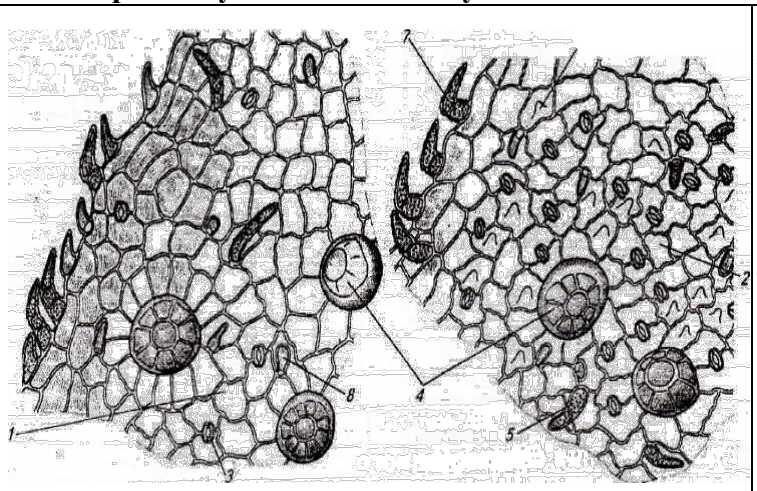
	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*thymol*

Macroscopic analysis of common thyme herb:

whole, cut, ground or pulverized		leaf dimensions	
stem cross section		leaf colour	
stem diameter		location of flowers on the stem, inflorescence	
stem colour		flower dimensions	
shape of leaf		flower colour	
the presence of petiole		flower pubescense	
leaf edge		odour when crushed	
type ofvenation		taste	
specific characteristics			
calyx: type		under magnifing glass:	
number of teeth		glands presence	
corolla: type		colour of glands	

Microscopic analysis of common thyme



The main diagnostic microscopic features of MPM:

1.
2.
3.
4.
5.
6.
7.
8.

Use in medicine _____

_____

_____

_____

Sample 18. Wild thyme herb

	Latin name	**English name**
MPM		
MP		
Family		

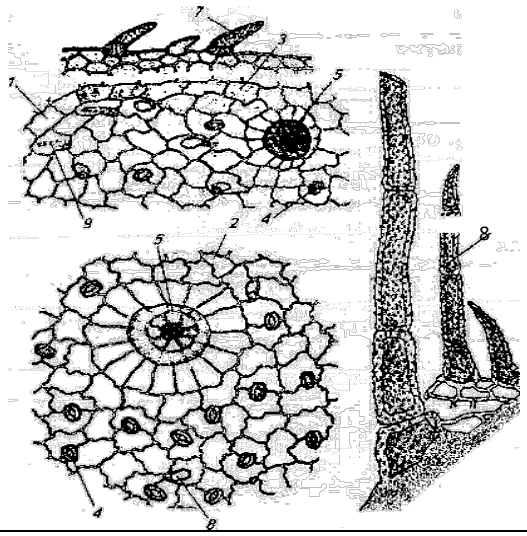
Dissemination of MP		*Structural formula*

Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*thymol*

Macroscopic analysis of wild thyme herb:

whole, cut, ground or pulverized		leaf pubecsence	
stem shape in cross section		leaf dimensions	
stem diameter		leaf colour	
stem colour		location of flowers on the stem, inflorescence	
shape of leaf		flower dimensions	
the presence of petiole		flower colour	
leaf edge		odour when crushed	
type of venation		taste	
specific characteristics			
calyx: type		under magnifing glass: presence of glands	
amount of teeth			
egde of teeth		colour of glands	
corolla: type		trichomes in a base of leaves	

Microscopic analysis of wild thyme herb



The main diagnostic microscopic features of MPM:

1.
2.
3.
4.
5.
6.
7.
8.

Use in medicine _____

_____

_____

Sample 19. Oregano herb

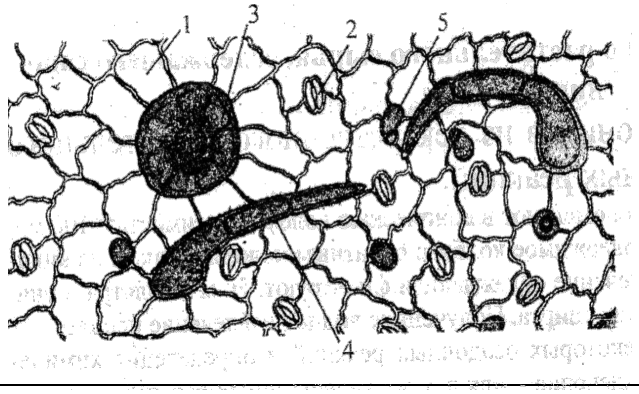
	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*carvacrol*

Macroscopic analysis of oregano herb:

whole, cut, ground or pulverized		leaf dimensions	
stem diameter		leaf colour	
stem shape in cross section		location of flowers on the stem, inflorescence	
stem colour		flower dimensions	
shape of leaf		flower colour	
attaching a leaf to a stem, the presence of petiole		flower pubescense	
leaf edge		odour when crushed	
type of venation		taste	
specific characteristics			
bracts: presence		shape of bracts	
top of bracts		colour of bracts	

Microscopic analysis of oregano herb

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5.

Use in medicine _____

_____

_____

Signature of teacher_____

INDEPENDENT STUDENTS WORK

MPM, which contain monoterpenoids

Sample 1. Lavender flowers

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

_____

_____

Sample 2. Caraway fruits

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	

Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

_____

_____

Sample 3. **Rosemary leaves**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

_____

_____

MPM, which contain sesquiterpenoids

Sample 4. **Arnica flowers**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

_____

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Sample 5. **Ginger rhizomes**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of ginger rhizomes:

shape		colour of fracture	
type of surface		colour of external surface	
characteristic of fracture		dimensions	
		odour	
presence of core		taste	

Use in medicine _____

_____

_____

Sample 6. Silver birch buds

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*betulin*

Macroscopic analysis of silver birch buds:

whole, cut, ground or pulverized		length	
shape		width	
type of top		colour	
location of scales		odour when crushed	
		taste	

Use in medicine _____

_____

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MPM, which contain arimatic compounds

Sample 7. Star anise friuts

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	

Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of star anise fruits:

whole, cut, ground or pulverized		**specific characteristics**
type of fruit		number of carpels location of carpels colour or seeds
shape		
surface		
number of seeds		
dimensions		
colour		
odour when crushed		
taste		

Use in medicine _____

_____

_____

Sample 8. Clove flower buds

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*eugenol*

Use in medicine _____

_____

_____

Sample 9. **Cinnamon bark**

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*cinnamaldehyde*

Use in medicine _____

_____

_____

Signature of teacher_____

TOPIC: TRITERPENOIDS. STEROIDS. SAPONINS.

Aim: to establish the identity of MPM containing saponins according to macroscopic and microscopic characteristics, and to determine the qualitative composition and quantitative content of BAS using methods of phytochemical analysis.
Objects for laboratory work: liquorice roots, horse chestnut seeds, ginseng roots, Japanese angelica tree roots, russian milkvetch herb, java tea leaves, yam rhizomes and roots.
Objects for independent study: common horsetail herb, jacob's ladder rhizomes and roots, fenugreek seeds, bindii herb (Tribulus terrestris).
Structural formulas of main BAS: lanostane, cycloartane, dammarane, lupane, ursane, oleonane, α-amyrin, β- amyrin, sterane.

I. Phytochemical analysis of MPM containing saponins

Task 1. Fill in Appendix 2 for the topic of this lesson.
Task 2. Determine the content of saponins in MPM using qualitative reactions.

Method: Place 5.0 g of powdered MPM in a 100 ml glass conical flask, and add 50 ml of 50% alcohol; heat with a reflux condenser in water bath for 15 min. Cool and filter the water-alcohol extract through paper filter. 20 ml of the extract is evaporated to 10 ml to remove alcohol. This water extract is used to test for foam formation, some precipitation reactions, and the detection of the chemical nature of saponins. The water-alcohol extract is used for other qualitative reactions and for chromatographic analysis.

Task 3. Conduct qualitative reactions on saponins. Make conclusions about the chemical nature of saponins.

Name of reaction	Methods	Observation
Test for foam formation	Shake 2-3 ml of water extract in a test tube for 1 min.	
Reactions of precipitation		
With barium hydroxide	Add 3-4 drops of barium hydroxide solution to the water extract in a test tube.	
With lead acetate	Add 3-4 drops of 10% lead acetate solution to 1 ml of water extract in a test tube.	
With cholesterol solution	Add 1 ml of 1% cholesterol alcoholic solution to 1 ml of water-alcohol extract in a test tube.	
Colour reactions		
Lafon's reaction	Place 2 ml of water-alcohol extract in a test tube. Add 1 drop of 10% copper sulfate solution and 1 ml of concentrated sulfuric acid; heat carefully.	
Salkovsky test	Place 2 ml of water-alcohol extract in a test tube. Add 1 ml of chloroform and 5-6- drops of concentrated sulfuric acid.	
Reaction with antimony (V) chloride solution	Add 0.5 ml of saturated antimony (V) chloride solution in chloroform to 1 ml of water-alcohol extract in a test tube.	
Sanje's reaction	Place 2 ml of water-alcohol extract in a test tube. Add 1 ml of 0.5% alcohol vanillin solution, 3-4 drops of concentrated sulphuric acid and heat in water bath at temperature 60°C.	

Conclusions:		
Determination of chemical nature of saponins		
Foam formation test	Take 2 graduated test tubes with glass stoppers. Add 5 ml of 0.1 mole/l hydrochloric acid to one of them. Add 5 ml of 0.1 mole/l sodium hydroxide to the other. Add 0.5 ml of water extract to each test tube and shake them vigorously for 1 min.	
Conclusions:		

Task 4. Carry out the chromatographic analysis of saponins in MPM according to monograph SPhU (2.0 T.3) "Licorice root".

Test solution. Place 0.50 g of raw material crushed into powder in a round-bottom flask with a capacity of 50 ml, add 16.0 ml of water and 4.0 ml of hydrochloric acid, heat in a water bath with a reflux condenser for 30 minutes, cool and filter. The filter and the round-bottomed flask are dried at a temperature of 105 °C for 60 min. Place the filter in a round-bottom flask, add 20.0 ml of ether, heat in a water bath at a temperature of 40 °C with a reflux condenser for 5 min, cool and filter. The obtained filtrate is evaporated to dryness, the residue is dissolved in 5.0 ml of ether P.

Comparison solution. 5.0 mg of glycyrrhetinic acid and 5.0 mg of thymol are dissolved in 5.0 ml of ether.

Plate: TLC plate with a layer of silica gel *F2S4* P.

Mobile phase: concentrated ammonia solution - water - ethanol (96%) - ethyl acetate (1:9:25:65).

Sample volume: 10 µl, in strips. Distance to be covered by the moving phase: 15 cm from the starting line.

Drying: in the air for 5 minutes.

Detection A: viewed in UV light at a wavelength of 254 nm.

Results A: the chromatograms of the test solution and the reference solution should show an absorption zone corresponding to glycyrrhetinic acid in the lower half.
Detection B: spray anisaldehyde solution, heat at a temperature of (100-105)°C for (5-10) minutes and view in daylight.

Results B: the chromatogram of the comparison solution should show: a purple zone in the lower half. corresponding to glycyrrhetinic acid, in the upper third — a red zone corresponding to thymol. The chromatogram of the test solution should show: in the lower half — a purple zone corresponding to the zone of glycyrrhetinic acid on the chromatogram of the comparison solution, and a yellow zone (isoliquiridigenin) — in the upper third below the thymol zone on the chromatogram of the comparison solution. Other zones may also be detected.

The upper part of the plate	
Red zone **(thymol)** **Purple zone** **(glycyrrhetinic acid)**	**Yellow zone** **(isoliquiritigenin)** **Purple zone** **(glycyrrhetinic acid)**
Comparison solution	**The tested solution**

Task 5. Determine the foam index for the MPM.
Classify the tested MPM into one of three groups by the value of foam index: over 5000 – a high foam index; 2000-5000 – middle foam index, less then 2000 – low foam index.

Method. Heat MPM in a drying box; grind it and sift through sieve 355. Make 1% water infusion from 1.0 g of MPM. Put 10 ml of infusion into a graduated cylinder with a stopper. Shake the cylinder containing the infusion for 15 sec.
Find the minimum concentration of the infusion which can make a foam that is stable for 1 min.

Calculation:

II. Macro- and microscopic analysis MPM containing saponins

Sample 1. Liquorice roots

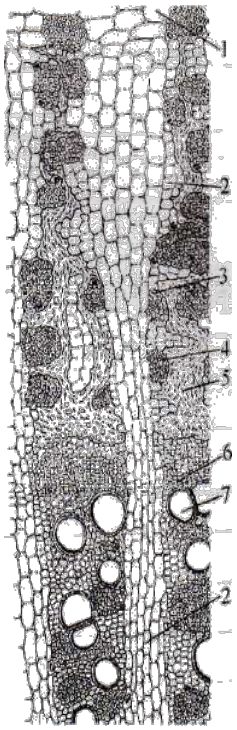
	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*glycyrrhizinic acid*

Macroscopic analysis of liquorice roots:

whole, cut, ground or pulverized		colour of fracture	
shape		colour of external surface	
type of surface		dimensions	
characteristic of fracture		odour	
presence of core		taste	

Microscopic analysis of liquorice roots



The main diagnostic microscopic features of MPM:

1.

2.

3.

4.

5.

6.

7.

Use in medicine _____

_____

_____

Sample 2. Horse chestnut seeds

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of horse chestnut seeds:

whole, cut, ground or pulverized	
shape	
type of surface	
colour	
dimensions	
odour when crushed	
taste	

Use in medicine _____
_____
_____
_____

Sample 3. Ginseng roots

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*panaxoside A*

Macroscopic analysis ginseng roots:

whole, cut, ground or pulverized		**specific characteristics**
shape		presence of branching
type of surface		
characteristic of fracture		characteristic of thickening
presence of core		location of thickening
colour of fracture		
colour of external surface		features of top
dimensions		rhizome
taste		
odour		

Use in medicine _____

_____

_____

Sample 4. Japanese angelica-tree roots

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the		

content of BAS		*araloside*

Macroscopic analysis of japanese angelica-tree roots:

whole, cut, ground or pulverized		colour of fracture	
shape		colour of external surface	
type of surface		dimensions	
characteristic of fracture		odour	
presence of core		taste	

Use in medicine _____

_____

_____

Sample 5. Russian milkvetch herb

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of russian milkvetch herb:

whole, cut, ground or pulverized		leaf dimensions	
stem shape in cross section		leaf colour	
stem diameter		location of flowers on the stem, type of inflorescence	
stem colour		flower dimensions	
shape of leaf		flower colour	
attachment of leaf to stem, presence of petiole		flower pubescence	
leaf edge		odour when crushed	

type of venation		taste	
specific characteristics			
type of inflorescence		length of flower spike	
location of inflorescence			

Adulteration (English and Latin names):
1._____
2. _____

Use in medicine _____
_____
_____

Sample 6. Java tea leaves

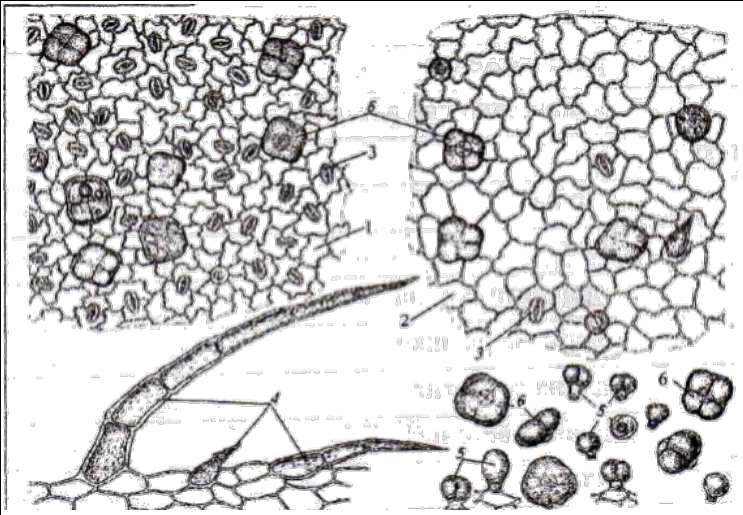
	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of java tea leaves:

whole, cut, ground, or pulverized		leaf edge	
shape of leaf		type of venation	
leaf blade division		leaf pubescence	
attachment of leaf to stem, presence of petiole		size of leaf blade and petiole	
leaf base		colour of upper and lower surface of leaf blade	
leaf apex		odour when crushed	

Microscopic analysis of java tea leaves:

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6.

Use in medicine _____

_____

_____

Sample 7. Yam rhizomes and roots

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of yam rhizomes and roots:

whole, cut, ground or pulverized		colour of fracture surface	
shape		colour of external surface	
type of surface		dimensions	

characteristic of fracture		odour	
presence of core		taste	

Use in medicine _____

_____

_____

Signature of teacher _____

INDEPENDENT STUDENTS WORK

Sample 1. Common horsetail herb

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

_____

_____

Sample 2. Jacob's ladder rhizomes and roots

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	

Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

_____

_____

Sample 3. Fenugreek seeds

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

_____

_____

Sample 4. Bindii herb

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____

_____

_____

Signature of teacher _____

TOPIC: CARDIAC GLYCOSIDES

Aim: to establish the identity of MPM containing cardiac glycosides according to macroscopic and microscopic characteristics, and to determine the qualitative composition and quantitative content of BAS using methods of phytochemical analysis.

Objects for laboratory work: purple foxglove leaves (common foxglove), woolly foxglove leaves (Grecian foxglove), yellow foxglove leaves (big-flowered foxglove), strophanthus seeds, spring adonis (pheasant's eye) herb; lily-of-the-valley herb, flowers, and leaves; treacle-mustard herb.

Objects for independent study: hellebores, sea squill

Structural formulas of main BAS: cardenolide, bufadienolide, purpureaglycoside A, lanatosid A, k-Strophanthin, gitoxin, digitoxin, convallatoxin, adonitoxin, eryhroside

I. Phytochemical analysis of MPM containing cardiac glycosides

Task 1. Fill in Appendix 2 on the topic of this lesson.

Task 2. Do the extraction of cardiac glycosides from MPM for qualitative reactions**.**

Method. Place 5.0 g of powdered plant material in a 100 ml flask, add 50 ml of 80% alcohol and infuse for 24 h. Remove alcohol under vacuum; the water residue is removed using a separating funnel; and extract lipophylic substances by adding 10 ml of carbon tetrachloride 6 times. Then the water residue is processed by adding 10 ml of chloroform, 4 times. The chloroform fractions are combined, filtered through 2 g of anhydrous sodium sulfate, and used for quality reaction.

Task 3. Conduct qualitative reactions on cardiac glycosides.

These experiments must be carried out in a fume cupboard!

Name of reaction	Methods	Observation
Reactions on steroidal part of cardiac glycosides		
Liebermann-Burchard test	Dissolve some dry residue in 1 ml of acetic anhydride, put in a dry test tube and add carefully down the walls 2-3 drops of concentrated sulfuric acid.	
Rosenheim test	Add 1 ml of trichloroacetic acid in ethanol to 1 ml of the chloroform extract.	
Conclusions		
Reaction on the unsaturated γ-lactone ring		
Kedde reaction	Dissolve some dry residue in 2 ml of 3% 3,5-dinitrobenzoic acid solution and add 1 ml of sodium hydroxide solution (1 mole/l).	
Raymond reaction	Dissolve some dry residue in 1 ml of 3% m-dinitrobenzole in benzole and add 2 drops of potassium hydroxide alcohol solution.	
Legal's reaction	Dissolve some dry residue in 1 ml of 5% sodium nitroprusside solution and add 1 ml of 10% sodium hydroxide solution.	
Conclusions		
Reaction on glycoside part of cardiac glycosides		
Keller-Kiliani reaction	Dissolve some dry residue in 1 ml of acetic acid with traces of iron (III) sulfate, add down the walls of test tube 1 ml of concentrated sulfuric acid. The contents of the test tube should not be shaken!	

Fehling reaction	Add 0.5 ml of 1% of hydrochloric acid solution to 2 ml of obtained extract and heat in a water bath for 1 h. Then add some drops of 10% sodium hydroxide solution, and 1 ml of Fehling reagent, and heat in a water bath.	
Conclusions		

Task 4. Do the chromatographic analysis of cardiac glycosides by TLC method for the MPM foxglove.

Method: Place 1.0 g of powdered plant material in a glass conical flask, add 20 ml of 50% alcohol and 10 ml of 10% lead acetate solution; heat for 2 min, cool, and centrifuge. Decant using a separating funnel, add 20 ml of chloroform, and shake. To create a stable emulsion the mixture in the separating should be centrifuged.Separate the chloroform layer and filter it through a layer of anhydrous sodium sulfate. 10 ml of the filtrate is evaporated to dryness in a water bath. Dissolve the dry residue in 1 ml of a mixture of equal volumes of chloroform and methanol.

Put 20 µl of the obtained solution on the chromatographic plate as a line (2 x 0.3 cm). Chromatograph in this mixture of solvents—ethyl acetate: methanol: water (75:10:7.5). A mixture of 2 ml of 1% chloramine and 8 ml of 25% trichloracetic acid alcohol solution is used to process the chromatogram. After processing, heat the chromatogram at 105^0C for 5-10 min. Analyze in UV light at a wavelength of 365 nm.

Chromatographic analysis

Sketch of chromatogram	Spots	Rf	Colour of spots

Solvents system	Derivatizing reagent

II. Macro- and microscopic analysis of MPM containing cardiac glycosides.

MPM which contain cardenolides

Sample 1. **Purple foxglove leaves**

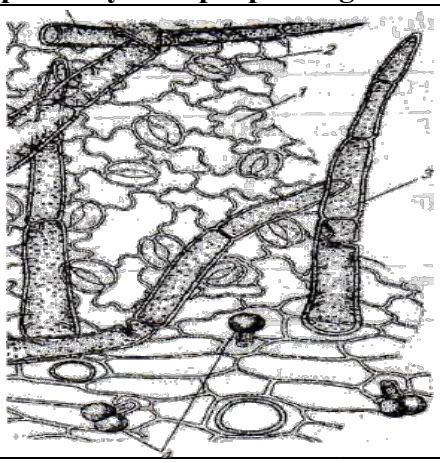
	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*digitoxin*

Macroscopic analysis of foxglove leaves:

whole, cut, ground or pulverized		leaf edge	
shape of leaf		type of venation	
character of leaf blade		leaf pubescense	
leaf blade division		dimensions of a leaf blade and a petiole	
attachment of leaf to stem, presence of petiole		colour of upper and lower surface of a leaf blade	
leaf base		odour when crushed	
leaf apex		taste	

Microscopic analysis of purple foxglove leaves:

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4.

Use in medicine _____

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Sample 2. Yellow foxglove leaves

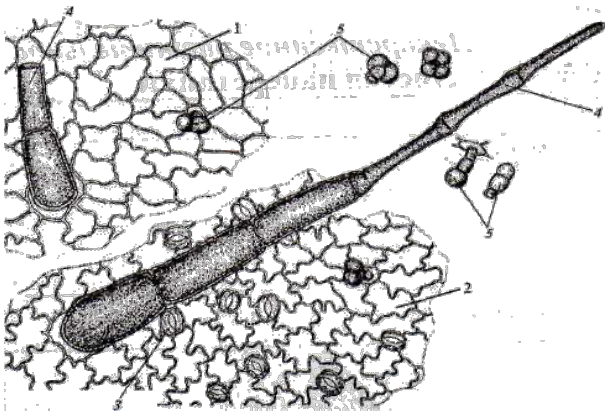
	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Macroscopic analysis of yellow foxglove leaves:

whole, cut, ground or pulverized		leaf edge	
shape of leaf		type of venation	
leaf blade division		leaf pubescense	
presence of petiole		dimensions of a leaf blade and a petiole	
leaf base		colour of upper and lower surface of a leaf blade	
leaf apex		odour when crushed	

Microscopic analysis of yellow foxglove leaves:

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5.

Use in medicine _____

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Sample 3. **Woolly foxglove leaves**

	Latin name	**English name**
MPM		
MP		
Family		

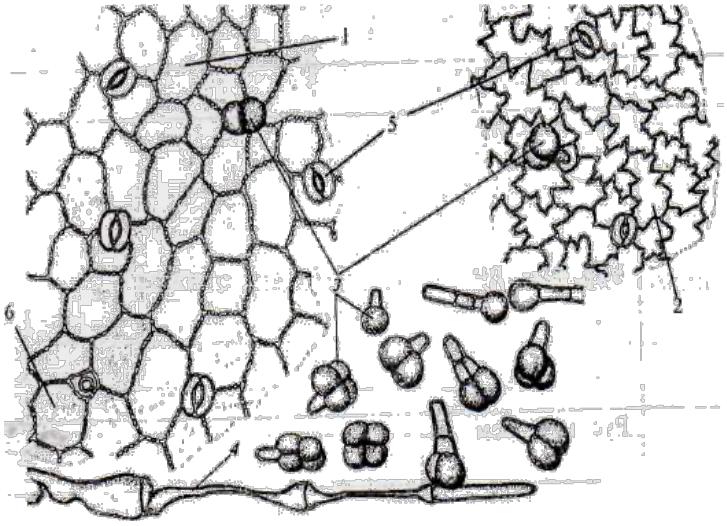
Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*gitoxin*

Macroscopic analysis of woolly foxglove leaves:

whole, cut, ground or pulverized		leaf edge	
shape of leaf		type of venation	
leaf blade division		leaf pubescense	
presence of petiole		dimensions of a leaf blade and a petiole	

leaf base		colour of upper and lower surface of a leaf blade	
leaf apex		odour when crushed	

Microscopic analysis of woolly foxglove leaves:

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6.

Use in medicine _____

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Sample 4. Strophanthus seeds

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*k-Strophanthin*

Macroscopic analysis of strophanthus seeds:

whole, cut, ground or pulverized		**specific characteristics**
shape		shape of upper end of seed features of upper end of seed feature of flat side
type of surface		
colour		
dimensions		
odour when crushed		
taste	**Don't, poisonous !**	

Use in medicine _____

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Sample 5. Lily-of-the-valley leaves

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*convallatoxin*

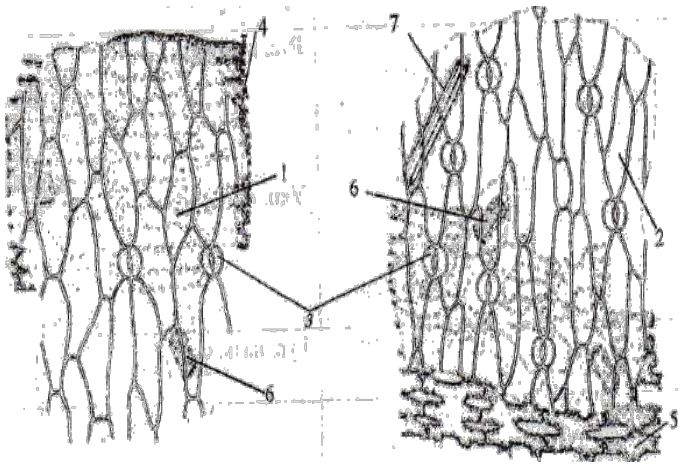
Macroscopic analysis of lily-of-the valley leaves:

whole, cut, ground or pulverized		leaf edge	
shape of leaf		type of venation	
leaf blade division		leaf pubescense	
presence of petiole		dimensions of a leaf blade and a petiole	
leaf base		colour of upper and lower surface of a leaf blade	
leaf apex		odour when crushed	

Adulteration (English and Latin names):

1. _____
2. _____

Microscopic analysis of lily-of-the-valley leaves:

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4. 5. 6. 7.

Use in medicine _____

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Sample 6. Lily-of-the-valley flowers

	Latin name	English name
MPM		
MP		
Family		

Macroscopic analysis of lily-of-the valley flowers:

whole, cut, ground or pulverized		shape of calyx	
type of inflorescence		shape of corolla	
pedicle, cm		dimensions	
type of perianth		colour of flower parts	
symmetry		odour when crushed	

Use in medicine _____

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Sample 7. Lily-of-the-valley herb

	Latin name	English name
MPM		
MP		
Family		

Use in medicine _____

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Sample 8. Spring adonis (pheasant's eye) herb

	Latin name	English name
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*adonitoxin*

Adulteration (English and Latin names):

1. _____
2. _____

Macroscopic analysis of spring adonis (pheasant's eye) herb:

whole, cut, ground or pulverized		**specific characteristics**	
stem shape in cross section		leaf: character of division	

stem diameter		character of division of lower part	
stem colour		character of division of upper part	
shape of leaf		shape of parts	
presence of petiole		shape of upper part	
leaf edge		edge of parts	
type of venation		flowers: number of sepals	
leaf dimensions		sepals: form	
leaf colour		shape of top	
location of flowers on the stem, inflorescence		number of petals	
flower dimensions		petals: shape	
flower colour		shape of top	
flower pubescense		shape of receptacle	
odour when crushed			

Use in medicine _____

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Sample 9. Treacle-mustard herb

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP		*Structural formula*
Harvesting time		
Drying conditions		
Storage conditions		
Basic group of BAS, %		
Other substances		
Standardization by the content of BAS		*eryhroside*

Macroscopic analysis of treacle-mustard herb:

whole, cut, ground or pulverized		leaf dimensions	
stem shape in cross section		leaf colour	
stem diameter		location of flowers on the stem, inflorescence	
stem colour		flower dimensions	
shape of leaf		flower colour	
attachment of leaf to stem, presence of petiole		flower pubescense	
leaf edge		odour when crushed	
type of venation		taste	
specific characteristics			
number of sepals		features of petals	
length of sepals		length of petals	
		number of stamens	

Microscopic analysis of treacle-mustard herb:

	The main diagnostic microscopic features of MPM: 1. 2. 3. 4.

Signature of teacher_____

INDEPENDENT STUDENTS WORK

MPM which contain bufadienolides

Sample 1. Hellebore rhizomes and roots

	Latin name	**English name**
MPM		
MP		

Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____
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Sample 2. Sea squill bulbus

	Latin name	**English name**
MPM		
MP		
Family		

Dissemination of MP	
Harvesting time	
Drying conditions	
Storage conditions	
Basic group of BAS, %	
Other substances	
Standardization by the content of BAS	

Use in medicine _____
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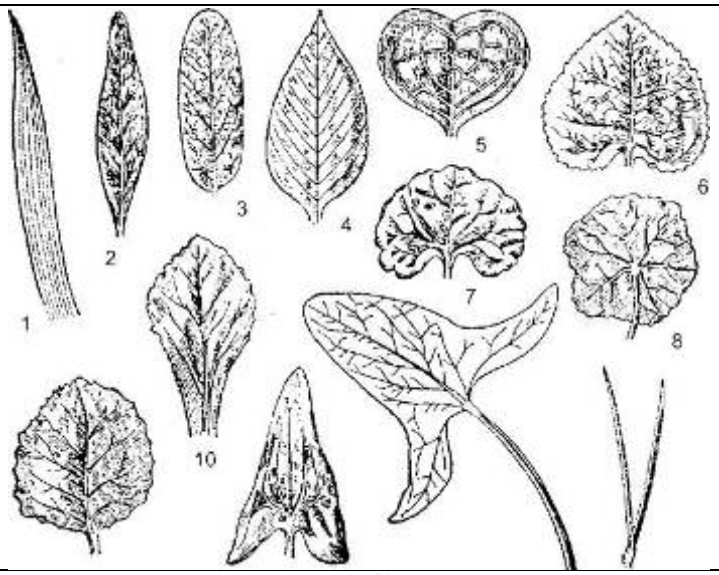
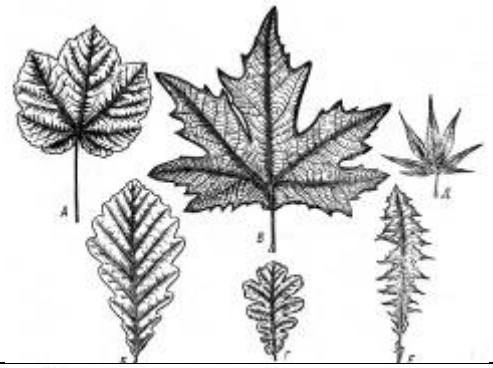
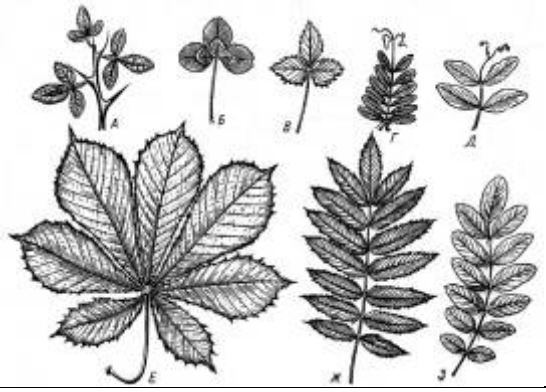
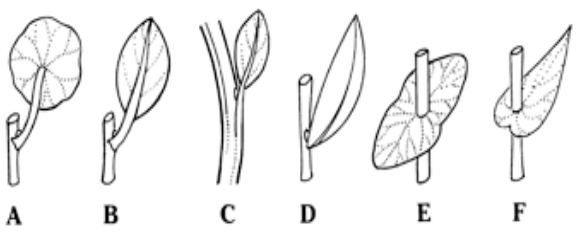
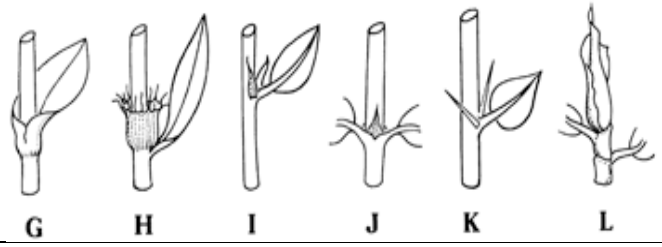
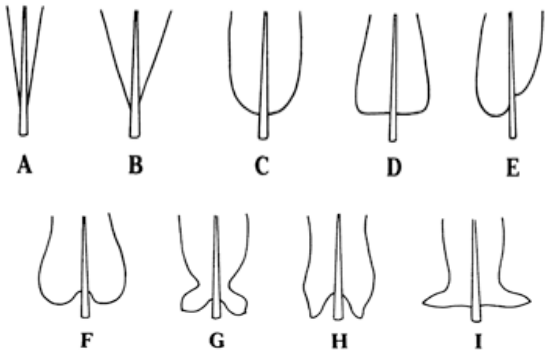
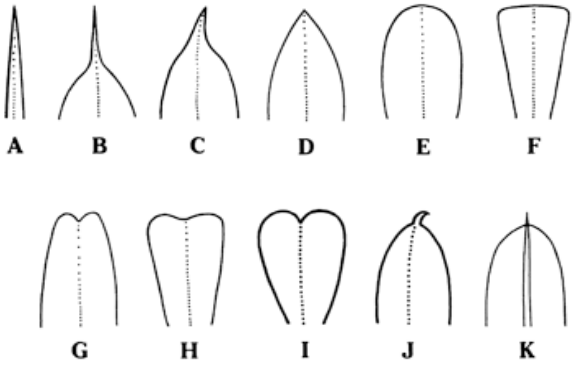
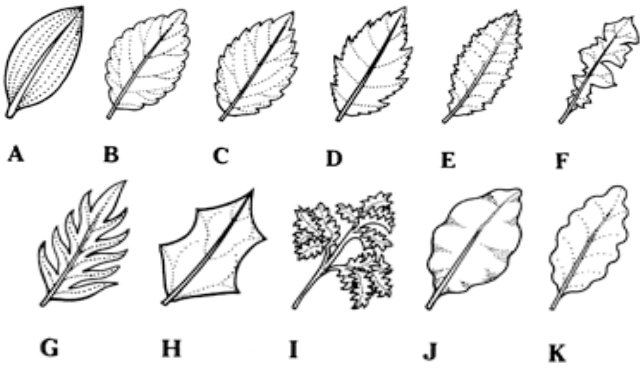
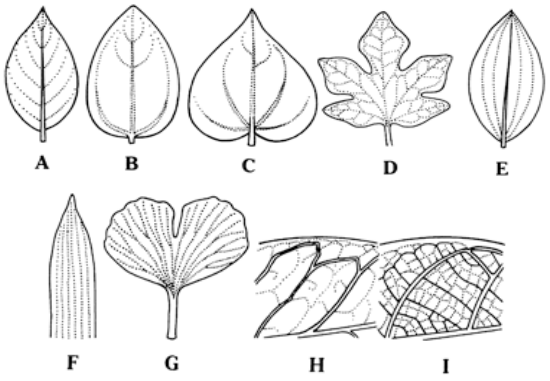
Signature of teacher_____

Appendix 1

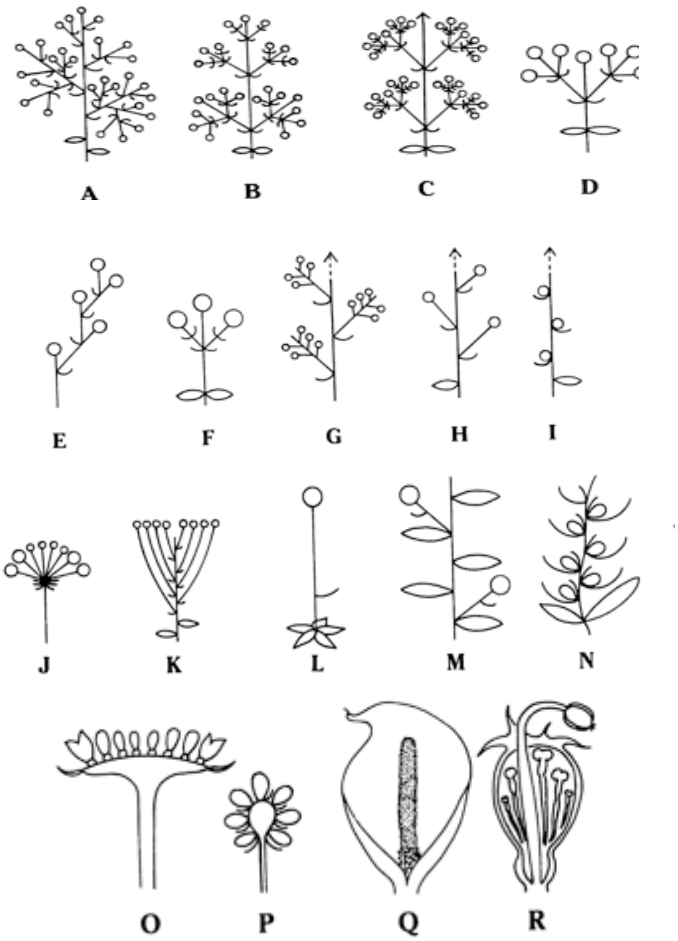
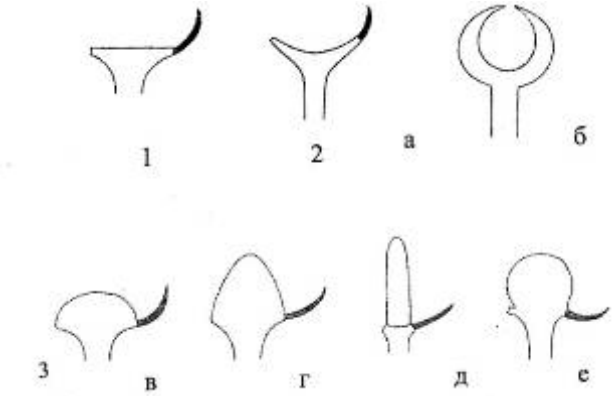
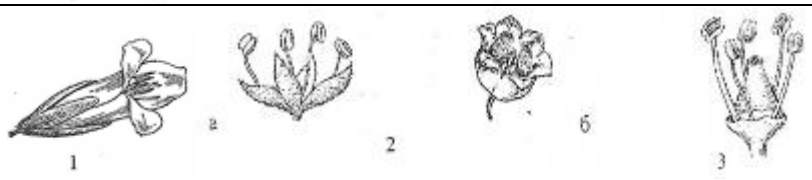
The morphological diagnostic features of MPM

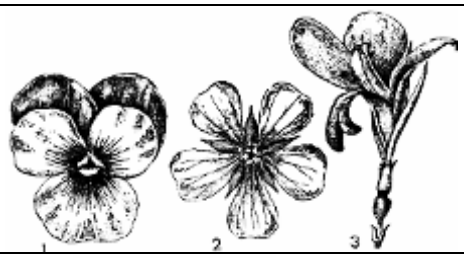
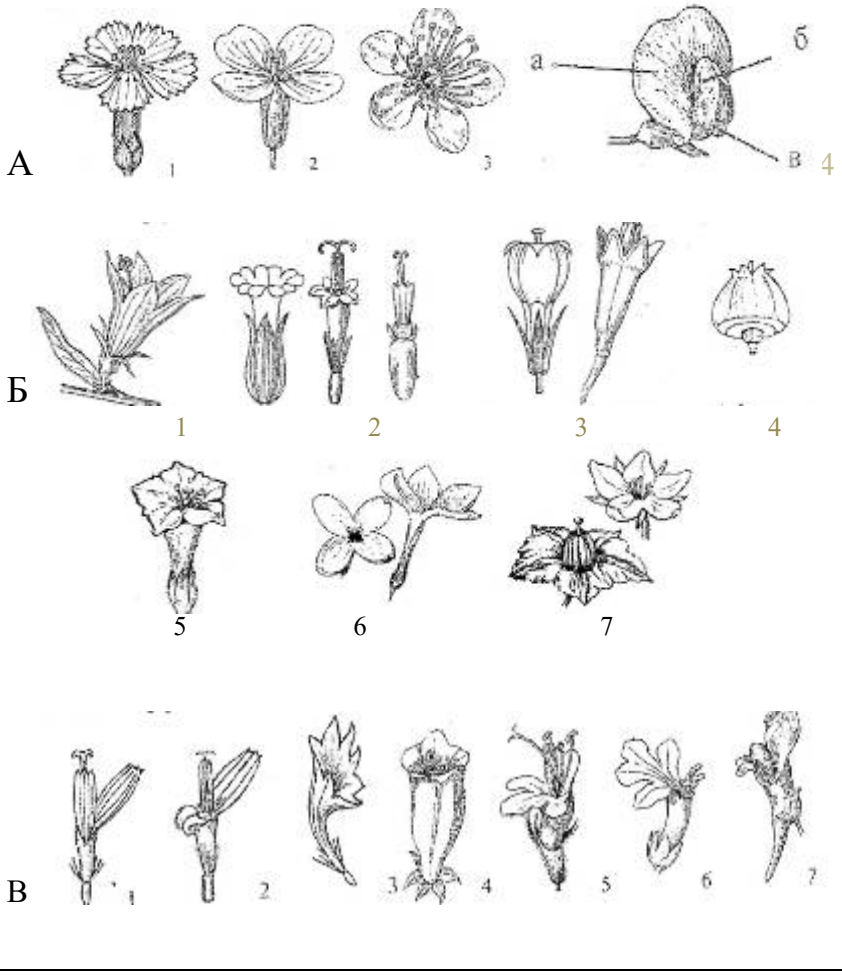
The morphological diagnostic features of leaf

Leaf shape: 1 – linear; 2 – lanceolate; 3 – elliptic; 4 – ovoid; 5 – obcordate; 6 – cordate; 7 – kidney-shaped; 8 – peltate; 9 – orbicular; 10 – spatulate; 11 – sagittate (or arrow-shaped); 12 – spear-shaped; 13 – needle-shaped (or acicular).	
Leaf blade division: А – palmatilobate; Б – pinnatilobate; В – palmatipartite; Г – pinnatipartite; Д – palmatisect; Е – pinnatisect.	
Compound leaves: А Б, В – trifoliolate; Г, Д – paripinnate compound; Е – palmately compound; Ж,З – odd-pinnately compound	
Lamina or Leaf Attachment, Stipules etc A, peltate; B, petiolate; C, decurrent; D, sessile; E, gamophyllous; F, perfoliate; G, sheathing; H, ocrea; I–L, stipules;	

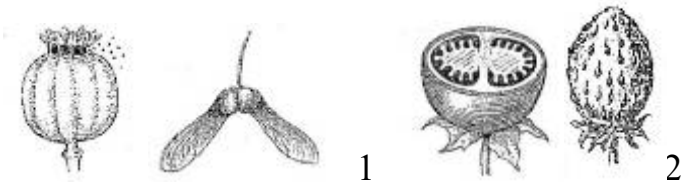
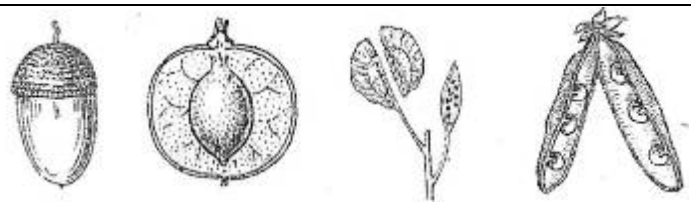
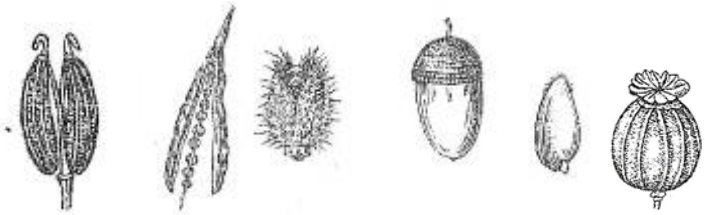
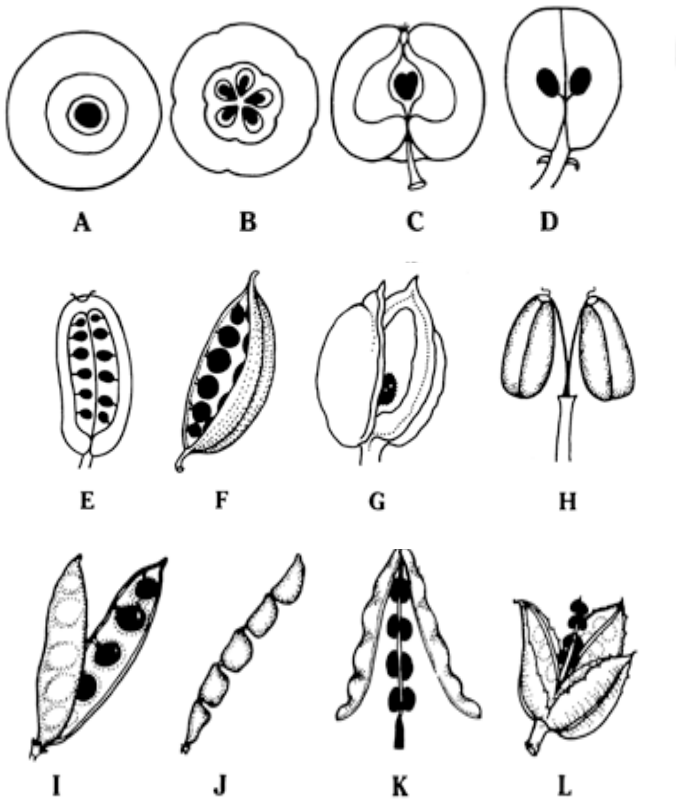
I, paired stipules; J, interpetiolar stipules; K, spinose stipules; L, rolled terminal stipules.	
Leaf bases: A, attenuate; B, cuneate (wedge-shaped); C, obtuse; D, truncate; E, asymmetric; F, cordate; G, auriculate; H, sagittate (arrow-shaped); I, hastate (spear-shaped).	
Leaf apex: A, subulate; B, aristate; C, acuminate; D, acute; E, obtuse; F, truncate; G, emarginate; H, retuse; I, obcordate; J, hooked; K, mucronate	
Leaf edge (margin): A, entire; B, crenate; C, toothed; D, doubly toothed; E, erose; F, lacerate; G. laciniate; H, angled; I, crisped; J, undulate; K, sinuate	
Type of venation A, pinnate; B, 3-veined from base; C, palmate; D, leaves palmately veined, lobes pinnately veined; E, longitudinal; F, parallel; G, dichotomous; H, reticulate; I, areolate.	

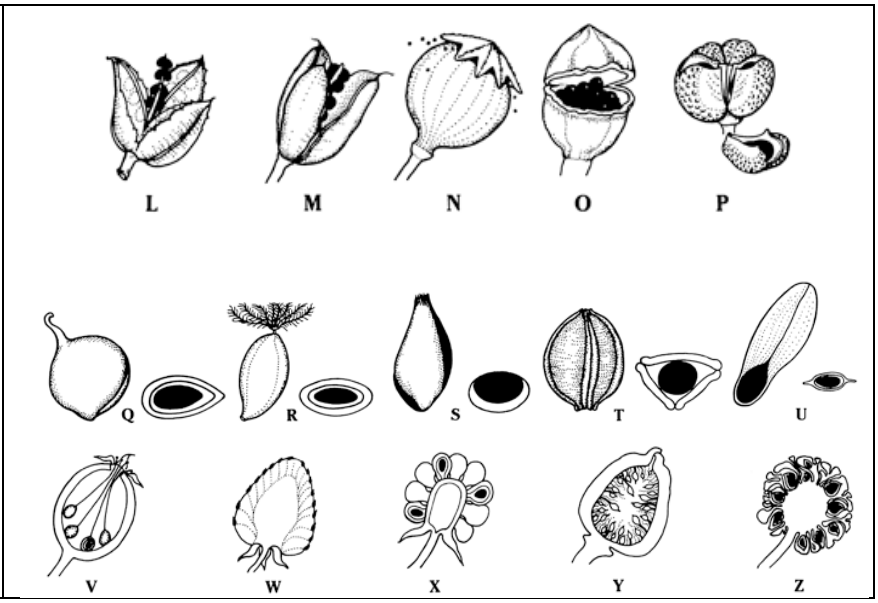
The morphological diagnostic features of flower

Type of inflorescence A, panicle; B, thyrsoid; C, thyrse; D, dichasium; E, monochasium; F, triad; G, panicle-like; H, raceme; I, spike; J, umbel; K, corymb; L, solitary on a scape; M, solitary in axils of leaves; N, spikelet; O, head with expanded receptacle; P, head with small; Q, spadix; R, cyathium (in L.S.).	
Shape of receptacle: 1 – flate; 2 – concave: а – patelliform; б – cyathiform; 3 – convex: в – subglobular; г – conical; д – oblong-conical; е - globular	
Type of perianth 1 – double perianth (Ca and Co) 2 – simple (Perigonium - P) calyciform (P ^{Ca}) corolliform (P ^{Co}) 3 – achlamydeous	

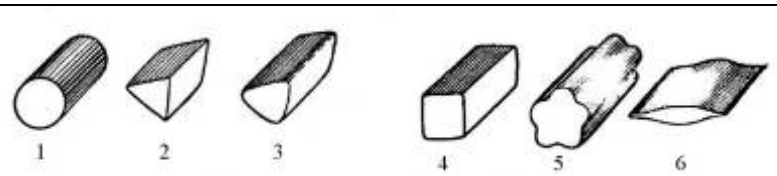
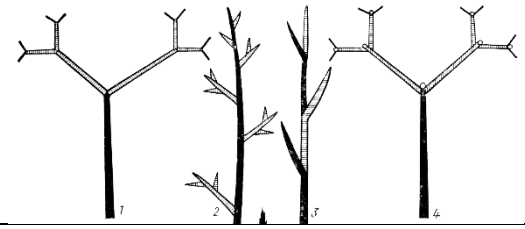
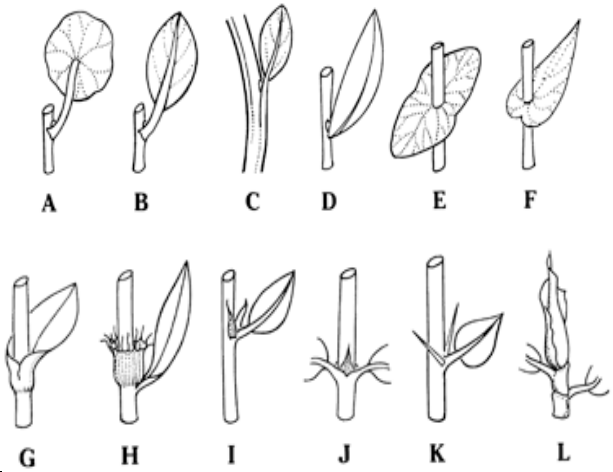
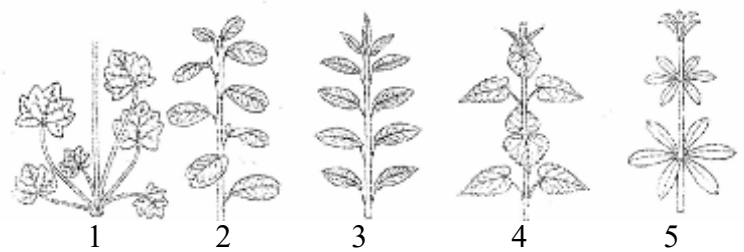
Symmetry: 1 – zygomorphous; 2 – actinomorphous; 3 – asymmetrical	
Shape of calyx: **А – polysepalous**: 1 – cruciform (or cross-shaped); 2 – stellar (or stellate). **Б – gamosepalous**: 3 – tubular; 4 – infundibuliform (funnel-shaped); 5 – campanulate (bell-shaped); 6 – bilabiate; 7 – campanulate-bilabiate	
Shape of corolla: **А – polypetalous**: 1 – caryophyllous; 2 – cruciate; 3 – stellar; 4 – papilionaceous. (а – vexilum; б – winsg petals; в–slipcover) **Б – gamopetalous actinomorphous**: 1 – campanulate; 2 – tubular; 3 – tubular-campaniform-shaped; 4 – ladybell-shaped; 5 – infundibuliform (funnel-shaped); 6 – patelliform; 7 – rotate; **В – gamopetalous zygomorphous:** 1 –ligulate; 2 – false-ligulate; 3 – funnelform; 4 – thimble-shaped; 5, 6 – bilabiate; 7 – bilabiate with spur	

The morphological diagnostic features of fruit

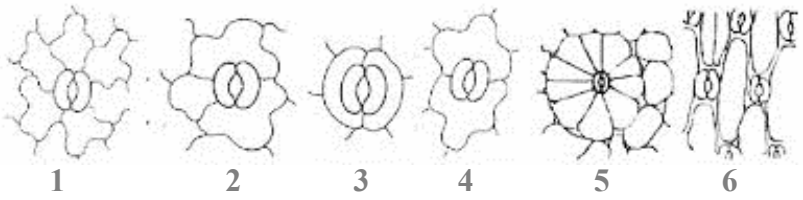
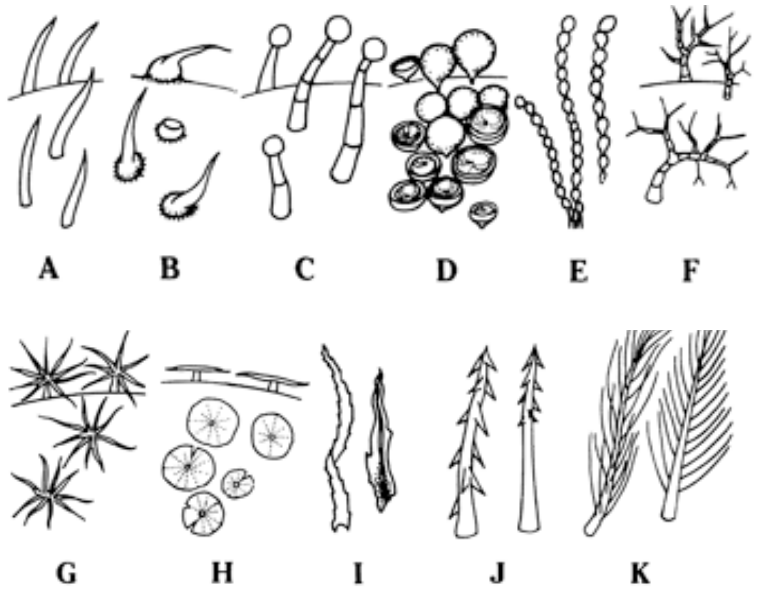
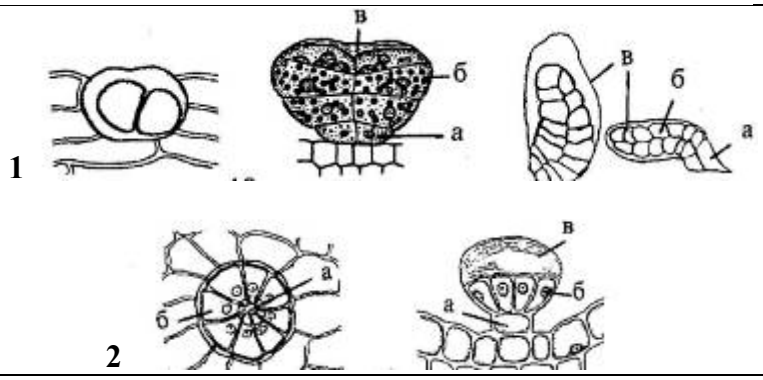
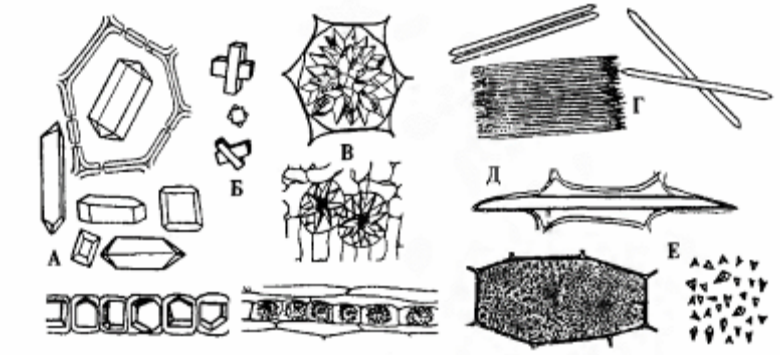
Type of fruit	
Morphological classification: **the consistency of the pericarp:** 1 – dry; 2 – fleshy	 1 2
number of seeds: 1 – one-seeds 2 – many-seeds	 1 2
method of seeds release: 1 – dehiscent; 2 – indehiscent	 1 2
Fruit Types (seeds black). *A–E, succulent indehiscent fruit*: A, drupe, 1-seeded; B, drupe, 5 seeded; C, pome; D, superior berry; E, inferior berry (in L.S.); *F–K, dry dehiscent fruits*; F, many-seeded follicle; G, follicle with 2-winged seeds; H, schizocarp; I, legume or pod; J, lomentum; K, siliqua; *L–P, capsules*: L, loculicidal capsule; M, septicidal capsule; N, poricidal casule; O, circumsciss capsule; P, schizocarp capsule; *Q–U, dry indehiscent fruits, with sections showing position of seed*: Q, achene from a superior ovary;	 A B C D E F G H I J K L

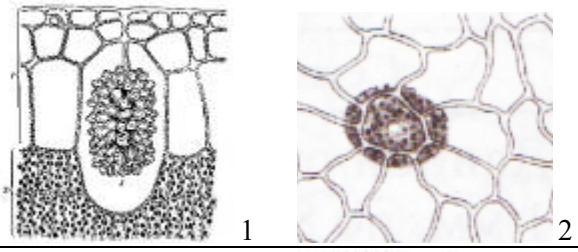
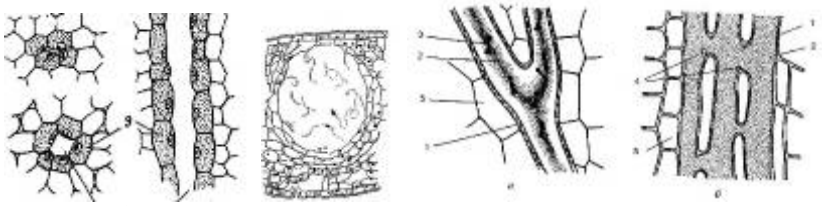
R, achene from inferior ovary with apical pappus; S, caryopsis; T, nut; U, samara; *V–X, aggregate fruits* V, rose ‘hip’, individual fruits drupelets; W, strawberry, individual fruits achenes; X, blackberry, individual fruits drupelets; *Y & Z, multiple fruits* Y, syconium or ‘fig’; Z, syncarp.	

The morphological diagnostic features of stem

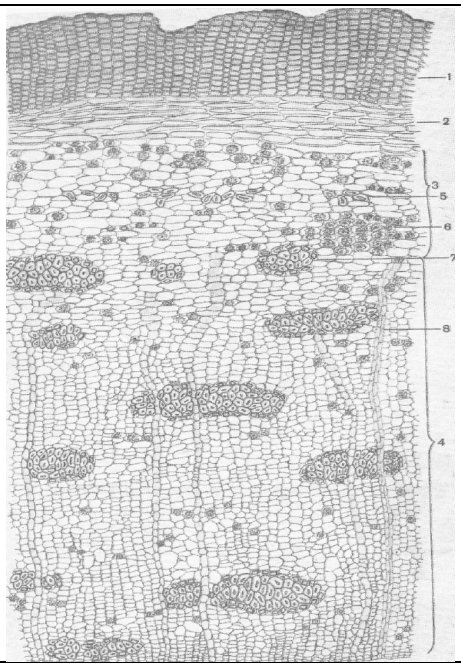
Stem form in cross section 1 – округле; 2, 3 – тригранне; 4 – чотиригранне; 5 – жолобчасте; 6 – крилате	
Типи галуження пагона: 1 – дихотомічне; 2 – моноподіальне; 3 – симподіальне; – несправжньо-дихотомічне	
Leaf Attachment to a stem A, peltate; B, petiolate; C, decurrent; D, sessile; E, gamophyllous; F, perfoliate; G, sheathing; H, ocrea; I–L, stipules; I, paired stipules; J, interpetiolar stipules; K, spinose stipules; L, rolled terminal stipules.	
Leaf arrangment: 1 – basal; 2 –alternate; 3 – opposite; 4 – spiral; 5 – whorled	

Anatomical diagnostic features of leaf

Types of stomata: 1 – anomocytic, 2 – anisocytic, 3 – paracytic, 4 – diacytic, 5 –actinocytic, 6 – tetracytic	
Trichome Type: A, simple hairs; B, tubercle-based hairs; C, glandular hairs; D, vesicular hairs; E, moniliform hairs; F, dendritic hairs; G, stellate hairs; H, peltate scales; I, elongate scales; J, barbed bristles; K, plumose hair.	
Secretory glandules: 1 – typical for Asteraceae familly (with biseriate stalk); 2 – typical for Lamiaceae family (with radial-located cells of head)	
Calcuim oxalate crystals: А – solitary crystals; Б –cross prisms; В – druses; Г – raphides; Д – styloids; Е – idioblast-cells with crystal sand **Calcium carbonate crystals:** Ж – cyctolite: 1 – tangent plane view; 2 – interior view	

	 ж 1 2
Internal secretory structures: 1 – schizogenous cavity; 2 – lysigenous cavity; 3 – non-branched(a) and branched (б) lacticifer	 1 2 3

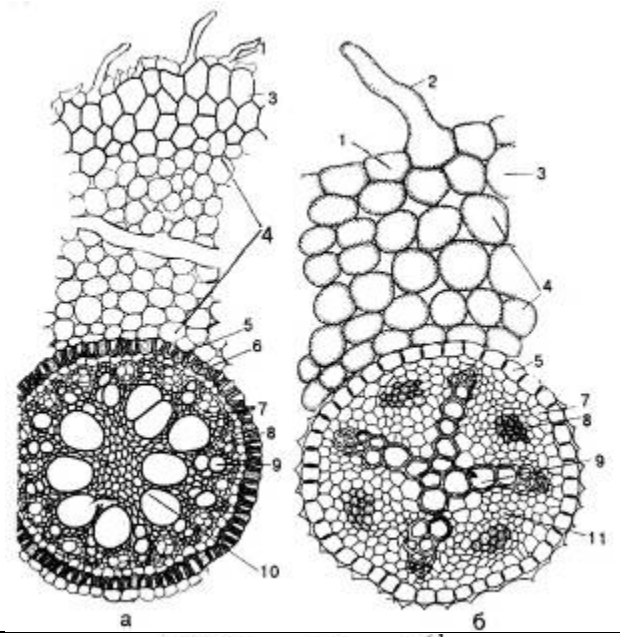
Anatomical diagnostic features of bark

A cross-section of a cortex: 1 – cork; 2 – lamellar collenchyme ; 3 – parenchyma of external cortex; 4 – parenchyma of internal cortex; 5 – druse of calcium oxalate; 6 – mechanical fiber with slightly thickened and weakly lignified 7 – groups of thick lignified bast fibers surrounded by crystalliferous facing that form concentric belts; 8 – uniseriate and multiseriate medullary raies;	
Cork	thickness, number of layers, colour
Main parenchyma	cells shape, cell inclusions (non-protoplasmic components)
Medullary rays	uniseriate, multiseriate
Mechanical elements	bast fibers, sclereides
Crystals inclusions	single crystals, druse, crystalliferous facing

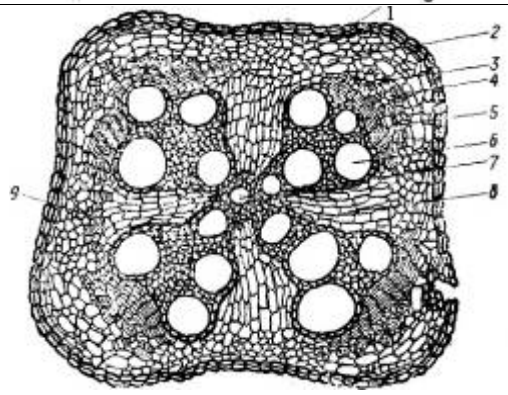
Anatomical diagnostic features of root

Structure	primary fascicular, secondary fascicular, nonfascicular, transition
Covering tissue	epiderm, periderm, cork

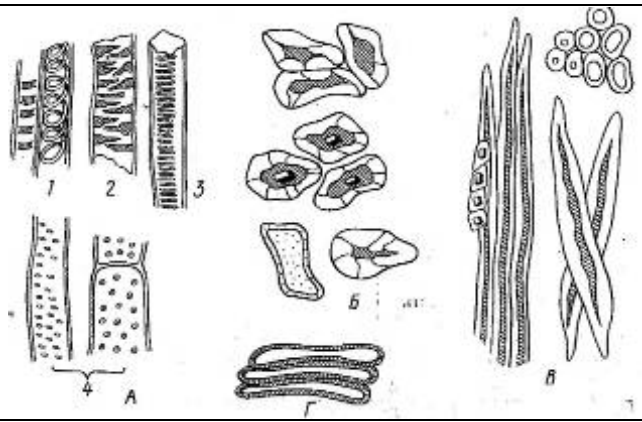
Root structure:
The primary structure of the root:
а – monocot plant;
б –dicot plant;
1 – epiblema; 2 – root hair; 3 – exoderm;
4 – mesoderm; 5 – endoderm;
6 – passage cell (3 – 6 primary cortex); 7 –pericycle; 8 – primary phloem; 9,10 – primary xylem;
11 – main p concentric
7 – 11 – central cylinder

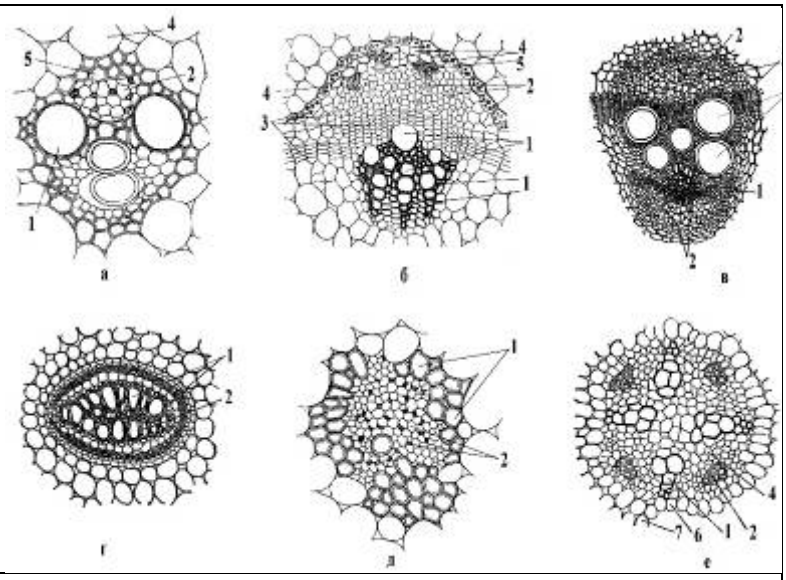
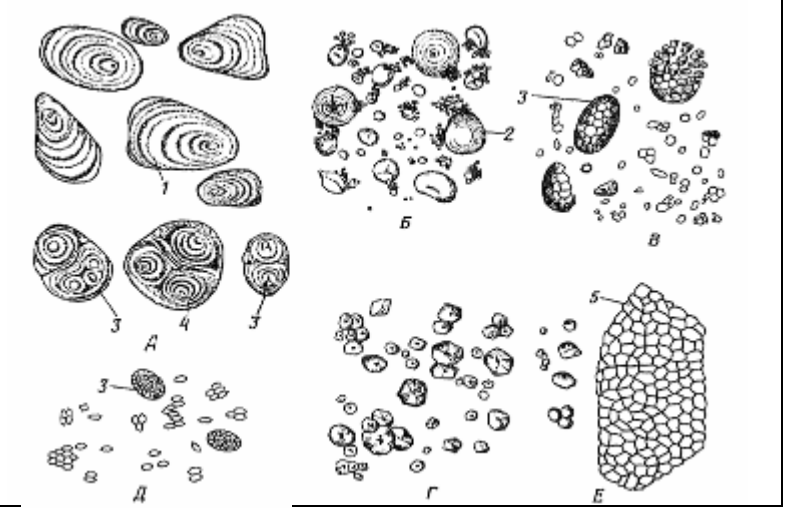


The primary structure of the root:
1 - periderm;
2 – secondary cortex parenchyma;
3 - primary phloem;
4 - secondary phloem;
5 - fascicular cambium;
6 – secondary xylem ;
7 - primary xylem;
8 – medullary ray;
9 - interfascicular cambium



Conductive and mechanical elements:
А – vessels:
1 – annulate and spiral;
2 – reticulate;
3 – ladder-shaped;
4 – pitted.
Б – stone cells (or brachysclereids);
В – fibers;

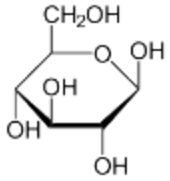


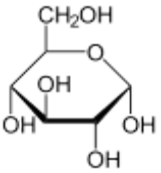
Types of conductive bundles: а – collateral close; б – collateral open; в – bicollateral; г – concentric centroxylem; д – concentric centrophloem; е – radial. 1 – xylem, 2 – phloem, 3 – cambium, 4 – main tissue, 5 – sclerenchyma, 6 – pericycle, 7 – endoderm	
Main parenchyma	palisade, spongy, aerenchyma
Medullary ray	uniseriate, multiseriate
Secretory structures	cavities, lacticifers, secretory cells
Crystals inclusions	single crystals, druse, crystalliferous facing
The grains of starch: А –potato; Б – wheat; В – oat; Г – corm; Д – rice; Е – buckwheat; 1 – simple eccentric grain; 2 – simple concentric grain; 3 – complex grain; 4 – semicomplex grain; 5 – cluster of simple grains 6 – starch layers	

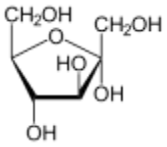
Appendix 2

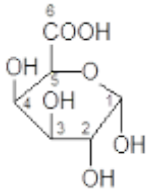
TOPIC: CARBOHYDRATES. GLYCOSIDES

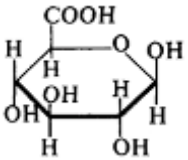
MONOSACCHARIDES



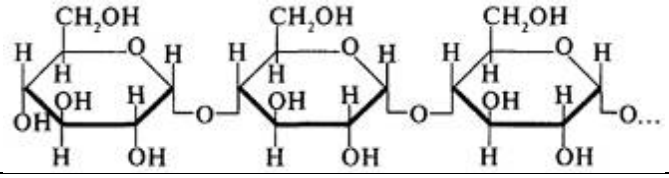


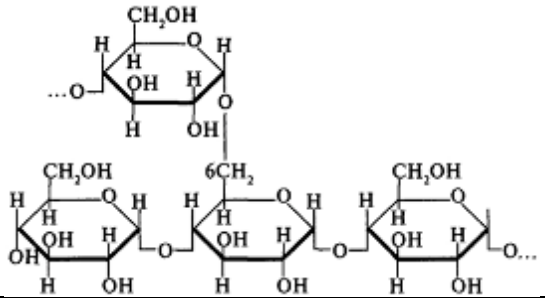


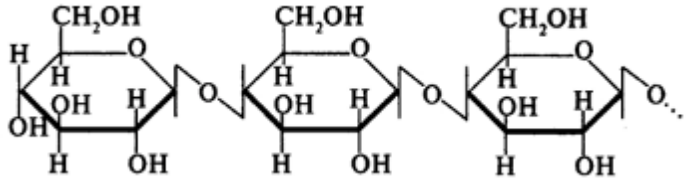




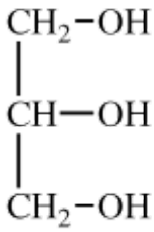
POLYSACCHARIDES

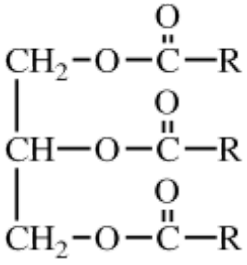


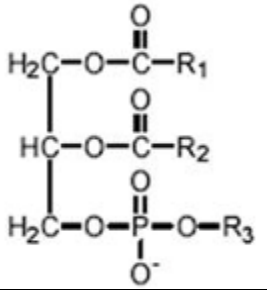




TOPIC: LIPIDS AND LIPOIDS

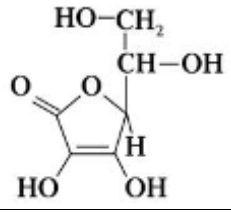


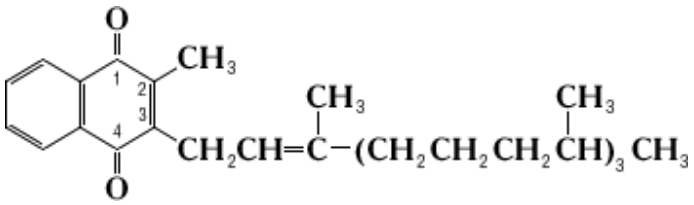


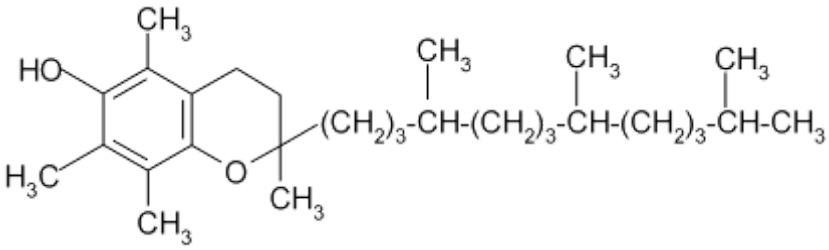


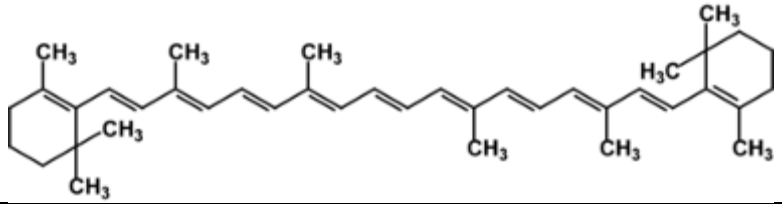
TOPIC: VITAMINS. MACRO- AND MICROELEMENTS. ORGANIC ACIDS

VITAMINS

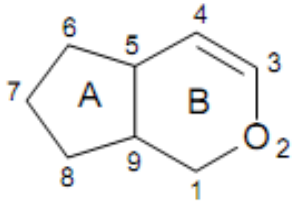


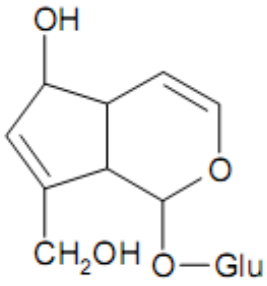


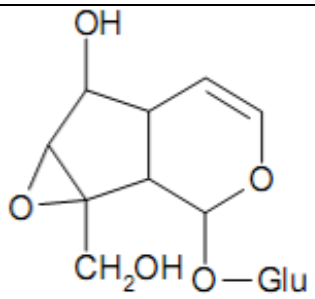


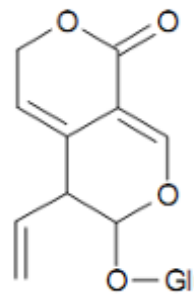


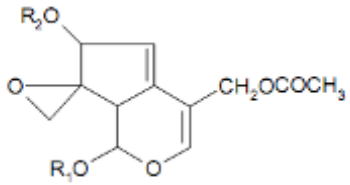
TOPIC: TERPENOIDS. IRIDOIDS

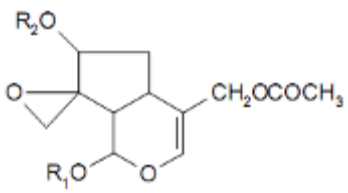


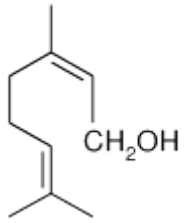
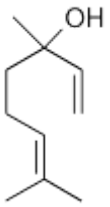
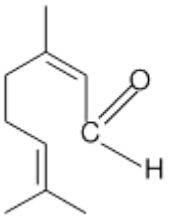
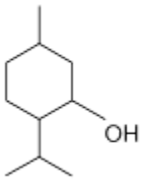
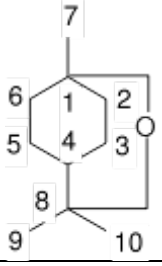
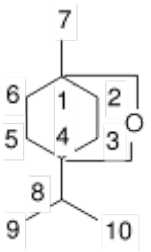
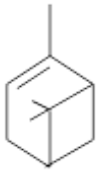
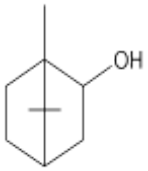
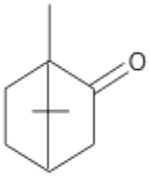
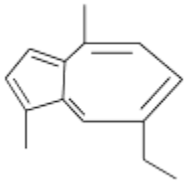
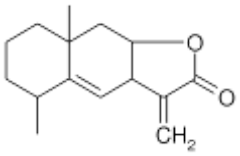










TEMA: VOLATILE OILS		
MONOTERPENOIDS		
		
		
		
SESQUITERPENOIDS		
		
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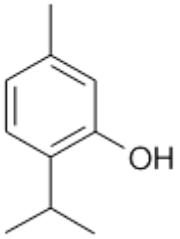	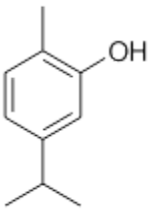	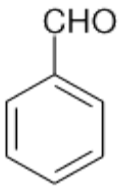
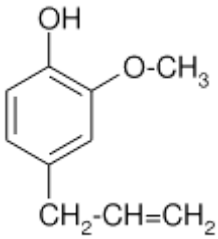	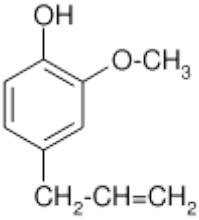	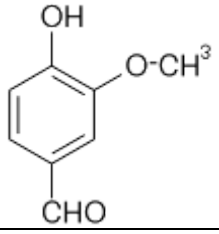

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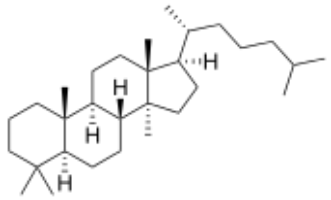	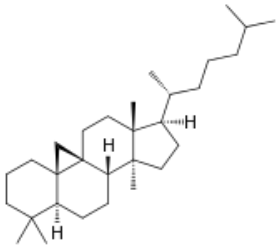	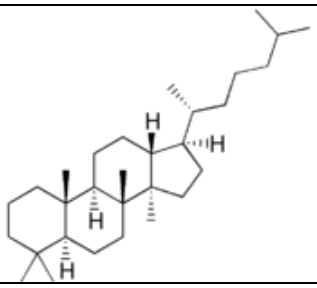
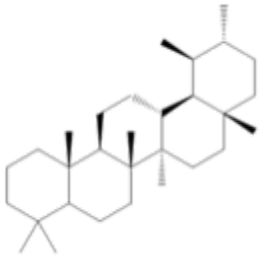	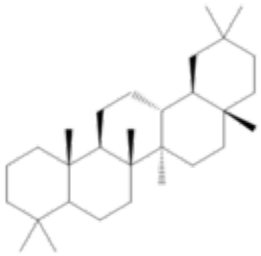	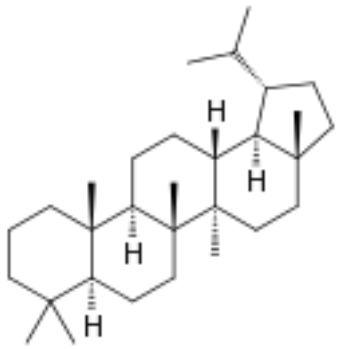
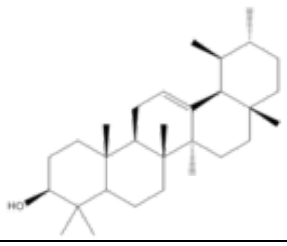	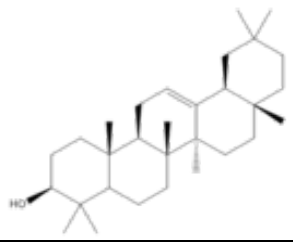	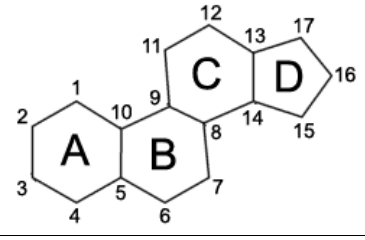

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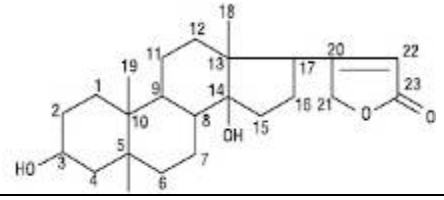	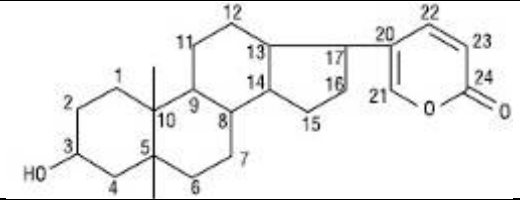
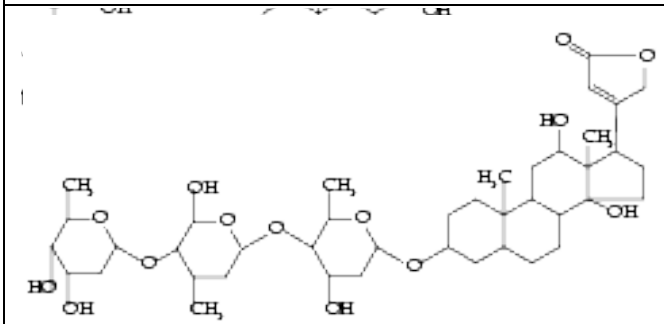	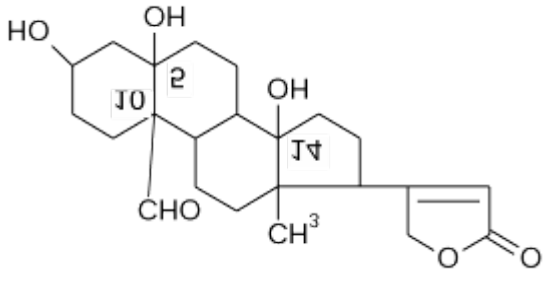

Appendix 3

Table 1.1. Physical and chemical quality of some oils

Oil	Density ρ[1]	Indicator refraction n	Saponification number	Iodine number	Unsaponifiable residue,%	Peroxide number
Peanut oil	0,911-0,926	1,460-1,472 1,460-1,463 at 40°C	185,6-197,0	93,0-105,0	0,3-0,5 to 1	<8
Castor oil	0,960-0,970 at 20°C	1,4774-1,4785 at 40°C	176,0-187,0	82,0-86,0	to 1	<10
Coconut oil	0,920-0,925	1,448-1,450 at 40°C	246,1-268,9	7,7-9,5	0,1-0,3	
Hemp oil	0,923-0,933 ρ[1]	1,470-1,479	185-195	145-175	to 2	
Corn oil	0,914-0,926 ρ[1]	1,471-1,475	188-203	111-131	<2,8	<10
Sesame oil	0,921-0,924	1,4707-1,4709 at 25°C	186,5-195,0	103,0-115,7	0,8-1,5	
Linseed oil	0,930-0,940	1,479-1,481	187,6-195,2	164,0-195,0	1,0-2,0	<12
Poppy seed oil	0,924-0,937	1,475-1,478	189,0-197,7	131,0-143,3	0,8-1,5	
Cacao butter	0,950-0,976	1,449 at 60°C	192,0-197,0	33,5-37,5	0,3-0,8	
Мигдальна oil	0,914-0,920	1,470-1,473	187,9-200,0	93,0-100,0	to1	
Olive oil	0,914-0,920	1,467-1,471	187,0-195,9	78,5-89,9	0,7-1,4	<10
Palm oil	0,921-0,925	1,453-1,459 at 40°C	196-210	48-50	0,3	
Palm kernel oil	0,925-0,935	1,449-1,452 at 40°C	240-257	12-20	0,5	
Sunflower oil	0,920-0,927 at 20°C	1,474-1,476	188,0-194,0	118,0-144,0	0,8-1,5	
Cod-liver oil	0,925-0,930	1,470-1,473 at 40°C	179,0-194,0	160,0-170,0	0,5-1,5	
Soybean oil	0,924-0,927	1,471-1,476	190,0-193,0	125,0-134,0	0,5-1,0	
Cotton oil	0,920-0,930	1,472-1,477	191,0-198,2	102,0-113,0	0,7-1,6	

Appendix 4

Quality indexes of essential oils

Essensial oil (plant name)	%	d_D^{20}	n_D^{20}	$[\alpha]_D^{20}$	Solubility - volume ratio of oil and ethanol (concentration of alcohol)	Main components
Essential oils which contain terpenoids						
Calamus Oil *(Acorus calamus)*	1- 3,5	0,945 – 0,970	1,500 – 10508	From +7 to +30	1:30 (90%)	Asarone, camphor, pinens, camphen, sesquiterpenea
Orange Oil *(Citrus simensi)* [*BPh*]	0,5–0,7	0,842 – 0,848	1,472 – 1,476	From +94 to + 99	1:7 (90%)	Limonen (~90%), decanal (0,9 - 3,2%)
Bergamot Oil from skin (*Citrus bergamia*)	To 0,5	0,875 – 0,883	1,464 – 1,468	From +4 to +28	1:1 (90%)	Linalilacetate (32 – 44%), limonen (18– 30%), linalool (12–15%), furocoumarin bergapten (5 – 6%)
Lemon [Limonis aetheroleum, *PhEur*]		0,850 – 0,858	1,474 – 1,478	From +56 to +70		Citral, bergamottin
Citronella Oil *(Cymbopogon winterianus)* [Citronellae aetheroleum, *PhEur*]	1,2–2,4	0,889 – 0,906	1,466 – 1,485	From –9 to –18	1:1 (80%)	Geraniol (20–25%), citronellal (30–45%), citronellol (9–15%), geranilacetate (3 - 8%), limonen (1 – 5%), citral
Orange Oil *(Citrus aurantium)* [Aurantii amari floris aetheroleum, *PhEur*]		0,866 – 0,880	1,468 – 1,474	From +1,5 to +11,5		Linalool (18 – 42%), limonen (9 – 18%), β– pinen (7 – 17%), linalilacetate (3 – 16%), furocoumarin bergapten
Rose Oil *(Rosa damascena)*	0,03 – 0,1	0,848 – 0,861	1,4530 – 1,4640	From– 2,2 to – 4,6	All proportions (90%)	Citronellol (30 – 35%), geraniol (~5%), nerol, phenylathyl alcohol (40 – 50%)
Geranium Oil *(Pelargonium roseum)*	0,1 – 0,15	0,884 – 0,900	1,4605– 1,4690	From –8 to –12	1:(2 – 3) (70%)	Citronellol (38 – 46%), linalool (10 – 12%), geraniol (15 – 18%), menthon and isomenthon (15 – 18%)
Lavender Oil *(Lavandula angustifolia)* [Lavandulae aetheroleum, *PhEur*]		0,878 – 0,891	1,455 – 1,466	From- 12,5 to– 7	Mixed with 90% alcohol, ether, fatty oils	Linalilacetate (25 – 46%), linalool (20 – 45%), cineole to 2,5% , 3 – octhanol to 2,5% , camphor to 1,2%
Peppermint Oil [Menthae piperitae aetheroleum, *PhEur*]		0,900 – 0,916	1,457 – 1,467	From – 10 to – 30		menthol (30 – 55%), menthon (14 – 35%), isomenthon (1,5 – 10%), menthylacetate (8 – 10%), cineole (3,5 – 14)

Spirmint Oil *(Mentha spicata)* [*BPh*,]		0,917 – 0,934	1,484 – 1,491	From – 45 to – 60	1:1 (80%)	Not less then 55,0% carvone
Sage Oil *(Salvia sclarea)*	0,1– 0,13	0,887 – 0,920	1,455 – 1,470	From– 4,5 to – 30	1:0,5 (90%)	Linalilacetate (to 75%), linalool (20%), cineole, borneol, camphor, thujone
Eucalyptus Oil [Eucalypti aetheroleum, *PhEur*]		0,906 – 0,925	1,458 – 1,470	From 0 to +10		Cineole (Not less then 70%)
Cumin Oil *(Carum carvi)* [*BPh*,CarawayOil]	Not less 3,5%	0,902 – 0,912	1,485 – 1,492	From +74 to +80	1:8 (80%)	Ketons content from 53,0 to 63% in terms of carvone
Dill Oil *(Anethum graveolens)* [*BPh*,]		0,895 – 0,910	1,481 – 1,492	From +70 to +80	1:1 (90%) 1:10 (80)	Content of carvone from 43,0 to 63%
Terpentine Oil *(Pinus spp.)* [*BPh*,]		0,855 – 0,868	1,467 – 1,477		1:7 (90%)	The residue after evaporation – not more then 0,5%
Fir Oil *(Abies sibirica)*	0,2 – 3	0,895 – 0,915	1,4690– 1,4720	From – 37 to – 46	1:5 (90%)	Bornylacetate (32 – 45%), borneol (3 – 5%), pinen, myrcene, limonen

Essential oils which contain aromatic compounds						
Anis Oil, Star anis Oil *(Pimpinella anisi* et *Illicium verum)* [Anisi aetheroleum, *PhEur*]		0,978 – 994	1,552– 1,561	From +15 to +19		*trans*- anethole (84 – 93%), *cis*- anethole (less then 0,5%), anise aldehyde (0,1 – 3,5%), estragol (0,5 – 6%), linalool (0,1 – 1,5%)
Basil Oil *(Ocimum gatissimum)*	0,3–0,7	0,995 – 1,402	1,514– 1,536		1:1,4 (70%)	Eugenol (52 – 82%), *cis*-β-O- cimen (10 – 16), linalool (10 – 16%), cadinenes (10 – 12%), santalens (6 – 8%), methylchavicol (to 6%)
Clove OIl [Caryophylli floris aetheroleum, *PhEur*]		1,030 – 1,063	1,528– 1,537	From 0 to – 2		Eugenol (75 – 85%), caryophyllene (5 – 14%), acetyl eugenol (4 – 5%)
Thyme Oil *(Thymus vulgaris)* [Thymi aetheroleum, *PhEur*]		0,915 – 0,935	1,490– 1,505			Thymol (36 – 55%), *n*-cimen (15 – 28%), linalool (4 – 6,5%), γ-terpinen (5 – 10%), carvacrol (1 – 4%)
Cinnamon Oil		1,030 –	1,0527–	From–		Eugenol (70 – 85%), linalool (1,5 – 3,5%), β-

(Cinnamomum verum) [Cinnamomi zeylanici aetheroleum, *EurPh*]		1,059	1,540	2,5 to 2,0		caryophyllene (1,5 – 7%), safrol less then 3%, cineole less then 1%, coumarin less then 1%
Cinnamon Oil [Cinnamomi zeylanici folii aetheroleum, *EurPh*]		1,000 – 1,059	1,572 – 1,591	From– 2,0 to +1		*trans*-cinnamon aldehyde (55 – 75%), eugenol – less then 7,5%, linalool (1 – 6%), β- caryophyllene (1 – 4%), cineole –less then 3% , safrol – less then 0,5%
Cinnamon Oil *(Cinnamomum cassia)* [Cinnamomi zeylanici cassiae aetheroleum, *EurPh*]		1,052 – 1,070	1,600 – 1,614	From – 1 to +1		*trans*- cinnamon aldehyde (70 – 90%), *trans*-methyl-cinnamon aldehyde (3 – 15%), coumarin (1,5 – 4%), cinnamoilacetate (1 – 6%), eugenol – less then 0,5%
Tea tree OIl *(Malaleuca aeterfolia)* [*BHPh*]		0,885 – 0,906	1,475 – 1,482	From +5 to +15	1:2 (85%)	1,8 cineal (4,5 – 16,5%), terpinen-4-ol (29 – 45%),γ-terpinen (10 – 28%), *n*- cimen (0,5 – 12%)

Note: *PhEur* – Europian Pharmacopea; *BPh* – British Pharmacopea; *BHPh* – British Herbal Pharmacopea;

Appendix 5

SAFETY PRECAUTIONS

Execution of educational and scientific experimental work at the Department of Pharmacognosy and Botany in educational and research laboratories associated with various chemicals (organic solvents, acids, alkalis), plant materials using, different chemical utensils, equipment and devices. Therefore, in the laboratory spaces there are continuously possible dangerous and harmful factors which can effect on students. These factors can lead to work-related injury and fire hazards.

Students are admitted to practical training in chemical lab only after a detailed briefing on safety and fire precautions.

Each student works in the laboratory must know the location of fire fighting and be able to know where the first aid kit and know how to provide first aid for various injuries.

Experimental part begins only after a thorough acquaintance with chemical dishes, technique experiments, properties, purpose reagents and solvents used, and the rules of work with devices. The workplace should only has necessary reagents, instruments and a notebook to record results.

Before using glass and porcelain tableware check its purity and integrity. Do not work with the dishes that have chips, cracks, deep scratches.

All transactions with flammable liquids, concentrated acids and alkalis, experiments with the formation of gases and work with metallic sodium should be performed only in a fume hood, if necessary, should use personal protective equipment (masks, goggles, mask, gloves, etc.). Smell of substance in a test tube or flask determine carefully directing couples to himself flick of the wrist. Mixing and dilution of chemicals, accompanied by heat, spend with heatproof bowl and porcelain.

Do not allow heat flasks with inflammable liquids over an open fire, avoid getting water on the heated external surface of glass vessels, gently and carefully to treat laboratory glassware and equipment.

Acids and bases to dial in using only the dropper rubber pear, forbidden to absorb acid and alkali liquid in the pipette mouth, because it can cause burns and poisoning.

Heating of substances in hermetically sealed vessels (safety explosion!)is strictly forbidden. To prevent the release of liquid from the reaction vessel should be carried out uniformly heating the to put to the bottom of the vessel 2-3 boiling stones (pieces of porous inorganic material).

Heating tubes of substances should be carried out at periodic shaking, vent tubes should be directed away from yourself and others that work.

Take and carry glass with substances should, covering them with hand from side, not the neck.

Do not leave without supervision laboratory installation, operating and equipment included.

It is strictly forbidden to drink water from the chemical dishes, eat, smoke at the laboratory.

After work should be thoroughly washed and put to dry dishes, cups and place shtanhlasy on their location, wipe the work surface of the table, close the gas and water valves, turn off appliances and exhaust ventilation.

In case of pouring concentrated acid, it must first fill with sand so that it is absorbed acid. Sand collect in the container and make out of room to place waste collection. Polluted place pouring rinse with water and wipe dry.

In case *of pouring concentrated and ammonia* - they can fill as sand and sawdust. Pouring place by weak solution of acetic acid after collecting sand or sawdust.

In case of fire in a laboratory situation should turn off gas appliances, exhaust ventilation and remove all flammable material from the fire area. Shouting loudly advertise on fire people working together and in neighboring areas.

It is necessary to take urgent measures to eliminate the fire using fire extinguishers or sand. Do not fill the flames with water that in many cases this leads to expansion of the fire. Only water-soluble substances (alcohol, acetone, etc.) quenched with water. In case of fire clothes should not run, you need to throw the victim's robe located in a prominent and accessible place.

Provision for first aid is the duty of everyone! In providing assistance priority should be to eliminate the cause of the injury, turn off the power grid, extinguish the flame, remove from the wound pieces of glass or substance that causes burns, etc .; the victim must create conditions for the most comfortable position and provide first aid.

If *cuts* must be removed with tweezers glass pieces of glass and wash the wound 3% solution of hydrogen peroxide. The skin around the wound grease 5% solution of iodine and apply a sterile bandage. In severe bleeding tourniquet and attach a note with precisely specified time imposing and send poterpitsloho doctor.

When I degree *thermal burns* (redness) burnt areas should be cool running water, while more severe burns to the provision of skilled care - apply dry aseptic bandage. Do not remove the skin from the burnt remains of clothes that burned.

Burns by concentrated acids affected skin should be washed with plenty of water for 10-15 minutes and then process the 2% solution of sodium bicarbonate and again rinsed with water.

Burns by concentrated alkalis affected area should be washed with plenty of water, and then - a 1% solution of acetic acid.

When *hit acids or alkalis to the eyes*, they should immediately wash with water for 10-15 minutes, then, if getting acid - 2% solution of sodium bicarbonate, and when it enters the meadow - isotonic sodium chloride solution for 30-60 minutes. After thorough rinsing eyes should consult a doctor.

After *burns by phenol* rub the affected area till restore the natural skin colour.

If poisoning by *gas substances* bring the victim to fresh air and create him complete rest and call a doctor.

When *electric shock* turn off power and, using a wooden or plastic objects poterpioho release from contact with electric wire. It is necessary to ensure the victim calm and bring it to life.

If *breathing or heartbeat stops* it's necessary to carry out artificial respiration and chest compressions and do not stop these operations to full functional recovery or the arrival of the medical workers.

Safety precautions at work, harvesting, drying, processing and storage of plant material that contains toxic and potent substances (alkadoides, cardiac glycosides, etc.):

1. Teenagers, students are allowed to collect only under the supervision of the responsible team leader or instructor. By collecting MP, which contain these substances, it is better to include the adult population to collect datura, henbane, hellebore teenagers do not allow!
2. During the assembly should not touch your eyes, the face, not to eat. After collecting wash hands thoroughly with soap and water.
3. During the processing, drying, sorting, packaging protecting mouth and nose with a respirator, wet gauze, eye - protective glasses. Do not take food or smoke.
4. After thoroughly shake out of clothes, wash clothes, wash the face with soap and water, wipe with a dust mask, goggles, gauze.
5. When the need to have a first aid kit.
6. To work with the potent and poisonous MP not allowed zhinkm pregnant and lactating.

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