ODESSA NATIONAL MEDICAL UNIVERSITY
Department of General and Clinical Pharmacology and Pharmacognosy

METHODOLOGICAL DEVELOPMENT
Course: “Pharmacognosy”

practical lesson for students on the topic:

"Coumarins and chromones. Methods of qualitative and quantitative determination. LR and LRS containing coumarins and chromones. Cranberry, horse chestnut, parsnip, but large, fig tree. "

Course: 3rd Faculty: Medical and Pharmaceutical

Approved on methodical meeting of the department
"30" 08.2024
Protocol № 1
Head department
MD, prof. JV Rozhkovsky

Odessa - 2024

1. Topic of the lesson:"Coumarins and chromones. Methods of qualitative and quantitative determination. LR and LRS containing coumarins and chromones. Cranberry, horse chestnut, parsnip, but large, fig tree. " - 4 years.

2. Relevance of the topic.

Coumarins are natural compounds based on benzo-b-pyrone (lacto cis-ortho-oxycinnamic acid). Chromones, unlike coumarins, are derivatives of benzo-g-pyrone.

In nature, they are most often found in the form of derivatives of oxy-, methoxy coumarins and chromones, as well as furocoumarins and furochromones in the free state, less often in the form of glycosides.

Coumarins are widespread in plants of the celery, legume, and root families. Their pharmacological properties are very diverse. They have anticoagulant, photosensitizing, antispasmodic and vasodilating effects. Their phytopreparations are used to treat thrombophlebitis and thrombosis, in the treatment of vitiligo, as cardiovascular drugs.

The knowledge and skills acquired by students in the study of this topic will be used by them in mastering some sections of pharmacology, herbal medicine, as well as in their professional activities.

3. Objectives of the lesson:

3.1. General goals: To study LR containing coumarins and chromones, as well as to perform work on macroscopic and chemical analysis of raw materials (ammi fruits, parsnips, figs, chestnut seeds, roots of butterbur, fruits of spring carrot).

3.2. Educational goals: formation of a professionally significant substructure of personality with relevant aspects of deontological, ecological, legal, psychological, patriotic, professional responsibility.

3.3. Specific goals:

- to know (level of assimilation according to Bezpalk - II):

1. Definition of "coumarins and chromones".
2. Distribution of coumarins and chromones in the plant world and resources of the studied raw materials.
3. Terms, methods of collection, rules of drying and storage of LRS.
4. External signs of the studied types of medicinal raw materials.
5. Preparations containing coumarins and chromones and their use in medicine.
6. The main qualitative reactions for the detection of coumarins and chromones, their chemistry.

Based on theoretical knowledge of the topic:

- Master the techniques (be able to) (level of mastering Bezpalkom - III):
- Recognize the external features of the plant (ami large, Siberian and hairy fruiting plant, spring carrot, fig, bitter chestnut, parsnip);
- Determine the identity and good quality of raw materials on external grounds;
- Detect coumarins and chromones by qualitative reactions.

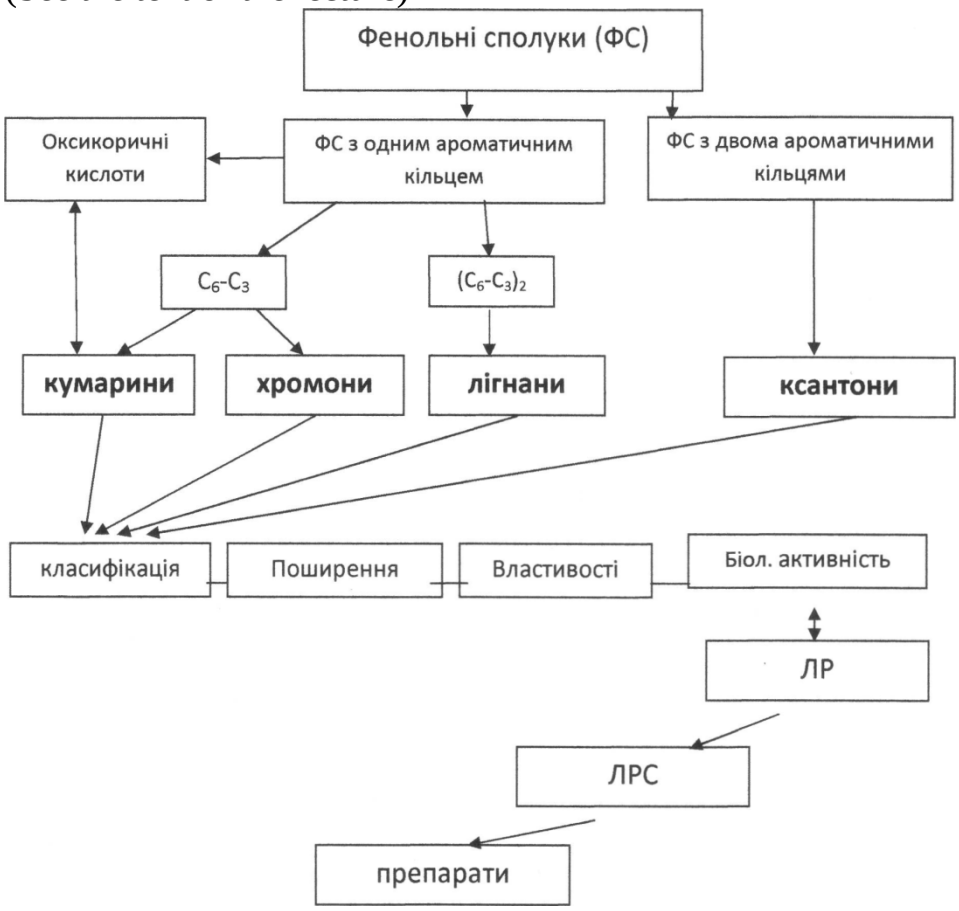
4. Interdisciplinary integration

№ p.p.	discipline	know	be able
1	2	3	4
1.	Previous disciplines: 1. Botany	Characteristic features of the families of the studied plants. Morphology of stem, bark, leaves, flower, fruit, root and rhizome. Anatomical structure of the leaf, bark, fruit, root, rhizome.	Use a microscope, prepare surface preparations and cross-sections.
	2. Organic chemistry	Physical and chemical properties of polysaccharides, glycosides, terpenoids, aromatic derivatives, heterocycles.	Carry out qualitative reactions; purification of organic compounds.
	3. Analytical chemistry	Methods of acid-base titration (neutralization) and permanganatometry	Work with analytical scales, measuring vessels, photoelectro-colorimeter, use methods of chromatography on paper and in a thin layer of sorbent.
2.	The following disciplines: 1. Physical and colloid chemistry	Solubility of solids and liquids in liquids. Distillation. Raoul's law. Konovalov's law. Vapor pressure and composition over mutually insoluble liquids. Buffer solutions. Polarography. Potentiometric titration. Adsorption. Ion exchange adsorption. Chromatography: paper, column, in a thin layer of sorbent, gel chromatography. Methods of measuring mass and volume. Preparation of powders or liquid drugs for internal and external use. Analysis of	

	2. Pharmacy technology of drugs 3. Industrial technology of medicines 4. Clinical pharmacology 5. Pharmaceutical chemistry 6. Organization and economics of pharmacy	prepared liquid drugs using a burette system. Conditions of industrial preparation of medicines. Principles of organization of pharmaceutical production of various dosage forms: liquid, solid, soft, injectable solutions, etc. Machines, devices, equipment for the production of medicines. Pharmacodynamics and pharmacokinetics of drugs. The pattern of action of drugs on the human body and its corresponding reactions. Basic principles of treatment in terms of drug selection, evaluation of their effectiveness and safety. Methods of qualitative and quantitative study of drugs. Pharmaceutical service management. Storage of medicines. Control and analytical service, organization of its work. Accounting for inventory and cash. Economic analysis of the pharmacy. Management and entrepreneurship. Organization as an object of management. Connecting processes in management. Human Resource Management Pharmaceutical Marketing Management. Pharmaceutical market research. International marketing.	

	7. Management and marketing in pharmacy		

5. Content of the topic (text and thesis), graphological structure of the lesson.
(See the text of the lecture)



6. Plan and organizational structure of the lesson.

№№ p.p.	The main stages of the lesson, their functions and content.	Learning objectives in the levels of mastery.	Means of training and control.	Materials on methodical forensuring the visibility of the lesson, control the knowledge of those who teachis.	Term (in minutes or in%) of the total class time.
1	**2**	**3**	**4**	**5**	**6**
1	*Preparatory stage* Organization of classes Setting learning goals Homework check	 II	 Oral interview on the topic	 Methodical works for students, album	 1% 2% 25%
2	*The main stage*				

	Conducting a practical lesson	III	Herbariums of medicinal plants, LRS, reagents		50%
3	*The final stage* Testing and assessment of practical skills Checking the final level of knowledge Providing homework with a reference to the literature	II- III II- III	Herbariums of medicinal plants, LRS, reagents	Methodical works for students, album Tests and situational tasks	5% 15% 3%

7. Materials on methodological support of the lesson.

7.1. Control materials for the preparatory stage of the lesson: questions, tasks, tests.

Tests.

1. Name the plant from the fruits of which tablets are prepared "Anetin". (Used for cardiovascular disease).
A. dill fruit
B. field honeycomb
B. wormwood grass
G. camphor tree
E. blackness black

2. From furocoumarins of this plant prepare the drug "Pastinacin", which is used as an antispasmodic in coronary insufficiency.
A. parsnip sowing
B. annual clown
B. Amur barberry
G. thermopsis lanceolate
D. sophora thick-fruited

3. For the treatment of white spots on the skin using tablets "Psoralen", they include a mixture of furocoumarins. Name the plant from the fruits of which these drugs are prepared.
A. psoralea drupe
B. parsnip sowing
B. ammi fruit is large
G. blood bread
D. scopolia light yellow

4. Coumarins are derivatives:
A. benzo-b-Piron

B. thymol
B. arbutin
G. hydroquinone
D. cyclopentane - perhydrophenanthrene

5. From the root of which plants make a phytopreparation that can enhance the antitumor effect of thiophosphamide when used together.
A. Russian yearbook
B. valerian medicinal
B. leukocyte jaundice
G. hawthorn blood-red
D. Amur barberry

6. From the fruits of which plants containing coumarins and furanochromones, get the drug "Avisan"
A. ammi dental
B. ordinary flax
B. creeping thyme
G. sumac tanning
D. cyanosis blue

7. The fruits, flowers, rays, umbrellas, stems and leaves of this plant contain kellin, which belongs to the furanochromon. Name this plant.
A. ammi dental
B. Amur barberry
B. fingerling straight
G. buckthorn brittle
D. dioecious nettle

8. The fruits of which plant contains a mixture of furocoumarins for the preparation of beverages "Pastinacin" and "Beroxan".
A. parsnip sowing
B. sage
B. eucalyptus ash
G. juniper
D. valerian medicinal

9. From which plant material containing coumarins and furanochromones, get the drug "Ammifurin", used to treat vitiligo and baldness.
A. ammi fruit is large
B. tansy flower
B. peppermint leaf
G. thyme herb
D. the root of the honeycomb

10. The laboratory received a batch of raw bearberry for analysis. Which method do you choose to quantify the content of arbutin:
A. iodometric
B. permanganatometric
B. photoelectrocolorimetric
G. weight
D. spectrophotometric

Question:
1. Definition of "coumarins and chromones", their classification.
2. Distribution of coumarins and chromones in the plant world.
3. Features of harvesting, drying and storage of raw materials
coumarins and chromones and measures for the protection of wild medicinal plants.
4. Latin and Russian names of raw materials that produce plants and families of all objects of the research topic.
5. External signs of the studied types of medicinal plant raw materials.
6. Habitats and resources of the studied plants.
7. Features of the chemical structure of coumarins and chromones and their classification.
8. The main qualitative reactions for the detection of coumarins and chromones, their chemistry.
9. Formulas of basic biologically active compounds.
10. Ways of use and medical application of LRS containing coumarins and chromones.

7.2. Materials of methodical support of the main stage of employment: professional algorithms, orientation maps for formation of practical abilities and skills, educational tasks.
The list of educational practical tasks that must be performed during the practical laboratory lesson:

Task 1. To study ami large, parsnip sowing, figs, Siberian and hairy butterbur, bitter chestnut, spring carrot-like and to analyze raw materials for AND
1. Examine ammi large, parsnip, figs, Siberian and hairy fruit, horse chestnut, spring carrot on herbarium specimens.

Scheme 1:

DETERMINATION OF DERIVATIVE PLANT BY EXTERNAL SIGNS

- Life form (herbaceous plant, shrub,
shrub, tree).
- Type of underground organs (root, rhizome, tuber, etc.)
- The structure of the stem (shape, nature of branching, pubescence,
diameter, etc.)
- Sheet placement (regular, opposite, whorled)
- Leaves (simple or complex. The shape of the leaf blade

or leaves, edge, veining, color, size).
- Flowers (single or inflorescences, flower structure, color, size, etc.)
- Fruit (type, shape, color, size).
- Bark (in woody species), (color, presence, shape and color) lentils, thorns, etc.).

1. To carry out the macroscopic analysis of fruits of ammi big, fruits of parsnip of sowing, a root of a poddutoplodnik, fruits of a spring of carrot, a fig leaf, a fruit (seeds) of a horse chestnut on an example of samples of raw materials.

Scheme 2:

ANALYSIS OF RAW MATERIALS "UNDERGROUND AUTHORITIES" BY EXTERNAL SIGNS

- Commodity type of raw materials (unharvested, cut, cleaned or uncleaned, etc.)
- Type of underground organs (roots, rhizomes with roots, rhizomes, tubers, bulbs, bulbs, etc.)
- Shape (cylindrical, conical, lumpy, twice curved, etc.)
- Dimensions
- Surface (smooth or wrinkled, the presence of longitudinal or transverse folds, scars from leaves, stems, traces lateral roots, etc.)
- Color on the outside, at the break.
- The nature of the fracture (granular, fibrous, smooth, rolling, bristly, etc.)
- The presence of the core
- Type of structure of the conductive system (beam, beamless).
- Odor when scraping or wetting with water.
- Taste (in non-toxic objects).

Scheme 3:

ANALYSIS OF RAW MATERIALS "FRUITS AND SEEDS" BY EXTERNAL SIGNS

- Commodity type of raw materials.
- Type of fruit (berry, box, perianth, drupe, achene, beans).
- Fruit shape (spherical, oblong, crescent-shaped, etc.)
- The nature of the surface (smooth, pitted, ribbed, wrinkled, shiny, matte, etc.)
- The form and features of the pericarp (pericarp).
- The number of seeds or seeds, their shape and structure, structure surface.
- Color.
- Dimensions (length, thickness).
- Smell (when rubbing or scraping).
- Taste (for non-toxic objects)

Write down the Latin and Russian names of raw materials produced by plants and families (give synonyms).

2. Note the compliance of the test sample of raw materials (on external grounds) to the requirements of the NTD.

Task 2. Carry out qualitative reactions for the detection of coumarins on samples of raw materials: ami fruits, parsnips, fig leaves, fruits and seeds of bitter chestnut, the root of Siberian and hairy fruit, carrot spring fruits.

Progress.

I. Preparation of the hood.

1. 3.0 g of raw material to grind to 1-3 mm.
2. Place the sample in a flask with a section of 100 ml.
3. Raw materials pour 30 ml of 96% ethyl alcohol
3. Close the flask with an air refrigerator and boil in a water bath for 20 minutes
4. After cooling, filter the extract. The resulting extract is used for reactions and chromatographic studies.

II. Reaction with alkali and diazo reagent.

1. To 3-5 ml of alcohol extract add 5 drops of 10% potassium hydroxide solution and heat in a water bath for several minutes.
2. Note the change in color (in the presence of coumarins, the solution turns yellow).
3. Add 3-5 drops of freshly prepared diazotized sulfanilic acid.
4. Note the change in color (in the presence of coumarins, the solution turns brownish-red to cherry color).
5. Record the results and chemistry of the reaction in a laboratory journal.

III. Lactone test

1. To 3-5 ml of alcohol extract add 5 drops of 10% alcohol solution of potassium hydroxide, heat in a water bath.
2. Add 5-10 ml of distilled water, mix well.
3. Add 10 drops of 10% hydrochloric acid. Note the turbidity or precipitation, which indicates the possible presence of coumarins.
4. Record the results of the reactions in a laboratory journal.

IV. Chromatography in a thin layer of sorbent.

1. Apply about 0.02 ml of an alcoholic solution containing coumarins or chromones to the capillary line of the Silufol plate.
2. In parallel on the starting line to apply samples of known coumarins and chromones ("Witnesses").
3. Dry the plate and place in a chamber with a solvent system of benzene-ethyl acetate 2: 1 or acetone - hexane 2: 8
4. After chromatography, dry the plate in an oven at 110-120oC for 2-3 minutes; treat with 10% alcohol solution of potassium hydroxide.
5. Examine the chromatogram in daylight and UV light before and after treatment with diazotized sulfanilic acid.

6. Note the nature of the color and fluorescence of the spots, calculate the values of Rf coumarins and chromones.

*Note:*Coumarins in UV light have blue, blue, purple, green fluorescence, which increases after treatment with alkali. After spraying the chromatograms with diazo reagent, coumarin spots acquire a color from brick red to purple, visible in daylight.

7. Draw chromatograms in the laboratory journal, number coumarin spots.

8. Compare the values of Rf, the nature of the color and fluorescence of the spots of the studied extract and "witnesses".

9. Record the results in a laboratory journal.

Instructional materials for mastering professional skills, abilities:

Methods of work performance, stages of performance:

a) get the necessary LRS

b) to study and describe the appearance of the obtained LRS, to draw LRS

c) to conduct LRS training

d) to study the anatomical and diagnostic features of roots and rhizomes

e) to study the anatomical and diagnostic features of fruits, leaves, bark

e) sketch observations and record them in a laboratory journal

7.3. Control materials for the final stage of the lesson: tasks, tasks, tests, etc.

Question:

1. What are the general morphological features of plants of the umbrella family?
2. On what external grounds are the fruits of ammi large and dental?
3. Which plant produces a source of raw parsnips?
4. What are the features of the morphology of the sedge parsnip?
5. What are the diagnostic features characteristic of the fruits of parsnip in the micropreparation?
6. What is the nature of the fluorescence of coumarin compounds in ultraviolet light?
7. What reactions can open coumarins in medicinal raw materials?
8. Distribution of coumarins and chromones in the plant world and resources of the studied raw materials.
9. Latin, Ukrainian and Russian names of raw materials produced by plants and families of all objects of the research topic.
10. Morphological characteristics of derived plants, their habitats (areas of cultivation).
11. External signs of the studied types of medicinal raw materials.
12. Features of the chemical structure of coumarins and chromones and their classification.
13. Physico-chemical properties of coumarins and chromones.
14. Formulas of coumarin, umbelliferone, esculetin, ostol, psoralen, bergapten, isopimpinellin, xanthotoxin, emperor, spundin, angelicin (isopsoralen), vysnadin, kellin.
15. The main qualitative reactions for the detection of coumarins and chromones, their chemistry.
16. Methods of isolation of coumarins from LRS.
17. Methods of chromatographic, spectrophotometric and colorimetric analysis.

18. Methods for quantitative determination of coumarins and chromones.
19. The relationship of chemical structure with biological action.
20. The chemical composition of LRS containing coumarins and chromones.
21. Ways of use and medical application of LRS containing coumarins and chromones.

Tests:
1. What biologically active substances of plant origin give positive reactions with solutions of iron-ammonium alum
A. tannins
B. saponins
B. polysaccharides
G. bitterness
D. fatty oils

2. The method of thin-layer chromatography is used to detect coumarins in vegetable raw materials. What is the physical property inherent in coumarin, allows them to be identified on chromatograms:
A. fluorescence
B. solubility in water
B. specific gravity
G. solubility in organic solvents
D. optical activity

3. Plant raw materials for the production of the drug Anavenol, which has a venotonizing effect, reduces the permeability of capillaries and improves microcirculation in blood vessels, are:
A. horse chestnut
B. ordinary flax
B. creeping thyme
G. sumac tanning
D. cyanosis blue

4. Fig leaf is a coumarin-containing raw material. To detect this class of compounds in the raw material using the reaction:
A. lactone test
B. cyanidin test
B. Wagner's reaction
G. Dragendorf reaction
D. reaction with methylene blue

5. Chromatographic analysis is a specific method of determining the authenticity of plant raw materials and phytopreparations. To identify individual substances in the chromatographic analysis determine the following value:
A. the value of Rf

B. melting point
B. boiling point
G. angle of refraction
D. angle of rotation [ba] 20

6. What qualitative chemical reaction is used to identify furocoumarins in the fruits of ammo large:
A. azo combinations
B. cyanidin test
B. sublimation reaction
G. reaction with Dragendorf's reagent
D. reaction with tannin

7. Fig leaves containing furocoumarins psoralen and bergapten, are a source of the drug "Psoberan". Caution should be exercised when harvesting fig leaves, as furocoumarins:
A. have a photosensitizing effect
B. cause irritation of mucous membranes
B. are poisonous substances
G. cause systolic cardiac arrest
D. is a keratolytic poison

8. The drug Escuzan has a venotonizing effect, reduces the permeability of capillaries and improves vascular microcirculation. Vegetable raw materials for the production of this drug are:
A. horse chestnut
B. clover medicinal
V. horsetail
G. sowing buckwheat
D. heart-shaped linden
9. Name the plant that contains furanochromones kellin, visnagin, pyrocoumarin. The main active ingredient is kellin, the amount of which can reach 2.5%.
A. ammi dental
B. Amur barberry
B. fingerling straight
G. buckthorn brittle
D. dioecious nettle

10. Which of these herbs is the raw material for the production of kellin, which is used as an antispasmodic in the treatment of coronary heart disease and asthma.
A. ammi dental
B. clover medicinal
V. horsetail
G. sowing buckwheat
E. belladonna grass

8. Literature for the teacher.

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Додаткова література:

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2. Сербін А.Г., Сіра Л.М., Слободянюк Т.О. Фармацевтична ботаніка. Підручник. – Вінниця: НОВА КНИГА, 2007. – 488 с.
3. Практикум з ідентифікації лікарської рослинної сировини: навч. посіб. / [В. М. Ковальов, С. М. Марчишин, О. П. Хворост та ін.] ; за ред. В. М. Ковальова, С. М. Марчишин. – Тернопіль: ТДМУ, 2014. – 250 с.

10. The topic of the next lesson:

Lignans. Xanthoni. LR and LRS containing lignans and xanthones. Chinese lemongrass, prickly eleutherococcus, milk thistle. Species of yarrow, St. John's wort.

Methodical recommendations were made by

associate professor Boyko IA