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## EC 12-VII Methods of Biochemical Analysis of Plants

Code	EC 12-VII
ECTS credits	5
Attendance time	7 Semester
Language of instruction	Ukrainian, English
Duration	1
Cycle	Each Winter Semester
Coordinator	Associate Professor, PhD, Andrii Schogolev
Instructor(s)	Associate Professor, PhD, Andrii Schogolev
Allocation of study programmes	Biology
Recommended prerequisites	<ul style="list-style-type: none"><li>Plant Physiology and Biochemistry</li></ul> Students must have basic knowledge of inorganic and organic chemistry and skills of working in chemical laboratory
Learning objectives	<ul style="list-style-type: none"><li>Gain proficiency in biochemical techniques commonly used in plant analysis.</li><li>Understand the principles behind different biochemical assays.</li><li>Learn to critically analyze and interpret experimental data.</li><li>Apply biochemical methods to address research questions in plant biology</li></ul>
Syllabus	Chapter 1: Determination of Carbohydrate Content <ul style="list-style-type: none"><li>Topic 1: Techniques of Laboratory Work<ul style="list-style-type: none"><li>Laboratory equipment and classification of reagents</li><li>Characteristics and production of distilled water, de mineralized water</li><li>Chemical dishes, calibrated dishes, heating devices</li><li>Scales and hanging, devices for measuring temperature</li><li>Shredding of reagents and plant material</li><li>Filters and filtering, extraction of substances, cleaning reagents</li><li>Recrystallization of chemical reagents</li></ul></li></ul>

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- Methods of growing plants for physiological and biochemical analyses
- Field method, vegetation method: sand and water cultures
- Selection of plant samples for chemical analysis, fixation, preservation
- Topic 2: Methods of Determining Soluble Sugars
  - Features of sugars and methods of their determination
  - Isolation of sugars from plant material, water, and alcohol extraction
  - Determination of soluble and reducing sugars
  - Determination of total sugar content
  - Determination of glucose, fructose
- Topic 3: Analysis of the Content of Polymeric Carbohydrates
  - Isolation and purification of starch
  - Volumetric and calorimetric methods of determining the amount of starch
  - Isolation, dissolution, and quantification of fiber content
  - Isolation and hydrolysis of fructoses
  - Quantitative determination of fructose content

#### Chapter 2: Analysis of Nitrogen- and Phosphorus-Containing Compounds

- Topic 4: Methods of Determination of Nitrogen-Containing Compounds
  - Determination of nitrogen according to Kjeldahl
  - Material preparation, incineration
  - Determination of protein and non-protein nitrogen by the Kjeldahl method
  - Colorimetric protein determination method (according to Lowry)
  - Protein analysis according to Lowry in Miller's modification
  - Analysis of ammonia content
  - Methods for determining the amount of nitrates
- Topic 5: Phosphorous Compounds in Plants, Methods of Their Determination
  - Determination of total phosphorus by the Fiske-Subbarow method
  - Determination of total phosphorus content in plant material
  - Material preparation, incineration, coloring, colorimetry
  - Extraction and quantification of various phosphorus compounds
  - Determination of acid-soluble phosphorus
  - Extraction and quantification of lipid phosphorus

	<ul style="list-style-type: none"> <li>• Determination of phosphorus according to the Lowry and Lopez method</li> <li>• Methods of determining nucleic acids</li> </ul> <p>Chapter 3: Methods of Plant Enzymology</p> <ul style="list-style-type: none"> <li>• Topic 6: Basic Principles of Enzyme Isolation and Purification <ul style="list-style-type: none"> <li>• Production of extract, grinding of tissues, composition of medium</li> <li>• Centrifugation, measurement of enzyme reaction speed</li> <li>• Techniques for working with protein solutions, concentration, salt removal</li> <li>• Buffer change, stabilizing factors for enzymes</li> <li>• Enzyme purification methods, detergents, precipitation of proteins</li> <li>• Salting out, denaturation, use of chromatographic columns</li> </ul> </li> <li>• Topic 7: Study of Catalytic Properties of Enzymes <ul style="list-style-type: none"> <li>• Definition of the Michaelis constant</li> <li>• Conditions for the enzymatic reaction</li> <li>• Dependence of the reaction rate on temperature and pH</li> <li>• Effect of activators and inhibitors on enzyme activity</li> </ul> </li> </ul>
Literature	<ol style="list-style-type: none"> <li>1. Aebi H (1974) Methods of enzymatic analysis. In Bergmeyer HU, Ed, Catalase. Academic Press, New York, pp 673–675</li> <li>2. Kar M, Mishra D (1976) Catalase, peroxidase, polyphenol oxidase activities during rice leaf senescence. Plant Physiol 57:315–319</li> <li>3. Lichtenthaler H (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148:350–382</li> </ol>
Teaching and learning methods	Laboratory (8 WH)
Workload	Classroom hours: 120 Individual study time: 30 <b>Total: 150</b>
Assessment	The assessments consists of the laboratory work performance and individual project accomplishment
Grading procedure	The module grade is the sum of study achievements during the semester and credit work
Basis for	<ul style="list-style-type: none"> <li>• Quality Control and Standarts of Biological Products</li> </ul> Elective courses and Course project